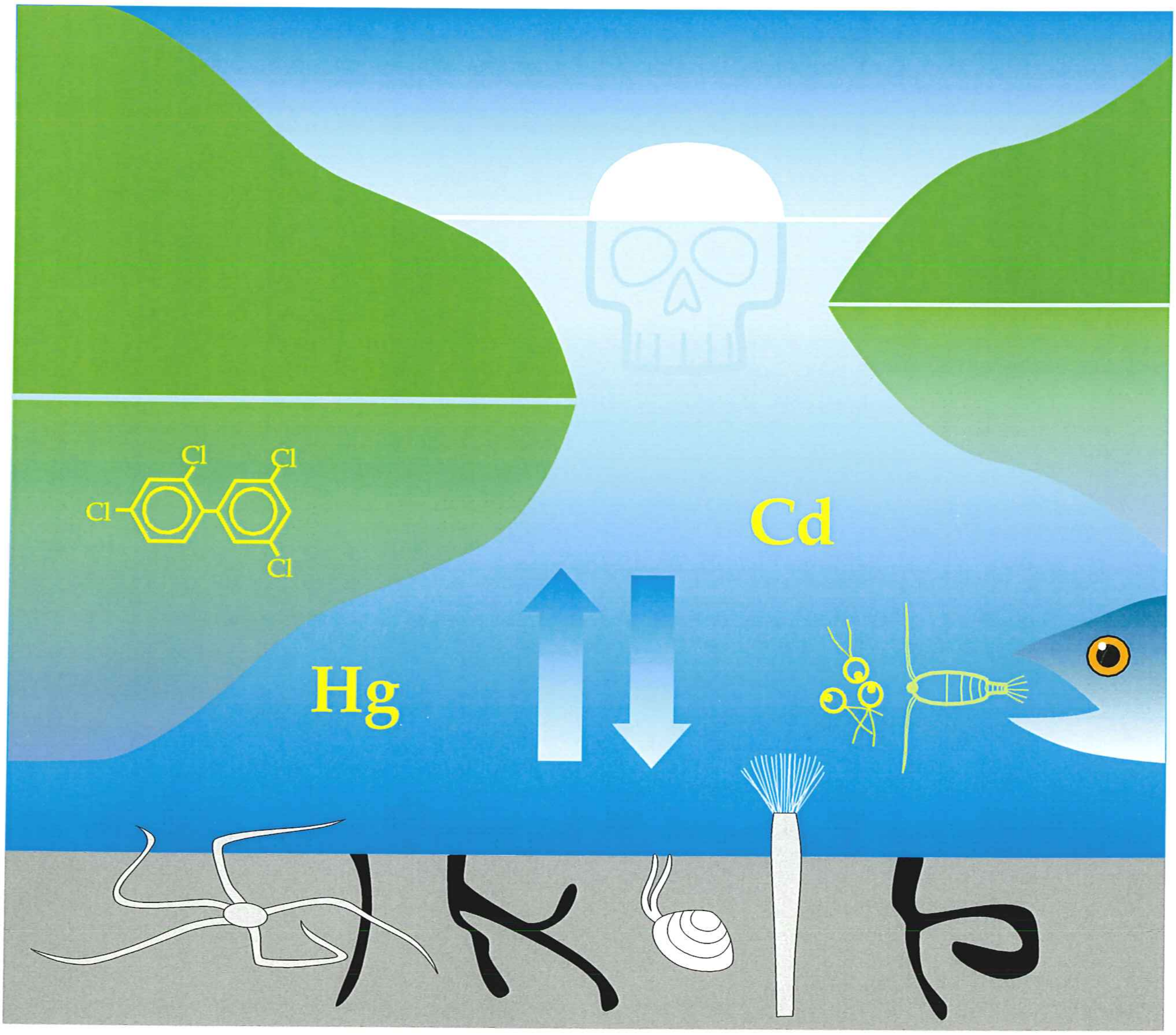
REPORT SNO 4129-99

Interaction between Contaminants and Eutrophication (MILE) 1995 - 1999



Final report
Strategic Research Programme
Norwegian Institute for Water Research
(NIVA)

Interaction between Contaminants and Eutrophication (MILE) 1995-1999

CONTENTS

Synthesis - MILE Research Programme (1995-1999)

The MILE research programme constitutes nine individual projects; six of them being devoted to benthic processes and three to pelagic processes. The contaminants of concern in these projects are heavy metals (Cd, Hg, Cu), metal organic compounds (TBT), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides (DDT).

Experimental work on the toxic effect of Cu on phytoplankton and the influence of variable amounts of nutrients, including metals like iron and manganese in the water, indicates a strong species dependence. However, generally phytoplankton is more sensitive with respect to copper toxicity in low nutrient regimes. The salinity has apparently rather limited influence on the toxicity. Both manganese and iron appear to have a detoxifying effect. From these experiments it may be concluded that the concentrations of nutrients (including iron) have a significant effect on heavy metal toxicity on planktonic species. *The results suggest that a reduction of inputs of nutrients to the marine environment without reducing the input of heavy metals (specifically copper) may have negative effects on phytoplankton.*

Re-oxidation of anoxic sediments in the marine environment may occur as an indirect consequence of reducing the inputs of nutrients and organic matter. A change in redox at the sediment-water interphase may have dramatic effects on the mobility and bioavailability of sediment bound contaminants. Trace metals like Hg and Cd are trapped in the sediments as metal sulfides. If the concentration of H_2S decreases, the level of Cd in the porewater increases. By re-oxidizing a Cd contaminated sediment the level of Cd in the porewater increases. At the same time the level of Cd in organisms exposed to the pore water increases.

Anoxic sediments are rich in organic matter and by re-oxidizing the sediment a large pool of metabolisable organic matter becomes available for organisms. *The conclusion is that a change from anoxic to oxic conditions at the sediment-water interphase increases the potential bioaccumulation in sediment living organisms.*

In deep fjord basins, eutrophication is frequently associated with increased input of organic matter and decreased availability of oxygen. Organic matter for food and oxygen for respiration are factors likely to affect bioaccumulation of toxic metals like Cd and Hg in sediment living organisms. Experiments on bioaccumulation of Cd and Hg in three test organisms living in sediments indicate that different feeding strategies play an important role with respect to metal uptake. *Bioaccumulation of both metals was primarily controlled by ingestion of contaminated sediment particles and stimulated by organic enrichment.*

Contaminated sediments, particularly in harbours, often contain high levels of oily substances. A hypothesis is that oil as a carbon source may interact with contaminants. *Experiments have shown that the presence of oil decreases the bioavailabilty of Cd, TBT and PCB in sediments. Furthermore, the carbon source of oil is apparently not utilized by organisms, at least not within the time frame of the experiment (3 months).*

Additionally, marine sediments in urban areas are often contaminated with PAHs. Experiments have been performed to investigate the effect of adding labile organic matter to such sediments. *Results have shown that the bioaccumulation increases with increased organic matter content in the sediments. The quality of organic matter appears to be of importance due to selective feeding on contaminated organic particles.*

The quality of organic matter also plays a role with respect to accumulation of Cd in sediment living organisms. An extensive input of organic matter to the sediments influences the redox conditions and Cd may be fixed in the sediment as a non-bioavailable metal sulphide ($CdS_{(s)}$). *If oxic conditions are maintained in the sediment, an increase of labile organic matter of algal origin leads to increased accumulation of Cd in the organisms. If the carbon source is wood fibres or sewage the bioaccumulation of Cd is less.*

An increase of labile organic matter input to sediments also appears to influence growth of sediment-dwelling organisms. *This implies that an increase in the state of eutrophication increases both the bioavailabilty and bioaccumulation and the growth of sediment-dwelling organism.*

One of the characteristics of eutrophic systems compared to oligotrophic systems is the increased biomass in the former. A study with an objective to clarify relationships between changes in biomass due to increased food availability and changes in bioaccumulation of contaminants showed that *both organic and inorganic contaminants bioaccumulate in benthic invertebrates to a greater extent under eutrophic conditions compared to oligotrophic conditions. The rate of bioaccumulation was species dependent.*

The effect of food particle quality (organic content) on bioaccumulation of PAH in mussels has also been investigated. *Apparently an increase in the organic content of the food particles elicited only a slight reduction in PAH accumulation, but with no significant difference between pre- starved and fed mussels. This implies that eutrophic status to a little extent influences the levels of PAH in mussels.*

Historical data of nutrients and levels of contaminants in fish and shellfish exist from some areas; i.e. Oslofjord. It was of interest to investigate if there is a link between changes in nutrient load or Secchi depth (as an indirect measure of eutrophication) and changes in the levels of contaminants in fish. Apparently it is difficult to identify such interactions as other factors than eutrophication affect contaminant levels in fish.

Interaction between Contaminants and Eutrophication (MILE) - the establishment of a strategic research programme (SIP).

Jens M. Skei

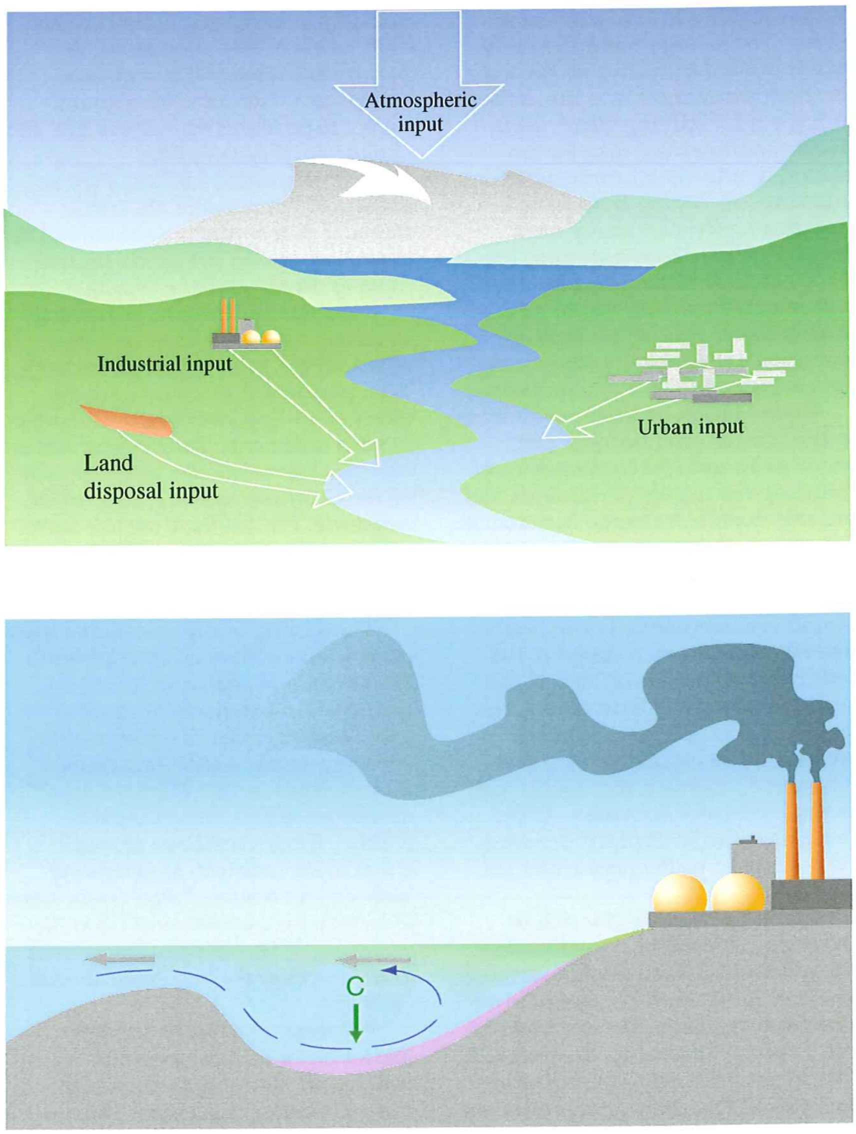
Eutrophication and persistant pollutants are the two main environmental problems in European marine and freshwater ecosystems. As they tend to co-occur, interactive processes between eutrophication and contaminants have been in focus for a long time in the scientific community (fig.1). However, the magnitude and the relevance of these interactive processes in natural aquatic ecosystems are not known.

In order to predict the consequences of remedial measures it is important to have tools that can predict ecosystem responses to changes in load. One question that needs to be answered in this context is what effect different combinations of reductions in the loads of nutrients, heavy metals and organic hazardous substances would have on the ecosystem.

Environmental risks will depend on the ultimate toxicity of the contaminants as well their speciation and association with other substances. Furthermore, the risks will depend on the mobility of the substances and their pathway in food chains. This implies that development of environmental criteria and classification of contaminants with respect to risks, based on total concentrations and no information about natural habitats and influencing factors, may be misleading. The ultimate consequence may be that wrong decisions are taken with respect to remedial actions for improving the environment. It is important to understand basic processes taking place in a natural ecosystem which influence the fate of contaminants with respect to transport, biogeochemical cycling, persistence, bioavailability, bioaccumulation, biomagnification and biological effects. This requires a multidiciplinary and holistic approach.

Norwegian Institute for Water Research (NIVA) has a long tradition in research and development in the fields of eutrophication and contaminants, both with respect to sources, transport, processes and effects. In 1995 NIVA presented a proposal to the State Pollution Control Authority (SFT) addressing the problem of interaction between eutrophication and contaminants. This resulted in two projects, partly financed by SFT and partly by the strategic program (SIP). Additionally, this topic was also adopted by Program on Marine Pollution (1992-1996) financed by the Norwegian Research Council and by the Nordic Council of Ministers. This implies that the SIP-projects have had several financial sources.

Figure 1. A schematic illustration of sources of contaminants (upper) and input of industrial waste containing contaminants and a phytoplankton bloom in a typical Norwegian fjord (lower).



Objectives.

The overall object is

- to assess and predict interactions between contaminants and eutrophication in marine ecosystems

Furthermore the programme intends

- to provide a scientific basis for the role which organic matter plays with respect to bioavailability, mobility of sediment bound contaminants and effects on growth or biomarkers in invertebrates
- to demonstrate the role of indirect effects of eutrophication on redox conditions and mobility of trace metals
- to assess the influence of organic matter quality on bioavailability of contaminants
- to assess the influence of level of eutrophication in a pelagic system with respect to toxic effects of metals on phytoplankton and bioaccumulation of PAHs in mussels

Utilization of the results.

The results are essential in environmental risk assessments and in the development of operational environmental criteria. The results are also relevant with respect to environmental monitoring and may be of importance regarding improvement of the quality of monitoring programmes.

The objective is to make results available for environmental decision makers and scientists in the field of environmental science. In practical terms the results may be used as a scientific basis for remedial actions (changes in loads). Furthermore, the results may give us insight in important mechanisms regarding the fate of contaminants in the aquatic environment. Finally, the results may have important implications with respect to predicting how changes in the eutrophic status of an aquatic ecosystem influence the environmental effects of contaminants.

Projects and activities - objectives, descriptions, highlights.

During the programme period the following projects have been defined:

1. The effects of heavy metals on phytoplankton in eutrophic environments (Evy R. Lømsland and Torbjørn M.Johnsen).
2. Bioavailability of Hg, Cd, PCB and DDT during reoxidation of anoxic sediments from the Drammensfjord (Morten Schaanning)
3. Interactions of oil and organic material with contaminants in marine sediments - distribution, bioaccumulation and biological effects (Ketil Hylland and Norunn Følsvik).
4. Interaction between eutrophication and contaminants - experimental work on contaminated sediments at NIVA Marine Research Station Solbergstrand (MFS). The following sub-projects were established:
   - Mobilization and bioaccumulation of Hg and Cd from marine sediments (Morten Schaanning, Ketil Hylland and Dag Ø. Eriksen)
   - Mobilization and bioaccumulation of benzo(a)pyrene from marine sediments (Jonas Gunnarsson, Morten T. Schaanning, Ketil Hylland, Mattias Sköld, Dag Ø.Eriksen, John Arthur Berge and Jens M. Skei)
   - Effects on sediment-dwelling organisms (Ketil Hylland)
   - Increased bioaccumulation and potential for trophic transfer of contaminants under eutrophic conditions (Ketil Hylland, Morten Schaanning, Mattias Sköld, Jonas Gunnarsson, Dag Ø. Eriksen, Jens Skei)
5. Effects of organic enrichment on the accumulation of PAH in blue mussels, *Mytilus edulis* (Torgeir Bakke)
6. Influence of organic enrichment on partitioning and bioavailability of Cd (Joanna Maloney)
7. Time-trend analysis of contaminants in cod (*Gadus morhua*) in two areas with different eutrophication regimes (Ketil Hylland, Birger Bjerkeng, Norman Green).
8. Workshop - MILE/EUCON, Tømte, November 1998.

The effects of heavy metals on phytoplankton in eutrophic environments

Evy R. Lømsland

Rationale and objectives.

Plankton algae both adsorbs and absorbs heavy metals. Besides the concentration of a specific metal there are to main factors, chemical and biological, controlling the toxicity of metals for plankton algae. The chemical factor involves chemical reactions between the specific metal and the other chemical components in the medium or in the natural environment. The biological factor involves the various ways of different species to cope with toxicity. The toxicity of a specific metal can be reduced by the alga itself by special handling of the metal within the algal cell or outside the cell by excreting organic components reacting with the toxic component. The sensitivity is therefore species related.

The main object has been to establish some knowledge of the effects of heavy metals on different marine phytoplankton species under varying concentrations of nutrients and at different salinities. Copper has been used as the toxic component.

Project description.

Growth conditions for the batch cultures were the commonly used seawater based medium *f* at a strength *f/2* and *f/40*. All experiments were performed without chelator because chelators have detoxifying effects. The algal species used was the flagellates cf. *Cryptomonas* sp., *Pseudopedinella pyriforme* N. Carter and *Pyramimonas disomata* McFadden et al., and the diatoms *Chaetoceros wighamii* Brightwell and *Skeletonema costatum* Greville, all unialgal isolates from the west coast of Norway.

Laboratory experiments with focus on the separate components of the medium were done for two of the species, *S. costatum* and *P. disomata*, and assess the effect of sublethal concentrations of the essential metals for algal growth: Zinc (Zn), copper (Co), manganese (Mn), molybdenum (Mo), iron (Fe), different components of nutrients: nitrate-N , phosphate-P, silicate-Si and vitamins to the toxicity of copper.

Table 1. Effect levels of copper (Cu) for different algal species at two growth medium concentrations.

Species	Salinity	Medium f/2 Reduced growth µg Cu/L	Medium f/2 No growth /Lethal µg Cu/L	Medium f/40 Reduced growth µg Cu/L	Medium f/40 No growth /Lethal µg Cu/L
Pyramimonas disomata	34	5-25	50	5-10	15
Pseudopedinella pyriformis	34	50-75	100	25	50
cf. Cryptomonas sp.	34	75-150	200	50	75
Skeletonema costatum	11	150-400	>400	5-100	150
Chaetoceros wighamii	34	100-400	>400	10-100	150

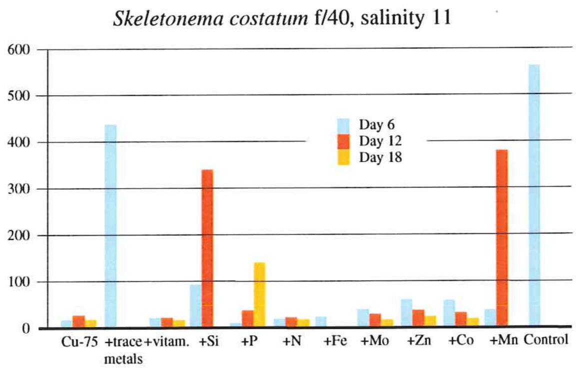


Figure 2. *Skeletonema costatum.* Detoxifying effects of different components added in a concentration of *f/2* to a growth medium of *f/40* enriched with copper (75 µg Cu/L).

Results - highlights.

The results showed that the toxic effect of copper was strongly species dependent. The green flagellate *Pyramimonas disomata* was the most sensitive species (table 1).

- All the species were more sensitive to copper at low nutrient concentration (*f/40*).
- The relative small effect of lowered salinity was both positive and negative dependent on the species involved.
- Iron immediately detoxified copper (fig. 2) with respect to both *S. costatum* and *P. disomata*.
- A detoxifying effect of manganese was recorded for both species.
- The detoxifying effect of silicate was most pronounced for *S. costatum*. Silica is an essential element for this organism.
- After a lag face, phosphate showed positive effect on toxicity for *S. costatum*.
- Zinc, nitrate and to some extent also molybdenum seemed to increase copper toxicity in *P. disomata*.

Gaps of knowledge

The above results indicate the complexity of the interaction of heavy metals and nutrients with respect to phytoplankton. This approach should be followed up incorporating both essential and not essential heavy metals. Components having positive effect on toxic levels of essential heavy metals may turn out to be a negative factor at low concentrations, reducing availability of essential trace metals for growth. In the view of the species specific effect of heavy metals and the occurrence of special blooming events in the heavy metal and nitrogen polluted fjord Sørfjorden in Hardanger it is of current interest to examine more algal species common in Norwegian coastal waters. (the results from this project are being published by Lømsland and Johnsen, in prep.)

Bioavailability of Hg, Cd, PCB and DDT during reoxidation of anoxic sediments from the Drammensfjord

Morten T. Schaanning

Rationale and objectives.

The Drammensfjord is a 20 km long and 122 m deep fjord separated from the open ocean by a shallow sill. For more than a century organic material and micro pollutants has been added to the fjord from upstream industrial and domestic sources. The pollution history culminated with the abatement of the pulp and paper industry at about 1970, but oxygen deficiencies still prevail at depth. Even though conditions may have improved over the last 10-20 years, anoxic waters are frequently observed below 40-60 m depth and the laminated sediment structure (fig. 3) indicated that the deep bottom areas had been devoid of bioturbators for several decades. If oxic conditions were to be restored in the bottom water, benthic animals will rapidly colonize the area and perturbate the fragile laminae. During this process, deposited contaminants previously unavailable to marine food chains may become remobilized. The objective of this project was to obtain some experimental evidence of the bioavailability of selected contaminants during recolonisation.

Project description.

Laminated, anoxic sediments were transferred from 115-m depth in the Drammensfjord to NIVA Marine Research Station Solbergstrand. For control purposes, oxic sediments were simultaneously collected from a near-by location at 25-m depth. In the mesocosm, the sediments were exposed to oxygenated seawater continuosly supplied from 60-m depth in the near-by fjord (fig. 3). As soon as hydrogen sulphide were no longer detected in the water downstream the anoxic boxcosms, polychaetes (*Nereis diversicolor*) were added and the outflow water was connected to mussel-aquaria. The polychaetes spread rapidly over the sediment surface and disappeared into the laminated sediment. Three months later, 265 of 450 added individuals were recaptured for chemical analyses, as compared to 280 of the 450 added to the oxic control. The polychaetes recaptured from the anoxic sediments had increased their fat content and the mussels downstrean had increased in biomass (fig. 4), indicating that the laminated sediment had offered favourable nutritional conditions for the experimental organisms.

Results - highlights.

The sediments were moderately polluted with cadmium and mercury and moderately to strongly polluted with PCB and DDT. Only Cd re-

Figure 3. Set-up at Solbergstrand. Separate flows of oxic fjord water was added to the two boxes in front, flushing the sediments from the oxic loaction (left-hand row) and anoxic location (right-hand row) and the mussel aquaria in the back. The core (inserted) subsampled from the anoxic boxes divided along the laminae, almost like a stack of cards.

Figure 4.
Final:initial concentration ratios of Hg, Cd, ΣPCB and ΣDDT in the polychaete *Nereis diversicolor* and the blue mussel (*Mytilus edulis*). The improved condition (c = fat content or biomass) of organisms exposed to the anoxic sediments tended to confirm the nutritional value of the simultaneous loss of organic carbon and nitrogen from the sediment (fig. 5). The polychaetes exposed to the anoxic sediment, accumulated PCB but lost metals. Accumulation of Cd from the oxic sediments was consistent with the observed increase of Cd in pore waters (fig.6). The trends observed in the blue mussels held in downstream aquaria were similar to the trends in the polychaetes, but because of the more remote sediment exposure changes were frequently smaller. DDT, however, accumulated strongly in both treatments and more strongly in the mussels than in the polychaetes. The inlet water was found to be the source of this DDT. Because local contaminant sources are not known or easy to imagine, the Oslofjord deep water may appear to be an important source of DDT.

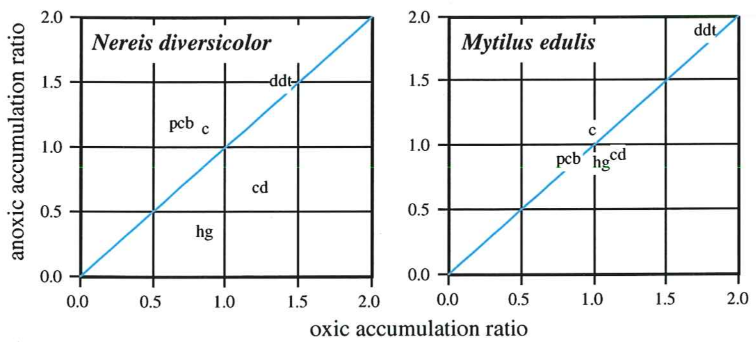
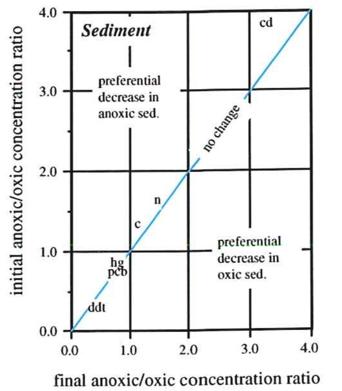


Figure 5.
Anoxic:oxic concentration ratios of C, N, Hg, Cd, ΣPCB and ΣDDT in the sediment at the start and end of the three-months experiment. Carbon and nitrogen were clearly lost from the anoxic sediment during the experimental period, whereas Hg, PCB and DDT were not. Cadmium was highly enriched in the laminated sediment, but random variation in both anoxic and oxic sediment analyses may have contributed to the apparent loss from the anoxic sediment during the experimental period.



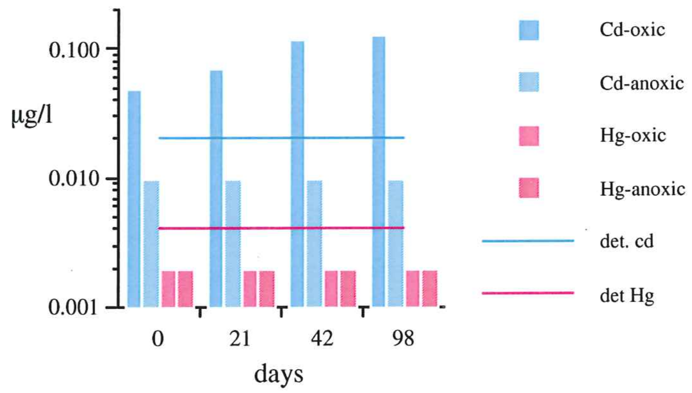


Figure 6. Cadmium in pore water extracted from the oxic sediments, was the only contaminant present at concentrations exceeding detection limits. Note log scale of concentration axis.

vealed a major difference in contaminant level between the two fjord locations. Consistent with a pathway proposed for *in situ* redistribution from oxic to anoxic sediments, Cd increased in the oxic pore waters during the experimental period (fig. 6). This mobilisation of Cd may also explain the accumulation of Cd in organisms exposed to the oxic sediments (fig. 4). In the anoxic sediments, however, both Hg and Cd appeared firmly bound as insoluble metal sulphides, resulting in metal stripping from the exposed organisms (fig. 5).

The experiment showed that the organisms utilized the organic material accumulated in the anoxic sediments for growth and increased fat content. A considerable production of new biomass must therefore be expected to follow reoxidation and recolonization of anoxic fjord sediments. Even though the bioaccumulation of PCB observed in the present experiment may appear moderate, a small uptake multiplied by a large new biomass may represent a significant transfer of contaminants to food chains in the fjord. Both longer periods of exposure, differences between species and biomagnification may result in higher concentrations in other parts of the biota than those obtained during the present experiment. As shown in another experiment reported in this volume, the sediment dwelling mussel *Abra alba* had a much higher potential for contaminant accumulation than the tube worm.

Gaps of knowledge.

DDT behaved differently compared to the other contaminants studied. The mother-compound pp-DDT was nearly absent in the anoxic sediment (and in polychaetes) whereas the concentration of the metabolites pp-DDD and pp-DDE was similar at the two locations. Also the unexpected result that the Oslofjord water appeared to be a major source of DDT needs further confirmation by investigations into the biogeochemical cycling and biodegradation of these compounds.

Interactions of oil and organic material with contaminants in marine sediments – distribution, bioaccumulation and biological effects

Ketil Hylland

Rationale and objectives.

In coastal areas impacted by human activity, it is rare to find single causes for environmental pollution. More commonly, such areas are affected by a range of pollutants, including eutrophication, oil, mechanical disturbance and input of contaminants. Especially in harbours, but also in other industrial areas, there will be a combined input of both oil and contaminants. It is highly likely that the presence of oil will modulate effects of contaminants in the marine environment, both through affecting physical and chemical processes and through effects on sediment-dwelling organisms. Although this relationship is fairly obvious, there are surprisingly few studies that concern such interactions. Oil does of course contain polycyclic aromatic hydrocarbons (PAHs), the effects of which are extensively covered in the scientific literature. There are also data from large-scale mesocosm studies in which whole harbour sediment has been employed. The design of the most relevant study was not such that the impact of oil in that sediment could be assessed, although PAHs in the sediment had clear impact on flounders.

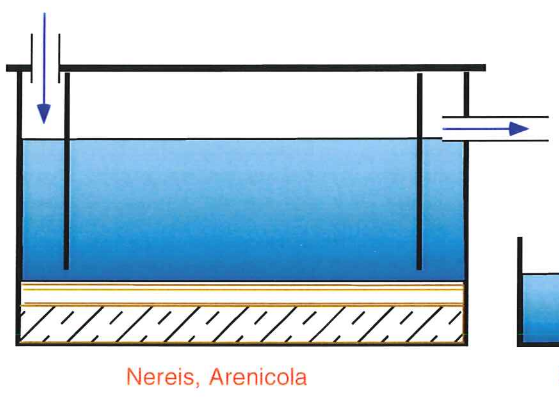
The most relevant contaminants in harbour sediments are, beside oil and PAHs, organic tin compounds (from antifouling treatment of ships), chlorinated compounds and metals (from various sources).

The objective of this study was to clarify whether the presence of oil in a typical marine sediment would affect the bioaccumulation of contaminants in the sediment, the vertical distribution of contaminants in the sediment or modulate effects of contaminants on sediment-dwelling organisms.

Project description.

The study was done at NIVA Marine Research Station Solbergstrand in the outer Oslo fjord (MFS). Sediment was collected from a clean site, sieved to remove all macrofauna and homogenised. Two sediment-dwelling polychaetes, *Arenicola marina* and *Nereis diversicolor*, were collected at the same site (Jeløya, near Moss). Both species are numerous in intertidal mudflats in northern Europe and are ecologically important. *Arenicola marina* is a subsurface deposit-feeder and is an active reworker of the sediment. The other species, *N. diversicolor*, will adapt its feeding strategy depending on the food source available. This species will construct burrows in the sediment, but does not rework the sediment to any greater extent. In addition to these sediment-dwellers, blue mussels *Mytilus edulis*, were kept in aquaria receiving effluent water from the aquaria with sediment (fig.7).

Figure 7. Overview of aquaria used.



Clean sediment was added to a depth of 5 cm in all-glass aquaria. On the top a 1.5-cm layer of treated sediment was added. Three replicate aquaria were used for each treatment. The top sediment was contaminated with tributyltin (TBT), aroclor 1254 and Cd at environmentally relevant concentrations. In a third of the aquaria, weathered diesel oil was added, in a third dried algae were added and in the final third no oil or organic material was added. Twenty individuals of the polychaete *N. diversicolor* was added to all aquaria and one individual of *A. marina* added to half (to assess the importance of bioturbation). The aquaria received a continuous supply of water from 60-m depth and were kept at ambient temperature through a water bath. The experiment was started in the autumn of 1996 and terminated in January 1997. All chemical analyses were performed at NIVA using accredited methods. For more details on design and methods, the reader is referred to Hylland (1997).

Results - highlights.

The presence of *A. marina* clearly affected the sediment in aquaria with no oil, resulting in a down-transport of organic carbon from the surface. This effect was not apparent in aquaria with oil added. Not surprisingly, Cd was transferred from the surface to deeper sediment to a larger extent in aquaria with the bioturbating polychaete present compared to aquaria with only *N. diversicolor* present.

All three contaminants accumulated to lower levels in N. diversicolor kept in sediment with oil added compared to *N. diversicolor* kept in control sediment or sediment with algae added. Surprisingly little Cd accumulated in this polychaete from sediment when oil was present (fig. 8). In addition, there was higher mortality of *N. diversicolor* in sediment with oil added compared to sediments with no oil.

Environmental factors affected bioaccumulation of Cd differently for the two polychaete species. In A. marina, the highest concentrations of Cd were found in individuals held in aquaria with organic material added, whereas the lowest levels were found in aquaria with no material added (control). Mean Cd concentrations in the tissues of *A. marina* held in aquaria with oil added were intermediate between algae and control (fig. 9). This polychaete appears to change feeding activity according to the amount of food available. Higher accumulation from sediment with oil compared to control could result from higher intake of sediment (not probable) or modulation of bioavailability of contaminants in sediments by oil.
The two polychaete species had different abilities to metabolise TBT. In *A. marina*, TBT appeared not to be very efficiently metabolised and was primarly degraded to DBT (dibutyltin), with low concentrations of MBP (monobutyltin). In contrast, most of the accumulated TBT was degraded to DBT and also MBT in *N. diversicolor*. As for Cd, oil inhibited accumulation of organotin in *N. diversicolor*, but there was still a significant accumulation compared to initial levels (fig.10).

The treatments had small effects on total biomass of *N. diversicolor*. There was a somewhat higher net biomass in treatments with algae added compared to treatments with oil or controls. Two of the six aquaria with oil added had high mortality and thus low net biomass, whereas the other two aquaria were similar to control. There were no obvious differences in bioturbation between the treatments (except for aquaria with *A. marina* added), although there was a tendency for aquaria with algae and oil to have higher bioturbation than control aquaria (fig.11).

The most important findings of this study were:

- The presence of oil decreased bioaccumulation from sediment of Cd, organotin and PCB in an omnivore polychaete, *N. diversicolor*.
- Accumulation and transformation of contaminants were very different in the two polychaete species studied, the subsurface deposit feeder *A. marina* and the omnivore *N. diversicolor*.
- The presence of oil did not cause increased net biomass compared to control in *N. diversicolor* and the increased amount of organic carbon (more than in treatments with algae) was not apparently used by this macroinvertebrate in within the 3-month experimental period.
- The presence of oil caused some, although not very strong, decrease in bioturbation of the sediment.

Gaps of knowledge.

There exist few studies on interactions between oil and contaminants (Bridges 1996; Levin et al., 1996). Some of the aspects of oil in sediments are intriguing, especially how it caused a decrease in bioaccumulation of Cd from sediments. There is still little knowledge of interactions between oil and other sources of organic carbon in sediments and how such interactions affect contaminant dynamics and effects. In addition, there is little knowledge of how oil would affect other taxonomic groups of sediment-dwelling organisms. In an another study, major differences were observed between polychaetes, brittle stars and bivalves (Hylland et al., 1997).

Literature references.

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Levin, L., Caswell, H., Bridges, T., DiBacco, C., Cabrera, D., Plaia, G. (1996). Demographic responses of estuarine polychaetes to pollutants: life table response experiments. *Appl. Ecol.*, 6, 1295-1313.

Figure 8. Concentration of Cd in *Nereis diversicolor* held in sediment with indicated treatments. Mean with standard error (n=3).

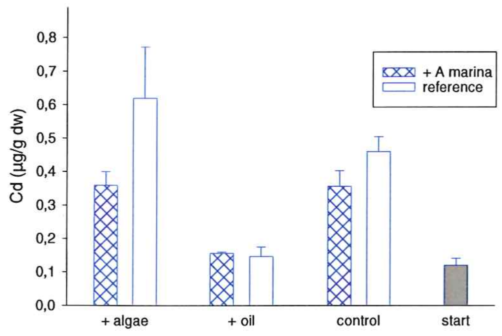


Figure 9. Concentration of Cd in *Arenicola marina* held in sediment with indicated treatment. Mean with standard error (n=3).

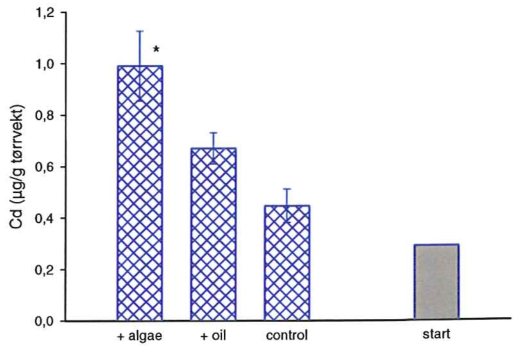


Figure 10. Concentration of organotin in pooled samples of *N. diversicolor* held in sediment with (+Am) or without (-Am) *Arenicola marina*.

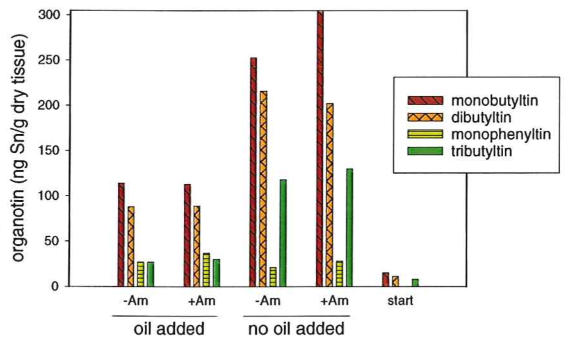
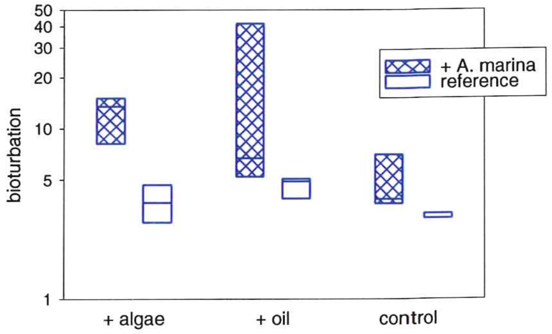


Figure 11. Bioturbation in sediment with indicated treatments. Median and range indicated (n=3).



Mobilization and bioaccumulation of Hg and Cd from marine sediments

Morten T. Schaanning

Rationale and objectives.

In deep basins and fjords, eutrophication is frequently associated with increased input of organic carbon and decreased availability of oxygen. Organic matter for food and oxygen for respiration are primary factors most likely to affect bioaccumulation of toxic metals in benthic invertebrates as in other animals. In order to test the relative importance and possible interactions between low oxygen and carbon enrichment, a two-factor designed experiment was performed at NIVA Marine Research Station Solbergstrand (MFS).

Project description.

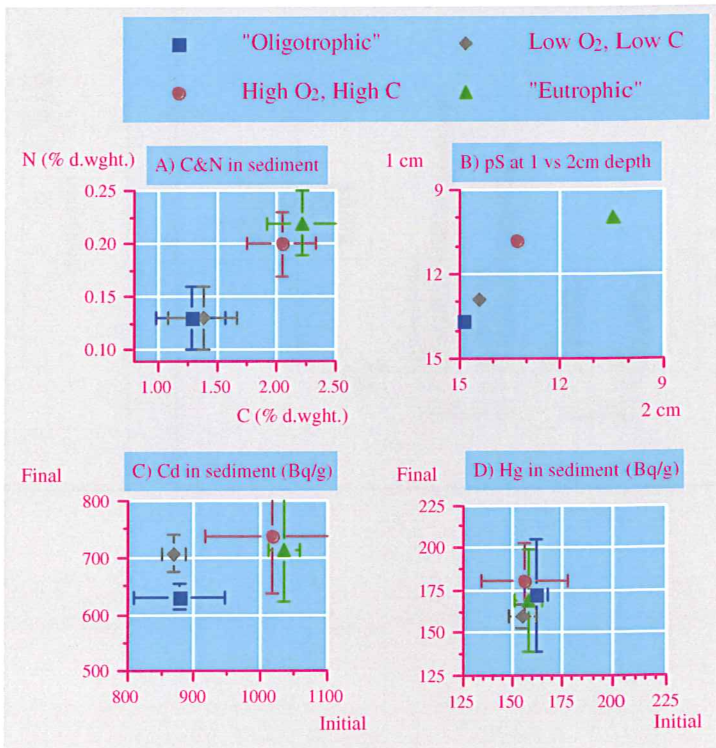
Three species of sediment-dwelling organisms, the polychaete *Nereis diversicolor*, the brittle star *Amphiura filiformis* and the small, white clam *Abra alba*, were kept in sediments spiked with ^{203}Hg and ^{109}Cd. The use of radioactive isotopes allowed precise analyses of many samples with small volumes. Mussels (*Mytilus edulis*) were kept in separate vessels downstream each experimental unit (see fig.7). Four different treatments were obtained by combination of two levels of organic carbon (enriched, not enriched) (fig. 12A) with two levels of oxygen (7-9 mg·l^{-1} and 2-3 mg·l^{-1}) in the water flowing through the aquaria. The required amounts of organic carbon was collected from a batch culture of phytoplankton algae (mostly *Skeletonema costatum*) grown in a large tank at the station area.

Dissolved isotopes were added to two flasks of sediment-seawater slurries and algae was added to one of them. The flasks were then allowed to age for a few days while stirring, before addition to each experimental unit (glass aquarium) by allowing the particles from the slurries to settle through a small column of seawater into a ca. 2 cm thick layer on top of a sediment bottom. The glass aquaria were continuously flushed with seawater from 60 m depth in the Oslofjord, but one line was deoxygenated in a N_2-tower before entering the low-oxygen aquaria. Initial isotope levels (fig.12C and D) were determined in sediment samples drawn from the aquaria shortly after addition. The grand mean of Hg-emission corresponded to 1.14 nmol Hg/g which is classified as "moderately polluted" marine sediment. Similarly, the mean Cd-emission corresponded to 41 nmol Cd/g or "markedly polluted". After an experimental period of three months, gamma emission from the two isotopes were counted in samples of sediments, porewater and biota (fig.13).

Results - highlights.

Quite unintentionally, the experiment demonstrated an important difference between Cd and Hg, which has significant impacts on the natural cycling of the two metals. As shown in fig. 12C and D, the addition of algae yielded increased retention of Cd in the carbon-enriched sediments. Since no such difference between treatments were found at

Figure 12.
Experimental conditions. A) Concentrations of organic carbon and nitrogen in the sediments. Bars represent standard deviation of the three replicate aquaria for each treatment. B) Sulphide ion activities were recorded on Ag-AgS electrodes at 12 h time intervals throughout the experimental period. Each pS-value is the mean -log[H_2S] calculated from 360 integrated readings within the spiked layer. The values are several orders of magnitude below "normal" detection limits for H_2S. C) Cadmium is shown to be enriched in the carbon-enriched treatments, but the initial excess was lost during the experimental period. D) Mercury settled in equal amounts in all treatments and no loss was observed during the experimental period.



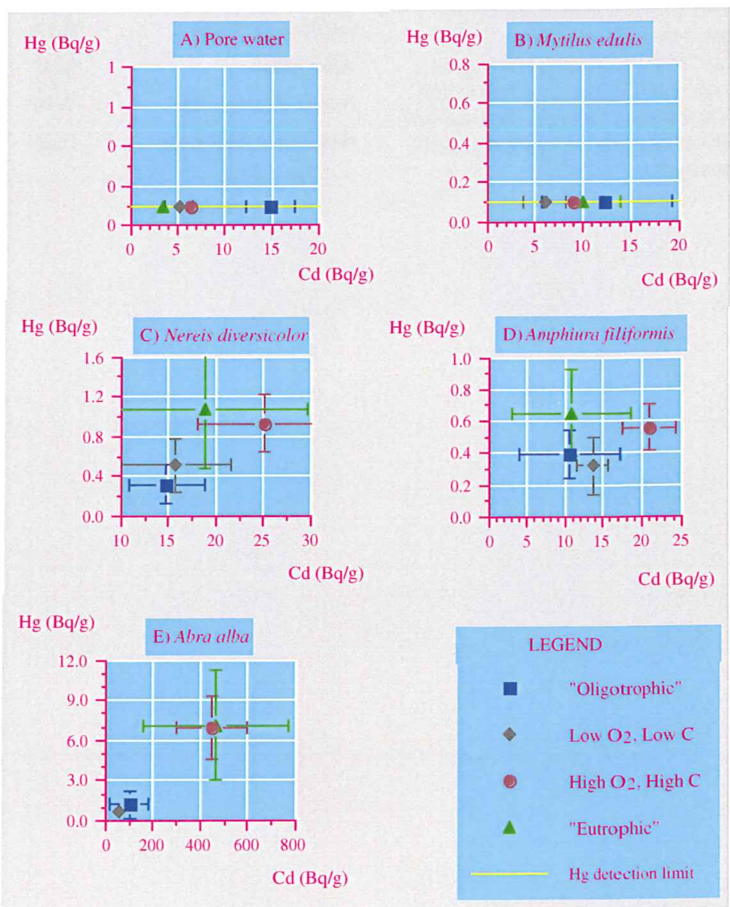
the end of the experiment, the excess Cd appeared to have been present in a labile association with algae material, which disappeared during the experimental period. In an anoxic fjord such as the Drammensfjord (ref. re-oxidation experiment, page 7) a similar mechanism may cause algae-associated Cd deposited in shallow, oxic sediments to be recycled until trapped as insoluble sulphides causing the Cd-enrichment observed in the deep anoxic sediments (fig. 5).

Another unintended result of the experiment was the systematic differences observed between the sulphide ion activities recorded on the Ag-AgS electrodes at fixed positions in the spiked sediment (fig. 12B). The results indicated that the activity of sulphate reducing bacteria may exert important control on metal cycling, not only in black sediments smelling of H_2S, but also in apparently oxic sediments with sulphide ion activities several orders of magnitude below "normal" detection limits at about pS = 6.

The experiment was designed to perform a two-way ANOVA (statistical analyses of variance) (table 2) on the effects of carbon enrichment and oxygen level on pore water distribution and bioaccumulation of the two metals. Based on the results of this analysis it was concluded that the bioaccumulation of both metals were primarily controlled by ingestion of sediment particles and stimulated by carbon enrichment. The large uptake in the clam was assumed to result from the ability of this organism to feed selectively on the added algae, onto which a significant fraction of the Cd apparently was adsorbed.

Because of the much higher concentrations of Cd in the pore water of the oligotrophic treatment, it might have been expected that the polychaete and the brittle star would also accumulate more Cd in this treatment. This was not found, possibly due to masking by the carbon factor stimulating uptake through feeding. The statistical analyses (table 2) showed that oxygen had stronger effects on the pore water and the brittle star, than on the polychaete. The polychaete is tolerant to low oxygen and able to survive severe sulphide events without leaving their burrows within the sediment. The brittle star is more sensitive and has been observed to respond to low oxygen by surfacing from their normal location inside the sediment. Similar behaviour has been observed for other organisms as well. They may do this for two reasons, either to obtain oxygen from the seawater, or to escape from the hydrogen sulphide which is highly toxic to all species. In the present experiment, sulphide concentrations were never severe and both species remained buried. If the polychaete has a better

Figure 13.
Metal distribution in pore water and organisms at the end of the experiment. Mercury was not detectable in the pore water (A) or in the blue mussels kept in the downstream aquaria (B). Cd was clearly elevated in the pore water of the most oligotrophic treatment and a corresponding Cd-signal occurred in the downstream mussels. Moderate activities of both metals were observed in the polychaete (C) and the brittle star (D). It appears that carbon stimulated uptake of both metals, but that the brittle star was more sensitive to oxygen than the polychaete. Diagramme E shows that the clam *Abra alba* has a much higher potential for metal accumulation than the other organisms and that the carbon factor was clearly dominant for this organism.



protection against H_2S from the pore water it might also be expected to reflect pore water Cd to an even lesser extent than the brittle star. We were not able to demonstrate any correlation between pore water and organism concentrations, but the fact that the oxygen factor was important for pore water and brittle star, but not for the polychaete, may suggest that sulphide avoidance is a primary motivation for this particular behaviour of the brittle star.

Gaps of knowledge

An obvious conclusion from this experiment was that each specie has a rather unique response with regard to metal accumulation. Physiological differences and feeding behaviour may both be important. We also feel that the performance of well-designed experiments with a high degree of realism with regard to dose levels and the environment of experimental organisms has a great potential for hypothesis testing as well as the provision of unexpected results obtained through "hands-on" exploration of remote systems such as the seabed.

Table 2.
Analyses of variance (ANOVA) on the effects of oxygen level, organic addition and oxygen . organic interactions on the activities of ^{109}Cd and ^{203}Hg in sediment, pore water and biota. R^2 = fraction of total variance explained by the model, n = number of analyses and p = probability. Shaded cells highlight significant effects (at the 95% level).

	R^2	n	p		
			oxygen	organic	interactions
Cd					
Sediment, initial	0.69	12	0.92	0.003	0.72
Sediment, final	0.32	12	0.54	0.20	0.26
Pore water	0.92	12	< 0.0001	0.001	0.08
M. edulis	0.13	37	0.01	0.10	0.003
Abra alba	0.81	12	0.95	0.0001	0.66
Nereis diversicolor	0.02	36	0.20	0.05	0.20
Amphiura filiformis	0.20	35	0.05	0.18	0.01
Hg					
Sediment, initial	0.06	12	0.75	0.82	0.56
Sediment, final	0.12	12	0.43	0.55	0.99
Abra alba	0.87	12	0.47	<.0001	0.38
Nereis diversicolor	0.14	36	0.53	0.003	0.33
Amphiura filiformis	0.24	35	0.95	0.06	0.14

Mobilization and bioaccumulation of benzo(a)pyrene from marine sediments

Rationale and objectives.

Sediments are frequently contaminated by PAH-compounds. An increase of the organic loading on the sediment surface may affect physio-chemical characteristics, such as water solubility, sorption capacity, fugacity and partitioning of PAHs, which again may influence bioavailability and bioaccumulation. In sediments, the effect on benthic fauna is complex due to different exposure routes, such as pore water and different types of particles loaded with contaminants.

The objective of this project was to conduct an experiment at NIVA Marine Research Station Solbergstrand (MFS) where the effects of two factors; organic enrichment and hypoxia, could be assayed on the distribution patterns of benzo(a)pyrene in the sediment and in associated benthic macrofauna.

Project description.

The experimental design is shown in figure 14. The experiment was run for 93 days, using 24 glass aquaria with sediment from the Oslofjord; 12 of them receiving oxygenated water (7-9 mg/l O_2) and 12 receiving deoxygenated seawater (2-3 mg/l O_2). Radio-labelled PAH (B(a)P) and organic matter (phytoplankton culture) was added to the sediments to simulate a situation where a PAH-contaminated sediment is exposed to increased eutrophication.

The brittle star (*Amphiura filiformis*), clam (*Abra alba*) and *a polychaete* were added to the sediment surface. Blue mussels were placed in down-stream aquaria (see fig. 7) to accumulate PAHs being released from the up-stream contaminated sediment.

Results - highlights.

- Results from this experiment show that addition of labile organic matter to the sediment increases the bioaccumulation of organic contaminants in benthic organisms. The accumulation was highest in the clam (fig.15).
- The reason may be high nutritional quality of the organic matter of planktonic origin which may enhance the contaminant uptake via selective feeding on contaminated organic particles.
- This suggests that the quality of organic matter may be of essential importance to explain the short term bioaccumulation patterns.
- Hypoxia had no significant effect on the bioaccumulation of PAH.

(results from this project has been published by Gunnarsson et al., 1996)

Figure 14.
The setup in two large water baths with separate header tanks containing water pumped from 60 m depth at Marine Research Station Solbergstrand. Sediment and sediment-dwelling organisms were added to the large aquaria , whereas the smaller aquaria were used for blue mussels (from Skei et al., 1996)

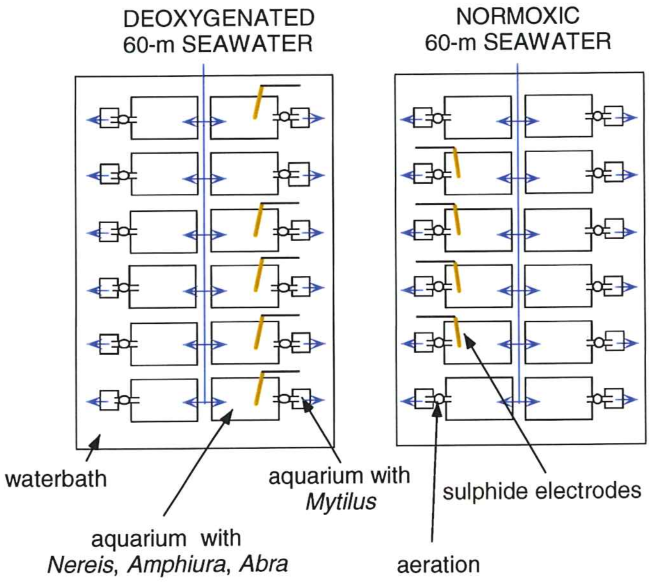
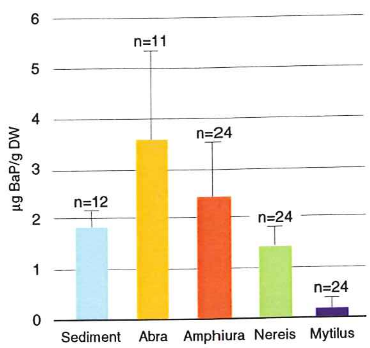


Figure 15.
Comparison between B(a)P in sediment and animals (from Gunnarsson et al., 1996).



Interactions between eutrophication and contaminants - effects on sediment-dwelling organisms

Ketil Hylland

Rationale and objectives.

Eutrophication and contaminant inputs are features of most estuaries and harbours in industrialised countries. Organic enrichment, the presence of contaminants and variable oxygen availability would be expected to interact in their effects on marine biota, but there are surprisingly few studies on whether and to what extent this is the case (Baxter et al., 1992; Gunnarsson et al.,1995).

Eutrophication could be expected to modulate effects of contaminants through decreased bioavailability in water and increased sedimentation and "co-precipitation" of contaminants. (Baker et al.,1985; Evans, 1988; Jonsson, 1992). On the other hand, the presence of contaminants may also affect the biological response to eutrophication. Increased food availability such as that found in more eutrophic systems would be expected to result in increased growth and reproduction (Dauvin & Gentil, 1989; Sköld & Gunnarsson, 1996), which may be decreased in the presence of contaminants (Herman et al., 1991). One of the The third factor referred to above, decreased oxygen availability, may affect the bioavailability of contaminants (e.g. Krantzberg, 1994), decrease growth and affect reproduction (Nilsson & Sköld, 1996) and have deleterious effects on benthic organisms (Rosenberg et al.,1991).

This study had three main objectives. Firstly, to identify effects of organic enrichment, decreased oxygen availability and moderate contamination for growth of selected sediment-dwelling invertebrates. Secondly, to identify interactions, if any, between these three environmental factors in the effects they exert on the sediment-dwelling organisms. Finally, to clarify whether bioaccumulation of selected contaminants affect responses at the cellular level (biomarkers) in sediment-dwelling organisms.

Project description.

To approach this problem we designed a microcosm experiment with three sediment-dwelling invertebrates, the polychaete Nereis (Hediste) diversicolor, the brittle star Amphiura filiformis and the bivalve Abra alba. These organisms were exposed to three environmental factors: organic enrichment, decreased oxygen availability and the presence of contaminants (sediment spiked with Cd, Hg and benzo(a)pyrene). Twenty-four glass aquaria were used in which a 4-cm bottom layer of standard sediment was similar, and the top 2 cm differed according to treatment. The aquaria were positioned in two water baths and received a continuous supply of filtered sea-water (25 µm filters) from 60 m depth. The temperature in water and sediments dropped from 10.1°C at the start (December 1994) to 6.1°C at the end (March 1995), and salinity varied between 33.6 and 34.6 psu during the 93-day experimental period. Two levels of each three treatments were used: organic enrichment ("C-enriched" and "C-low"), oxygen availability ("deoxygenated" and "normoxic") and contamination ("contaminated" and "uncontaminated"). The top 2 cm of sediment was manipulated as described below and then allowed to settle for 2 days in all aquaria through 15 L of seawater. Before adding organisms, the sediment was allowed to equilibrate for 7 days with a continuous flushing of water from 60-m depth. The flow of water through the aquaria varied between 114 and 166 ml/min through the experimental period.

Three replicates were used for each treatment. The selected contaminants, Cd, Hg and benzo(a)pyrene, were added as non-radioactive substances mixed with appropriate amounts of 109Cd, 203Hg, 14C-benzo(a)pyrene, respectively.The levels of contaminant in the top cm of "contaminated" sediments at the start of the experiment were: Cd 4.8 ± 0.2 mg/kg dry sediment, Hg 0. 23 ± 0.01 mg/kg dry sediment, benzo(a)pyrene 1.32 ± 0.19 mg/kg dry sediment (0-1 cm). In treatments with organic enrichment, approx. 40 g C/m2 were added, 20 g C/m2 to sediment and 20 g C/m2 to water after 2 months. The water in treatments with low oxygen availability kept 2.4-3.5 mg/l oxygen during the entire period, whereas water in the control aquaria was above 7 mg/l during the experiment.

All three selected sediment-dwelling species are ecological key-species, in intertidal (*N. diversicolor;* Möller *et al.*,1985) or subtidal (*A. alba* Rainer, 1985, *A. filiformis;* Sköld *et al.*,1994) soft-bottom communities. Effects on the experimental organisms were evaluated by measuring growth, arm-regeneration and biomarkers (metallothionein-like proteins, glutathione reductase, glutathione *S*-transferase) at the end of a 93-day experimental period. The design of the experiment is described in more detail elsewhere (Hylland et al., 1997).

Results - highlights.

There were no systematic differences between groups regarding survival of the three experimental species through the experimental period and sediment redox values remained high throughout the experimental period. Mean recovery ranged from 77% (*Amphiura filiformis*) to 85% (*Nereis (Hediste) diversicolor*) and 86% (*Abra alba*).

The biomass of *N. diversicolor* increased in aquaria with organic material added compared to aquaria without organic material (fig. 16). In addition, the biomass of this species was negatively affected by decreased oxygen availability. There was also a trend towards decreasing biomass with the presence of contaminants, but this effect was not statistically significant (fig.16). *Nereis diversicolor* is known to be tolerant to low oxygen levels (Vismann, 1990; Miron & Kristensen,1993 a,b) and was able to use available organic material for somatic growth even under hypoxia. The presence of contaminants did not affect growth in *N. diversicolor* in this study, although exposure to similar concentrations of benzo(a)-pyrene in sediment has been found to decrease growth in *N. diversicolor* previously (Hylland, unpublished).

Growth (disk diameter) in the brittle star A. filiformis was increased in the organically enriched treatment, but there were no significant effects from oxygen availability, the presence of contaminants or interactions between the three factors. Arm regeneration in A.

Figure 16.
Effects of oxygen availability, organic enrichment and the presence of contaminants on biomass change in the polychaete Nereis diversicolor; mean ± standard deviation (n=3). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontaminated groups are unmarked.

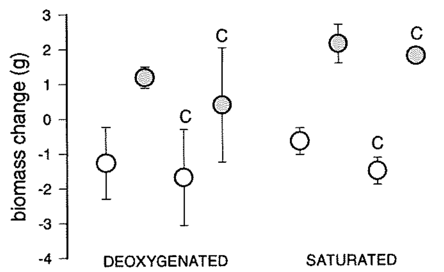


Figure 17.
Effects of oxygen availability, organic enrichment and the presence of contaminants on arm regeneration in the brittle star Amphiura filiformis;. mean ± standard deviation (n=10). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontaminated groups are unmarked.

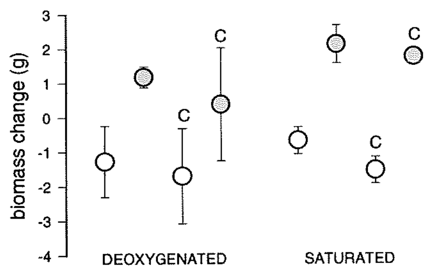
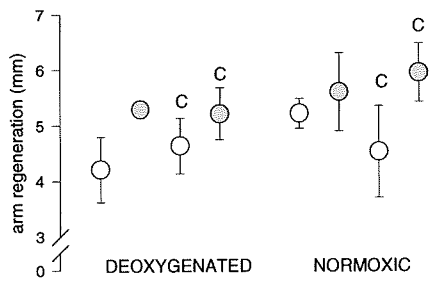


Figure 18.
Effects of oxygen availability, organic enrichment and the presence of contaminants on growth (area change of shell) of the bivalve Abra alba; mean ± standard deviation (n=2-10). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontami-nated groups are unmarked.



filiformis increased signficantly in aquaria receiving organic enrichment and oxygen availability also affected this growth variable (fig. 17). Both disk size and arm regeneration in A. filiformis increased under enriched conditions. Similar results were found by Sköld & Gunnarsson (1996) in their study of A. filiformis and A. chiajei. Whereas disk size was not affected by other treatments in the present study, hypoxia negatively affected arm regeneration, in accordance with previous observations (Nilsson & Sköld, 1996). The only indication that the presence of contaminants may have affected arm regeneration in A. filiformis was by a weak (non-significant) interaction between all three factors, apparently due to a contaminant-related decrease in arm regeneration which was only detectable when there was sufficient oxygen but little food.

Organic enrichment increased growth in the bivalve A. alba (fig. 18). In addition, there was a significant interaction between organic enrichment and oxygen availability in their effects on growth of A. alba. The interaction between organic enrichment and oxygen availability was due to a smaller increase in growth in A. alba from enriched compared to non-enriched aquaria in the deoxygenated treatment relative to the normoxic treatment. There was no effect of contamination on the growth of A. alba.

Metallothionein-like proteins, a biomarker for metals such as Cd, Zn and Cu (Roesijadi, 1992), appeared to primarily respond to growth or growth-related processes and organic enrichment for N. diversicolor and A. alba, but to contaminants (benzo(a)pyrene) in tissues for A. filiformis.

It is not clear why glutathione reductase activity in N. diversicolor increased in response to tissue Cd. Glutathione reductase is involved in free radical metabolism through its reduction of oxidised glutathione, an important cellular scavenger of radicals. The activity of this enzyme may therefore to some extent reflect cellular levels of either oxyradicals or chemically generated radicals (Halliwell & Gutteridge, 1989). Cd may affect glutathione reductase activity indirectly by depleting intracellular glutathione (cf Cartaña et al.,1992). Tissue levels of Cd and benzo(a)pyrene in N. diversicolor correlated strongly, however, and effects from individual contaminants could not be separated in the present study. Increased activity of glutathione reductase a response to benzo(a)pyrene is not unexpected as exposure to this contaminant is known to induce antioxidant enzymes in other invertebrates (Livingstone et al.,1990; Ribera et al.,1991; Schlenk et al.,1991). Moreover, increased glutathione reductase activity also related by tissue benzo(a)pyrene in A. filiformis.

The third biomarker, glutathione S-transferase, encompasses a family of enzymes involved in conjugation reactions with both endogenous and exogenous lipophilic compounds (Clark, 1989). This biomarker has previously shown some promise as a marker for organic contaminants in invertebrates (Lee et al.,1988; Egaas et al.,1993). Although not relating directly to tissue levels of contaminants, glutathione S-transferase in N. diversicolor appeared to be weakly affected by contaminant exposure.

Biomarker responses in the sediment-dwelling species of this experiment were expected to be driven by the presence or absence of contaminants in sediments. Such differences in biomarker responses were weak to non-existent. One of the major reasons for this was presumably the large variability found for all biomarkers, especially in organisms not exposed to contaminants.

The most important findings of this study were:

- An addition of diatom organic material, equivalent to approximately 40 g C/m2 and resulting in an increase in sediment organic carbon from 1.21% to 1.92%, caused increased growth in the three sediment-dwelling species N. diversicolor, A. filiformis and A. alba.

- Hypoxia (2.4-3.5 mg O2/l) decreased growth all three species, but the interactive effects between organic enrichment and hypoxia differed between the three species. Whereas N. diversicolor and A. filiformis appeared to be able to adjust their metabolism in response to low oxygen levels, the growth decrease in A. alba indicated that this species was inefficient in its use of the available organic material under hypoxia.

- The presence of contaminants had minor effects on growth in these organisms, although there were indications of growth reduction in N. diversicolor and of effects of interactions between all three factors on arm regeneration in A. filiformis.

- Biomarker responses in A. filiformis and to some extent N. diversicolor were affected by tissue contaminant levels, whereas there were no such responses in A. alba.

- There were only weak direct interactions between eutrophication-related processes and sediment-bound contaminants in their effects on growth in the sediment-dwelling invertebrates studied here. However, biomarker responses in these species, especially A. filiformis, reflected interactions between eutrophication and contaminants through their response to bioaccumulated contaminants.

Gaps of knowledge.

Perhaps the most obvious question mark that remains following this study are the minor effects from contaminant exposure compared to the impacts of decreased oxygen availability and increased food availability. Thus, there is a requirement for studies that focus on how (and if) sediment-dwelling organisms are affected by contaminants in sediment or overlying water. Such studies would need to investigate effects that range from the cellular level to individual health, growth and reproduction.

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Increased bioaccumulation and potential for trophic transfer of contaminants under eutrophic conditions

Ketil Hylland og Morten T. Schaanning

Rationale and objectives.

An increase in eutrophication would be expected to affect contaminant dynamics in aquatic systems, through increased sorption of contaminants to organic material and through altered energy flows. One of the characteristics of eutrophic systems compared to oligotrophic systems is the increased biomass in the former. Thus, a given contaminant load could conceivably be "diluted" into more individuals and more biomass in an eutrophic system than in an oligotrophic system, resulting in lower contaminant level in individual organisms (Olsson & Jenssen, 1975).

Due to the complex nature of the processes involved, it is not obvious how different species will be affected. It is well established that different sediment-dwelling species accumulate contaminants to levels varying by orders of magnitude (Bryan and Hummerstone, 1977; Bryan et al. 1980). There has not been many comparative studies under controlled conditions.

The objective of this study was to clarify relationships between changes in biomass due to increased food availability and changes in bioaccumulation of contaminants due to interactions between contaminants and organic material.

Project description.

Three macroinvertebrate species in marine sediments, the bivalve *Abra alba*, the polychaete *Nereis diversicolor* and the brittle star *Amphiura filiformis* were exposed for 93 days to sediment-bound contaminants in a flow-through system. The system is described in detail elsewhere (Hylland et al., 1997). Briefly, a 5-cm layer of clean marine sediment was evenly spread in the bottom of all aquaria. On top of this sediment, a 2-cm layer of treated sediment was introduced. The treated sediment was contaminated with Cd (4.8 mg/kg dry sediment; ^{109}Cd added to specific activity of 88.83 kBq/mg), Hg (0.2 mg/kg dry sediment; ^{203}Hg added to specific activity of 199.21 kBq/mg) and benzo[a]pyrene (1.3 mg/kg; ^{14}C-b[a]p added to specific activity of 36.56 kBq/mg). In half the aquaria, the treated sediment was also spiked with organic material both in sediment (20 mg C/m^2) and through water after two months (20 mg C/m^2). Half the aquaria were kept under deoxygenated conditions (2.4-3.6 mg/l) throughout the period. There were 3 replicate aquaria for each treatment.

Following a 7-day equilibration of the system, 10 *Abra alba*, 20 *Nereis diversicolor* and 10 *Amphiura filiformis* were introduced to each aquarium. Prior to addition, the bivalves were marked individually and the size determined. Similarly, the polychaetes were allowed to empty their guts and weighed before addition. At the end of the exposure, all macroinvertebrates were retrieved, the size of the bivalve determined, the biomass of the polychaete determined and both size (disc diameter, biomass) and arm regeneration determined for the brittle star.

Animals were pooled from each aquarium for tracer analyses and concentrations in tissues determined. A contamination index was calculated for each species and aquarium in the following manner. For *A. alba*, a growth index was computed using the square root-transformed change in shell area. Maximum growth observed for one aquarium was set to 1, all others would be in the range 0-1. There was net growth for *A. alba* populations in all aquaria. This growth index was then multiplied by the mean concentration of contaminants in tissues of *A. alba* from each aquarium to arrive at a contamination index for Cd, Hg and benzo[a]-pyrene. For *N. diversicolor*, the biomass in each aquarium was divided by the original biomass to derive a measure of relative change. This figure ranged from 0.6 to 1.4. In some aquaria there was thus a net biomass decrease during the exposure period. The index of relative biomass change was multiplied by mean contaminant concentration for polychaete populations from each aquarium to derive the contamination index.

Different measures of growth were available for *A. filiformis*. Brittle stars were distributed at random at the start of the experiment, but were not measured. The contamination index was computed by multiplying the total biomass in each aquarium at the end of the exposure with the mean concentration of each contaminant.

Results - highlights.

It has earlier been proposed that the measured bioaccumulation of contaminants would be lower in eutrophic compared to oligotrophic systems due to growth dilution of accumulated contaminants. In the present study, all three organisms had higher growth in treatments with organic material added. In contrast to earlier observations however the concentration of contaminants (Hg, Cd and to some extent benzo-[a]pyrene) increased in organisms under eutrophic conditions (addition of organic material). This effect was most pronounced for the bivalve *Abra alba* (fig.19), but was also evident for the polychaete *Nereis diversicolor* (fig. 20) and for the brittle star *Amphiura filiformis* (fig. 21).

It is probable that increased food availability increases general metabolism, which leads to higher uptake and accumulation of contaminants. The results are highly relevant for environmental management as increased eutrophication could lead to much higher mobilisation of sediment-bound contaminants.

The major findings from this study were:

- both organic and inorganic contaminants bioaccumulate in benthic invertebrates to a greater extent under eutrophic conditions compared to oligotrophic conditions.
- the rate of bioaccumulation varied greatly between species, the highest accumulation was found for metals (Cd, Hg) in the bivalve *Abra alba*, followed by metals and benzo[a}pyrene in *Nereis diversicolor* and benzo[a]pyrene in *Amphiura filiformis*.

Gaps of knowledge.

The results from the present study were contrary to what was expected based on results from previous

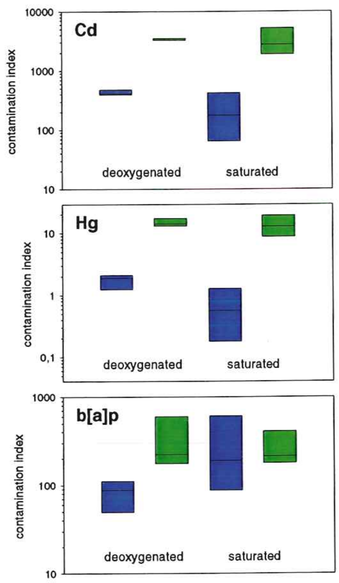


Figure 19. Contamination index for Cd (upper panel), Hg (middle panel) and benzo[a]pyrene (lower panel) in ***Abra alba***, n=3. Organic material added (green), reference (blue). The two left boxes from treatments with low oxygen levels, the two right boxes from normal oxygen levels (saturated). Note logarithmic axis.

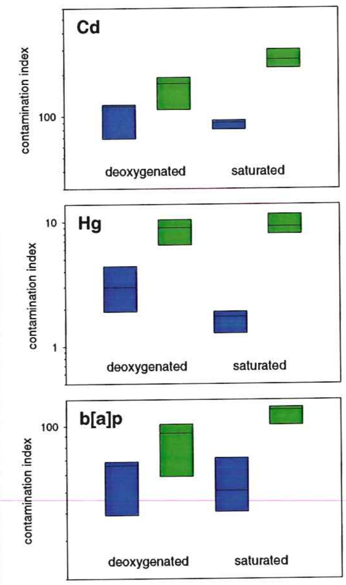


Figure 20. Contamination index for Cd (upper panel), Hg (middle panel) and benzo[a]pyrene (lower panel) in ***Nereis diversicolor***, n=3. Organic material added (green), reference (blue). The two left boxes from treatments with low oxygen levels, the two right boxes from normal oxygen levels (saturated). Note logarithmic axis.

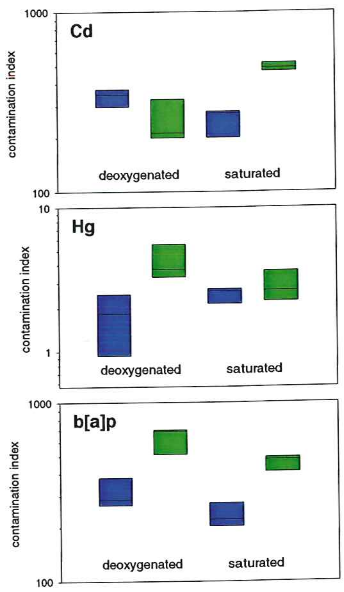


Figure 21. Contamination index for Cd (upper panel), Hg (middle panel) and benzo[a]pyrene (lower panel) in ***Amphiura filiformis***, n=3. Organic material added (green), reference (blue). The two left boxes from treatments with low oxygen levels, the two right boxes from normal oxygen levels (saturated). Note logarithmic axis.

studies. Some questions arise:

- what is the mechanism by which uptake is increased under eutrophic conditions?
- is uptake solely from the water phase or is partitioning of contaminants onto food particles and subsequent uptake also relevant?
- are bioaccumulated contaminants partitioned similarly in organisms under both oligotrophic and eutrophic conditions?

Literature references.

Bryan, G.W. and Hummerstone, L.G. (1977) Indicators of heavy-metal contamination in the Looe estuary (Cornwall) with particular regard to silver and lead. *J.mar.biol.Ass.U.K.* **57**, 75-92.

Bryan, G.W., Langston, W.J. and Hummerstone, L.G. (1980) The use of biological indicators of heavy-metal contamination in estuaries. *Occ.Publ.Mar.Biol.Ass.U.K.* **1**, 1-73.

Hylland, K., Schaanning, M., Skei, J., Berge, J.A., Eriksen, D.Ø., Sköld, M., Gunnarsson, J. (1997). Interaction between eutrophication and contaminants – partitioning, bioaccumulation and effects on sediment-dwelling organisms. NIVA-rapport 3700, 53 s.

Olsson, M., Jenssen, S. Institute of Freshwater Research. (1975). Pike as test organism for mercury, DDT and PCB pollution. A study of the contamination in the Stockholm archipelago. Drottningholm, pp. 83-106.

Effects of organic enrichment on the accumulation of PAH in blue mussels, *Mytilus edulis.*

Torgeir Bakke

Rationale and Objectives.

The rationale for this subproject was to test a hypothesis which had emerged during a previous experiment on the biavailability of smelter derived PAH in contaminated sediments to mussels (Næs et al. 1995). The hypothesis suggested that the accumulation of PAH in mussels was stimulated by elevated organic content in the available food particles. Other authors have shown that PAH-accumulation is stimulated by elevated denseties of algal cells in the water. A mechanism proposed was stimulation of the mussel filtration rate, and hence of PAH uptake, from the increase density of food particles. The present hypothesis was that the organic quality of the food particles alone (i.e. high TOC:TSM ratio) would enhance PAH accumulation, possibly through a similar mechanism. A supplementary hypothesis, related to the mechanism, was that the effect of organic enrichment would be stronger during periods when the mussels have low food availability, e.g. in winter. In other ways the effect would be stronger in pre-starved than in normally fed mussels.

The aim of the subproject was to test these hypotheses experimentally. A factorial design was applied involving

- two groups of mussels; one pre-starved for one month and one normally fed,
- two levels of organic content on the suspended particulate matter, high and low,
- two levels of exposure to particulate PAH from a smelter residue, PAH enriched and control.

Project description.

This gave 8 experimental conditions and within each condition 3 replicate groups of 20 individuals were tested. The experiment was run in a flow through system at NIVA Marine Research Station Solbergstrand with exposure lasting for 30 days followed by 1 day in clean seawater to empty the gut content. The soft tissues of all individuals within a group were pooled before PAH analysis, which gave 3 replicate analytical results for each of the 8 treatments. The experiment was performed during September-November 1997, with subsequent analytical work during 1998.

PAH and food particles were fed to the test aquaria from pre-mixed stock slurries mixed continuously into the incoming water. The net nominal addition of PAH (mostly particulate) to the high PAH treatments was 6.5 µg/l. Nominal addition of TOC was 0.22 – 0.32 mg/l in the high organic treatment and 0.14 – 0.16 mg/l in the low organic treatment. This gave a TOC:TSM ratio of 0.09 – 0.12 in the high organic treatment and 0.04 – 0.05 in the low organic treatment. This treatment difference appeared to be somewhat lower than aimed at during planning.

Results - highlights.

- There was no significant difference in accumulation of sum PAH between pre- starved and fed mussels after 30 days (fig.22).
- There was also no difference between these two groups in accumulation of the subset of PAH compounds which are regarded as cancerogenic (KPAH).

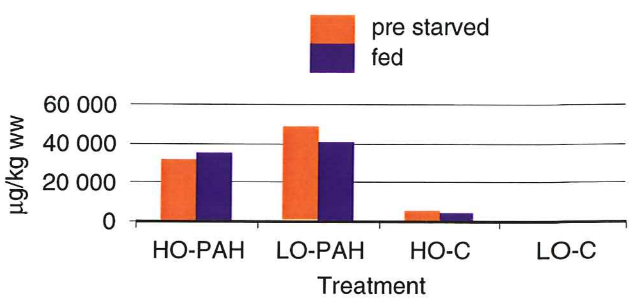
The % KPAH was, however, significantly higher in pre-starved than in fed mussels, indicating that accumulation selectivity was dependent on nutritional status of the shells.

- Low TOC:TSM ratio gave slightly higher accumulation of sum PAH than high TOC:TSM (fig. 23). The effect was only marginally significant.
- Likewise the highest accumulation of KPAH and highest % KPAH was also found in the low TOC:TSM ratio regime. This difference was slightly more pronounced (but not significantly) in pre starved than in the fed mussels (fig. 23).

The experiment thus gave the conclusion that organic enrichment of the food particles resulted in lower total accumulation of PAH, and lower accumulation of the potentially most harmful PAH components (the KPAH), than without such enrichment. This is the opposite effect than postulated from the *a priori* hypothesis.

All mussels exposed to PAH developed similar PAH profiles characterised by high levels of fluorantene, pyrene and benzo(a)antracene, and low levels of benzo(a)pyrene, benzo(e)pyrene,

Figure 22. Net tissue change (mean of 3 replicate groups) in sum PAH after 30 days exposure to 4 experimental regimes. HO-PAH: PAH and high TOC:TSM; LO-PAH: PAH and low TOC: TSM; HO-C and LO-C: corresponding control groups without PAH.



chrysene/triphenylene and metylphenantrene. The nutritional value of the food particles did not affect this picture. Furthermore the control mussels receiving high TOC:TSM showed the same profile, whereas the low TOC:TSM control groups (fed and pre starved) developed different and more variable PAH profiles.

The PAH profiles of the mussels differed clearly from that of the PAH source. This supports the conclusions of Næs et al (1998) showing that natural mussels in Norwegian smelter affected recipients attain a profile distinct from the nearby contaminated sediments. The results, however, contradict the findings of Bakke and Helland (in prep) showing clear profile similarity with source after exposing mussels to contaminated harbour sediments for 1 to 11 days. An explanation may be that the source used by Bakke and Helland (in prep) was more influenced by petroleum derived PAH, as opposed to the smelter PAH studied by Næs et al (1998) and in the present experiment.

The profiles of fed and pre starved groups were slightly different at the start of the experiment, and this difference was retained in the control groups after 30 days, most pronounced after the low TOC:TSM treatment. The profiles were characterised primarily by high phenantrene in the fed mussels and high antracene in the pre starved mussels. The 30 days exposure to PAH was not sufficient to mask the profile signal imposed by the initial nutritional status of the mussels.

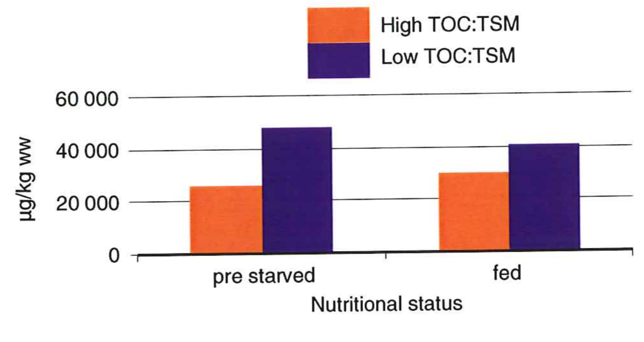
Literature references.

Bakke, T. and Helland, A., (in prep). The application of mussels (Mytilus edulis L) as indicator organisms for episodic short term exposure to micropollutants.

Næs, K., Bakke, T., and Konieczny, R., 1995. Mobilization of polycyclic aromatic hydrocarbons (PAHs) from polluted seabed and uptake in the blue mussel (*Mytilus edulis*). J. Mar. Freshwater Res., 46: 275-285.

Næs, K., Oug, E. and Knutzen, J., 1998. Source and species-dependent accumulation of polycyclic aromatic hydrocarbons (PAHs) in littoral indicator organisms from Norwegian smelter-affected marine waters. Mar. Environ. Res., 45: 193-207.

Figure 23. Net accumulation of sum PAH (difference between PAH-exposed and control groups) as function of mussel nutritional status at start of exposure and food particle organic content



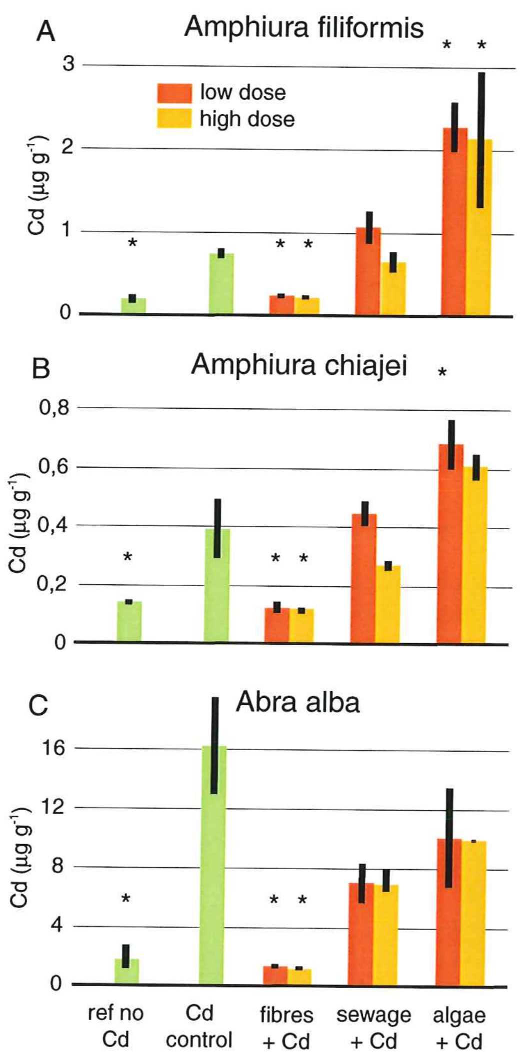
Influence of organic enrichment on partitioning and bioavailability of Cd

Rationale and objectives.

This study examined the influence of different sources of organic matter on the partitioning and bioavailability of Cd within a model microcosm system. The establishment of the interactions between organic enrichment and the fate of Cd is especially important due to influence on speciation. The subsequent bioaccumulation of Cd in benthic macrofauna is dependent on the metal partitioning within the sediment-pore water matrix.

The objective of this study was to study the interactive processes between eutrophication and Cd additions in marine benthic ecosystems, using three types of organic matter with different quality (wood fibres, primary treated sewage and ground cultured benthic algae).

Figure 24. Cadmium concentrations (mg g^{-1} dry wt) in the 3 benthic species, *Amphiura filiformis* (A), *Amphiura chiajei* (B), and *Abra alba* (C), after organic enrichment treatments of fibres, sewage and algae, with constant cadmium additions. * Concentrations significantly different from the Cd control (+Cd) when a < 0.05. Error bars denote ± SE.



Project description.

The microcosm system consisted of 24 glass aquaria with a flow-through system at NIVA Marine Research Station Solbergstrand (see fig.14). Each aquaria contained a sediment, spiked with Cd (0.1 mg/g). All aquaria contained the same population of macrofauna selected on the basis of their different trophic strategies. Two types of brittle stars, a bivalve and a polychaete were added to the sediments, containing different concentrations of Cd and different concentrations and qualities of organic matter. The redox conditions in the sediments were continually recorded. The experiment lasted for10 weeks.

Results - highlights.

- Additions of Cd without organic enrichment resulted in a 3-fold increase of Cd in the porewater and a subsequent increase in the benthic organisms.
- Additions of Cd combined with different organic enrichment regimes of cellulose fibres, sewage and benthic algae, significantly altered the Cd partitioning in the sedimentary matrix.
- Both algal treatments and high sewage levels significantly decreased the sediment redox potential, with a corresponding increase in sulfide activity.
- Bioaccumulation of Cd in the three benthic species examined showed significant variation between the different types of organic matter, with an increasing gradient of Cd bioaccumulation in the sequence: fibres<sewage<algae (fig.24).
- Consequently, high quality, potential "benthic food", such as algaes, causes increased Cd bioaccumulation.

(the results from the project have been published by **Maloney, 1996.**)

Time-trend analysis of contaminants in cod (*Gadus morhua*) in two areas with different eutrophication regimes

Ketil Hylland

Rationale and objectives.

Since the end of the 1960s, contaminant levels in herring from the Baltic has decreased markedly. It is not probable that inputs have decreased sufficiently to explain this and increased eutrophication has been brought in as a possible explanation. In theory, increased eutrophication should lead to higher concentrations of organic material in the water column, decreasing bioavailability and increasing sedimentation of hydrophobic contaminants. If this is a general situation, similar decreases should be found in other enclosed coastal areas with increased eutrophication, or the opposite, increased contaminant load if eutrophication decreases.

To be able to detect such long-term changes, there is a need for extensive time-series of monitoring data, including both contaminants and eutrophication. The Oslofjord is one area for which such data are available. The eutrophication of the inner Oslofjord increased until around 1980. Following implementation of a new sewage treatment plant, inputs of nutrients have decreased and a concomitant improvement of the eutrophication of the fjord was expected. There are no known industrial point sources of hydrophobic contaminants in the Oslofjord-area and there are no known changes in the input of e.g. PCBs and DDEs in the period between 1981 and 1997. The objective of this study was to clarify whether changes in eutrophication in the inner Oslofjord would affect contaminant concentrations in Atlantic cod (Gadus morhua) in the area. The outer Oslofjord is an area with smaller changes in eutrophication status during the same time period and was used as a reference.

Project description.

Since 1984, cod has been sampled in the inner and outer Oslofjord in September/October every year. In most years, 25 individual cod have been analysed for chlorinated organic contaminants and trace metals. One of the quantitatively most important PCBs, CB 153, was chosen to represent levels of CBs in tissues. Due to the use of different quantification methods, determinations before 1987 had to be transformed to CB 153. This was attained using a regression over an established relationship between total PCB (by the old method) and CB 153 (current methods). In addition, DDE (a metabolite of DDT) had been determined during the entire period and was included as an additional persistent hydrophobic contaminant.

Results - highlights.

There were significant differences in both length and conditions between years, but the differences are presumably not biologically important. Cod collected in different years had significantly different hepatic lipid concentrations. Liver lipid correlated strongly with liver somatic index (LSI). There was a trend towards increasing lipid levels (and LSI) at the site in the inner Oslofjord over the period studied. In general, the material from the outer Oslofjord appears reasonably homogenous with regard to the quality of fish sampled, whereas the cod collected in inner Oslofjord in the years 1984-1987 were markedly smaller and had lower lipid levels than cod collected in the same area 1988-1996.

Liver concentrations of CB 153 at both sites differ between the years, but appeared to be following a similar pattern until 1990 (fig. 25). In the following years, the level of CB 153 in cod from the outer Oslofjord appeared to decrease whereas levels in cod from the inner Oslofjord remained constant. In the two last years of the series, 1995 and 1996, levels of CB 153 in cod from the outer Oslofjord appeared to increase. An analysis of covariance with CB 153 concentration as dependent variable, year and station as factors and lipid, length, condition and LSI as covariates, indicated that all factors except condition contributed to explaining variation in CB 153 . There were very clear differences between the two areas, but liver lipid and size also had a strong influence.

A similar pattern was observed for p,p-DDE, although for this OC there appeared to be a decreasing trend in cod from both sites (fig. 26).

There were no obvious relationships between tissue concentrations of OCs and eutrophication status for the inner Oslofjord (fig. 27). Despite improved sewage treatment, there was not an obvious upward trend in Secchi depth, a coarse measure for primary production and hence eutrophication. One observation can be made from the results. Other environmental influences appear to have a larger impact than eutrophication. In 1995 there was a major flood in southern Norway causing low visibility in water (fig. 27) and presumably also increasing contaminant load in cod (figures 25 and 26). It is unclear why this effect is most obvious for cod from the outer Oslofjord.

Major improvements in the eutrophication status for the inner Oslofjord presumably took place before 1981 and it is thus difficult to assess their effects. There appear to have been only minor changes in eutrophication status since 1981 (assessed from Secchi visibility) and year-to-year variability is not sufficient to affect a long-term process such as accumulation of persistent contaminants.

The main findings of this project are:

- There is no obvious link between changes over time in eutrophication status (measured as Secchi visibility) and contaminant levels in cod from the inner Oslofjord.
- Environmental influences other than eutrophication affect contaminant levels in cod, e.g. the flood in 1995.

Gaps of knowledge.

Bioaccumulation of contaminants is a multifaceted process and it is not sufficient to study single parameters. The processes in question all have different development rates and scopes. It would nevertheless be interesting to compare data for cod with data for a species with a shorter "turn-over", such as blue mussel. There is presumably also a need for more high-resolution environmental data.

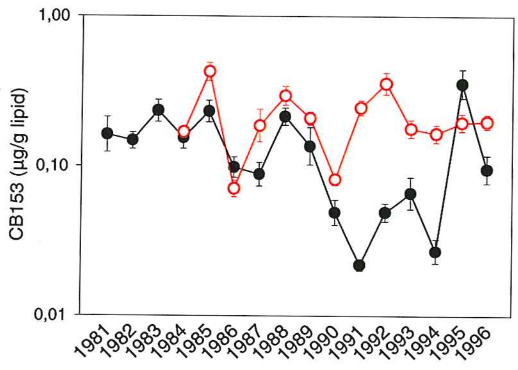


Figure 25. Concentrations of CB 153 in livers from cod collected in the inner Oslofjord (red) and outer Oslofjord (black). Mean and standard error, n=18-50. Note logarithmic scale.

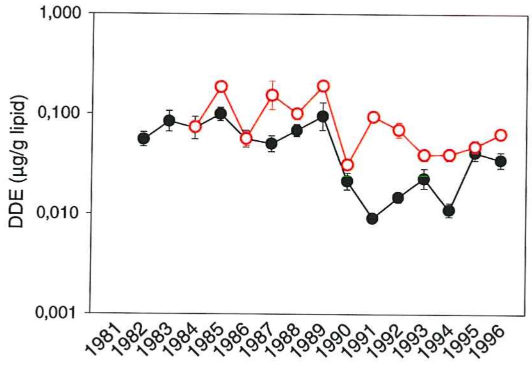


Figure 26. Concentrations of DDE (a DDT-metabolite) in livers from cod collected in the inner Oslofjord (red) and the outer Oslofjord (black). Mean and standard error, n=18-50. Note logarithmic scale.

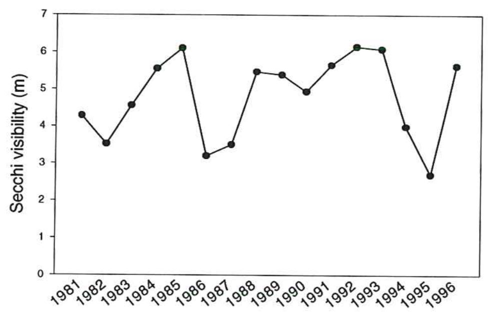


Figure 27. Mean Secchi visibility at a site in the inner Oslofjord, averaged over all seasons. Note that data from 1985 and 1986 only constitute one data-point.

Workshop - MILE/EUCON, Tømte, Hurdal, November 1998

Jens M. Skei

Rationale and objectives.

Parallel to the MILE strategic programme, a five year Swedish research programme, financed by the Swedish Environmental Protection Agency (SNV), was launched in 1995 and titled "Interaction between Eutrophication and Contaminants" (EUCON). During this time period, there has been a substantial exchange of ideas between individuals of the two Scandinavian programmes and between the steering committees of the programmes.

The Swedish EUCON programme established in the initial phase some hypotheses and questions to be addressed in the programme:

1. Will the concentrations of bioaccumulative substances in marine biota decrease as a result of dilution in a lager biomass caused by eutrophication?
2. Has eutrophication caused decreasing concentrations of contaminants in the water mass and pelagic biota in the sea?
3. Has eutrophication caused an increased sedimentation of contaminants?
4. Has eutrophication caused an increased trapping of contaminants in the sediments during the last decades?
5. Will the concentrations of contaminants in the water mass and pelagic biota increase if the nutrient input to the sea is reduced, without a simultaneous reduced input in contaminants?
6. May a decreased eutrophication induce changes in the accumulation bottoms that may lead to decreased denitrification capasity?

These questions are relevant for both EUCON and MILE. Figure 28 illustrates the varies compartments in the ecosystem being influenced by eutrophication.

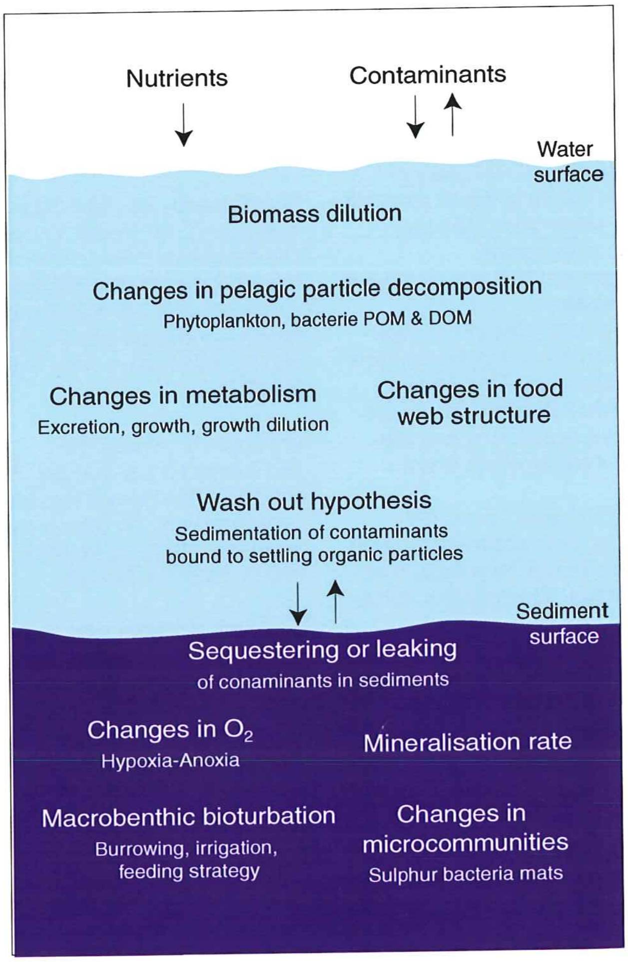
It was decided to arrange a workshop at Tømte, a biological field station of the University of Oslo, with participants from both research programmes, to discuss matters of mutual interest. A number of 14 persons participated in the workshop (November 9-10, 1998). Presentations from the various projects were given, including results from pelagic and benthic studies (experimental or use of field data) as well as modelling.

At the end of the workshop gaps of knowledge was discussed, establishment of networks and further cooperations in proposals to NMR (Nordic Ministry Council) and EU.

Outcome.

Valuable contact between Norwegian and Swedish researchers was established. Results from the two research programmes were compared and evaluated. Ideas about future cooperation was discussed and a joint NMR-project was later established (Large scale comparisons of field data on eutrophication and contaminants).

Figure 28. The boxes illustrate different levels where contaminants and eutrophication processes may interact in the aquatic environment (POM: particulate organic matter, DOM: dissolved organic matter (from Gunnarsson et al., 1995).



Interaction between contaminants and eutrophication - Gaps of knowledge

It is evident that all research programmes uncover gaps of knowledge and new challenges which have not been addressed within the time frame and the economic resources made available. The objectives of experimentally designed programmes are to mimic complicated natural processes by simplification. In many instances it is a question of trying and failing.

Attempts to understand the interaction of heavy metals and nutrients and its effect on phytoplankton should be followed up, incorporating both essential and non-essential metals, and perhaps mixtures of metals which is often the natural situation.

One of the contaminants tested (DDT) behaved very differently from the others studied, with respect to anoxic sediments. The mother substance pp-DDT was almost absent in the anoxic sediments; the presence of the metabolites pp-DDD and pp-DDE was dominating. More research is needed to understand the biogeochemical cycling and metabolization of DDT in oxic versus anoxic sediments.
There exist few studies of the interaction between oil and contaminants in marine sediments. The reason why bioaccumulation of Cd in sediment-dwelling organisms is inhibited by the presence of oil is not understood.
Some of the results from the MILE-projects were contrary to what was expected, based on results from previous studies. This raises a series of questions:

- what is the mechanism by which uptake of contaminants is increased under eutrophic conditions?
- is uptake solely from the water phase or is partitioning of contaminants onto food particles and subsequent uptake also relevant?
- are bioaccumulated contaminants partitioned similarly in organisms under both oligotrophic and eutrophic conditions?

An overall impression of the results from the MILE-projects is that the level of contaminants in sediments appears to have minor effects on organisms compared to the impact of low oxygen levels and the availability of food. This has implications for risk evaluations and the establishment of sediment quality criteria. It appears that information on redox conditions and the quality of organic matter in sediments are essential factors to predict the biological effects.

List of publications and reports (MILE)

Bakke, T., (in prep). The influence of food particle quality and nutritional status on the accumulation of smelter-derived polycyclic aromatic hydrocarbons (PAHs) in the blue mussel (*Mytilus edulis*).

Gunnarsson, J.S., M.T.Schaanning, K.Hylland, M. Skøld, D.Eriksen, J.A.Berge and J.Skei, 1996. Interactions between eutrophication and contaminants. III. Mobilization and bioaccumulation of 14-C-B(a)P from marine sediments. Mar. Pollut. Bull., 33, 80-90.

Hylland, K., Skøld, M., J.S.Gunnarson and J.Skei ,1997. Interactions between eutrophication and contaminants. IV. Effects of sediment-dwelling organisms. Mar. Pollut. Bull.,33, 90-100.

Hylland, K., Schaanning, M. and Skei, J., 1997. Samspill mellom ulike typer forurensning (in norwegian). NIVAs Årbok 1996, 18-19.

Hylland, K., M. Schaanning,., J. Skei, J.A.Berge, D.Ø.Eriksen, M. Skøld, og J.S.Gunnarsson, 1997. Interactions between eutrophication and contaminants. - partitioning, bioaccumulation and effects on sediment-dwelling organisms. NIVA-rapport, 3700-97, 53 s.

Hylland, K., 1998. Interaksjoner mellom olje og miljøgifter i marine sediment. NIVA- rapport 3766-97, 42 s.

Hylland, K., Følsvik, N., Interactions of oil and organic material with contaminants in marine sediments: distribution, bioaccumulation and biological effects (in prep)

Lømsland, E.R & T.M. Johnsen, in prep. Effects of copper on marine phytoplankton at different nutrient levels.

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