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MARINE ECOTOXICOLOGICAL CHARACTERIZATION OF AN INDUSTRIAL EFFLUENT - A CASE STUDY

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ABSTRACT

The aim of this study was to predict the effects of discharging wastewater from a herbicide manufacturing plant into a marine environment. The fate and effects on the marine environment of the wastewater and its five major components (MCPA, MCPP, 2,4-DP, 4-chloro-2-methylphenol, and 2,4,6-trichlorophenol) have been studied.

The processes of evaporation, bioconcentration, and adsorption onto sediments have been evaluated on the basis of literature studies on physicochemical properties of the five major components. Biodegradability has been estimated on the basis of simulation tests, performed in the laboratory as die-away tests, with inocula taken from the receiving water. No biodegradation of phenoxyacids was observed, while chlorophenols showed to be biodegradable.

The toxic effects of the wastewater and the five substances have been estimated on the basis of laboratory screening-tests with various marine organisms including fish, crustaceans, algae, bacteria, and higher plants. When possible, both acute and chronic effects were studied. The toxic effects of the wastewater could be accounted for assuming an additive effect of the phenoxyacids, while the chlorophenols - due to their low concentrations - did not contribute significantly to the overall toxicity.

In situ studies of off-flavouring and bioaccumulation with transplanted eel, placed in cages near the wastewater outlet and at a reference station during 6 weeks, showed no difference in taste nor in content of chlorophenols.

KEYWORDS

Marine ecotoxicology, Case study, Industrial effluent, Herbicides, Phenoxyacids, Chlorophenols, Chemical fate, Biodegradability, Off-flavouring, Bioaccumulation.

INTRODUCTION

Direct discharge of industrial wastewater is one of the major routes for chemicals to reach the aquatic environment. In order to assess the possible impact on the aquatic ecosystem of industrial chemicals, there is an increasing need to make use of predictive methods. In recent years, the Water Quality Institute (WQI) has conducted a large number of assessment studies of marine industrial discharges, and valuable experience has been gained on the stepwise study comprising :

- inventories of used chemicals and processes ;
- ecotoxicological and chemical characterization of the effluent ;
- biological and chemical monitoring in the vicinity of the outlet ;
- bioaccumulation and toxicity studies on blue mussels or eel grown in cages in the vicinity of the discharge ;
- dilution and chemical fate modelling based upon hydraulic dispersion models (usually established by a sister institute, the Danish Hydraulic Institute).

Recently, a similar procedure to characterize industrial effluents has been proposed by Linden et al. (1983). This paper presents an impact study on the biological effects on a marine environment (Bay of Køge, Denmark) receiving a.o. wastewater from a herbicide manufactoring plant (Kemisk Vaerk Køge A/S (KVK)). Chemical analyses showed phenoxyacids and chlorophenols to be major components of the wastewater from KVK. The industrial wastewater is discharged into a sewage-treatment plant receiving both domestic and industrial wastewater ; the effluent from this sewage-treatment plant is then discharged into the Bay of Køge.

MATERIALS AND METHODS

CHEMICALS

The phenoxyacids MCPP, MCPA, and 2,4-DP of technical grade were delivered by KVK. The 4-chloro-2-methylphenol and 2,4,6-trichlorophenol were of analytic grade.

SAMPLING

Wastewater was sampled at three sites (i) at the discharge point of the wastewater pools of herbicide plant, which led to the sewage-treatment plant ; (ii) at the secondary effluent of the treatment plant, and (iii) at the secondary effluent of the treatment plant during a period of no discharge by the herbicide plant.

All samples were taken as flow-proportional 3-week samples. The daily subsamples were frozen (- 20 °C). At the end of the sampling period, all subsamples were thawed in a water bath and mixed proportionally to the daily discharged amount of wastewater. During the period of sampling, the average influent (i) amounted to 600 $m^3.day^{-1}$, the secondary effluent (ii) to 16 000 $m^3.day^{-1}$, and the secondary effluent with no discharge from herbicide plant (iii) to 13 600 $m^3.day^{-1}$. All samples were analyzed without pretreatment, at a pH of 8.1 of the recipient water, a salinity of 9 ‰ of brackish water, and a temperature ranging from 15 to 20° C (in the biodegradability study also 4 °C).

TEST MATRIX

Tests were performed on wastewater samples i, ii, and iii ; and the five major components, MCPA, MCPP, 2,4-DP, 4-chloro-2-methylphenol, and 2,4,6-trichlorophenol.

For practical (and economical) reasons, a test matrix (Table I) was developed. This matrix presents the tests performed on the samples in question.

Table I. Test matrix

Tests	Wastewater samples			Major compounds				
	(i)	(ii)	(iii)	MCPA	MCPP	2,4-DP	4-chloro-2-methylphenol	2,4,6-trichlorophenol
Chemical analysis								
Chlorophenols	+	+						
Phenoxyacids	+	+						
Ecotoxicological tests :								
Biodegradation in seawater	+			+	+	+	+	+
Natural phytoplankton	+		+	+	+	+	+	+
Cultured algae (*growth*)	+	+	+					
Heterotrophic microorganisms (oxygen consumption)	+		+	+	+	+	+	+
Crustacea (mortality)	+	+	+					
Crustacea (reproduction)	+	+	+					
Fish (mortality)	+	+	+					
Fish larvae (mortality, growth)	+	+	+					
Eelgrass (growth)	+	+	+	+	+	+		

CHEMICAL ANALYSIS

Wastewater samples were extracted with hexane/ether (2 + 1) at pH 2 just after thawing. The isolated chlorophenols of the sewage samples were extractively acetylated and monitored by GC/MS at selected ion monitoring (SIM) using specific standards. The isolated phenoxyacids were esterified with concentrated sulphuric acid and 2,2,2-trifluoroethanol. The mixture was stocked at 50 °C in a water bath for 30 min, then 1 M dipotassium hydrogen phosphate was added. The hexane phase was isolated and analyzed by GC/MS using specific standards.

MARINE ECOTOXICOLOGICAL TESTS

Biodegradation studies on the five major components and a wastewater sample (i) were performed as shake flask die-away tests with natural seawater from the receiving water. Before use, the seawater was filtered through coarse paper and enriched with nutrients (solution a, b, c, and d in the MOST test, OECD's Guidelines, 1980). Tests were carried out at 15 and 4 °C in climatic rooms in diffuse light at two concentrations : 10 $mg.l^{-1}$ measured as dissolved organic carbon (DOC), and 0.1 $mg.l^{-1}$ measured as specific compound. The degradation was detected by a combination of DOC and specific analysis. Sodiumbenzoate served as a reference compound of ready biodegradability.

On the basis of literature data on the physicochemical properties of the five major components, evaporation processes were evaluated based on correlations shown by Liss and Slater (1974), the bioconcentration according to correlations shown by Veith *et al.* (1979), and the adsorption on sediments based on correlations shown by Karickhoff *et al.* (1979). Phenoxyacids and some chlorophenols ionize in the receiving water depending on the pK_A of the substances and the pH of the recipient. Consequently, bioaccumulation (P_{ow}) and adsorption onto the sediment were corrected for ionization of the substances using the following distribution coefficient :

$$D = P_{ow}/(1 + 10^{pH - pK_A}), \text{ (Freese } et\ al.\text{, 1979).}$$

Acute toxicity tests on heterotrophic microorganisms were performed according to the ISO (1982) proposed standard.

Inhibition of photosynthesis (^{14}C-uptake) by marine phytoplankton, taken from the receiving water, was studied. The test duration was 6 h and the methods were described by Damgaard and Nyholm (1980).

A growth inhibition test with the marine diatom (Phaeodactylum tricornutum) kept in culture at the WQI was conducted. The medium was natural seawater, with a salinity of 20 ‰ enriched with nutrients. The test algae were grown in Erlenmeyer flasks on a shaking table under a light intensity of 6.7 - 7.3 quanta x $10^5.cm^{-2}.s^{-1}$ (4 200 lux) and a temperature of 19 °C ± 2 °C. The initial cell density was 10^4 cells.ml^{-1}. The cells were counted every 24 h during 96 h, and again after 168 h with a particle counter. The test was performed according to the principles of the ISO standard TC147/SC5/WG5/N67 (1982b).

A 96 h acute toxicity test with the marine invertebrate (Nitocra spinipes) kept in culture at the WQI, was performed as a static test. The tests were performed in the dark in natural seawater with a salinity of 9 ‰ at 20 °C, according to the test method described by Bengtsson (1981).

Reproduction studies with Nitocra spinipes were carried out as static tests. The tests were also done in the dark in natural seawater with a salinity of 9 ‰ at 20 °C. Adult females with an egg sac were transferred to the test ion containers, and the development of the larvae was followed throughout a period of 12 days when the number of offspring was determined. The test thus provided information on the effects on egg hatching as well as on larval development. Test method was modified after Renberg et al. (1980).

Acute toxicity tests of 96 h with female guppies (Poecilia reticulata) (total length 4.4 ± 0.4 cm, weight 0.89 ± 0.09 g) were conducted at room temperature. The test was performed according to the ISO standard, TC147/SC5/WG3/N37 (1982c).

Tests on fish larvae were conducted with 2-months-old turbot (Scophthalmus maximus) (total length 15 ± 4 mm, weight 0.4 ± 0.1 mg). The tests were performed as semistatic tests in natural seawater (salinity 28 ‰) in 15 l black plastic containers at 15 °C under a light/dark regime of 16 : 8 h. The larvae were fed daily. The test duration was 1 month and mortality and growth (by weight) were used as effect criteria.

Laboratory tests on eelgrass (Zostera marina) were performed as semistatic tests. The plant material collected in the field was cultured in 10 l plexiglass containers in two ways : 1) with the roots and rhizomes exposed directly to the water ; 2) with the roots and rhizomes covered by fine-grained sandin pots. The plants were continuously illuminated by fluorescent tubes and kept at 15 °C. The growthmedium was continuously aerated and the test lasted for 6 weeks. Once a week the following program was carried out :

- the growth of the leaves was measured by means of a marking system ;
- number of new leaves were registered, new leaves were measured and marked ;
- the number of new shoots were recorded ;
- samples for chemical analysis were collected ;
- the growth containers were changed ;
- the medium was changed.

FIELD INVESTIGATIONS

Field investigations on eelgrass were performed as a transplantation experiment. Mats of plants were placed in plastic boxes, 20 plants in each box were marked, and the boxes were placed at various distances from the wastewater outlet at a depth of 3 m. The growth of the leaves was measured every 2 weeks during 6 weeks.

In situ studies on off-flavour and bioaccumulation were performed with transplanted eel (Anguilla anguilla) caught near the Swedish Baltic sea coast. The eels were put in cages and placed at six stations in a north-south direction around the wastewater outlet. A total of 180 eel was used (30 at each station), and 70 at a reference locality. After 2, 4, and 6 weeks five eels from each station were collected for macroscopic clinical examination, tasting, and chemical analysis. The off-flavour was determined by an organoleptic triangle test, in which 12 persons subsequently tasted one eel from the reference locality, and one eel from each of the exposed stations.

Table II. Biodegradation (in %) of the initial test concentration after 14, 28, and 56 days at 4 and 15 °C, corrected for blank concentration

Compound	Initial test concentration ($mg.l^{-1}$)	% Degradation					
		Test temperature 15 °C			Test temperature 4 °C		
		14 d	28 d	56 d	14 d	28 d	56 d
MCPA	13	0	0	0	0	0	0
MCPP	15	0	0	0	0	0	0
2,4-DP	13	0	0	13	0	0	14
2,4,6-trichlorophenol	14	0	10	10	0	0	0
	0.18[a]	45	99	99	80	99	99
4-chloro-2-methylphenol	16	0	2	2	0	0	0
	0.15[a]	93	93	99	49	n.a.[b]	99

[a]Measured as specific chemical compound. All other concentrations measures as DOC

[b]Not analyzed

Table III. Biodegradation (in %) of initial test concentration after 14, 28, and 56 days at 15 °C in wastewater sample (i) diluted 40 times with natural seawater from the receiving water

Compound	Initial test concentration ($mg.l^{-1}$)	% Degradation		
		14 d	28 d	56 d
MCPA	9.4	0	0	0
MCPP	2.8	0	0	0
2,4-DP	0.5	0	0	0
4-chloro-2-methylphenol	0.003	10	30	98
2,4,6-trichlorophenol	0.003	10	10	20
2,6-dichlorophenol	0.0005	15	40	90
2,4-dichlorophenol	0.0002	15	40	95
4,6-dichloro-2-methylphenol	0.0001	0	0	0

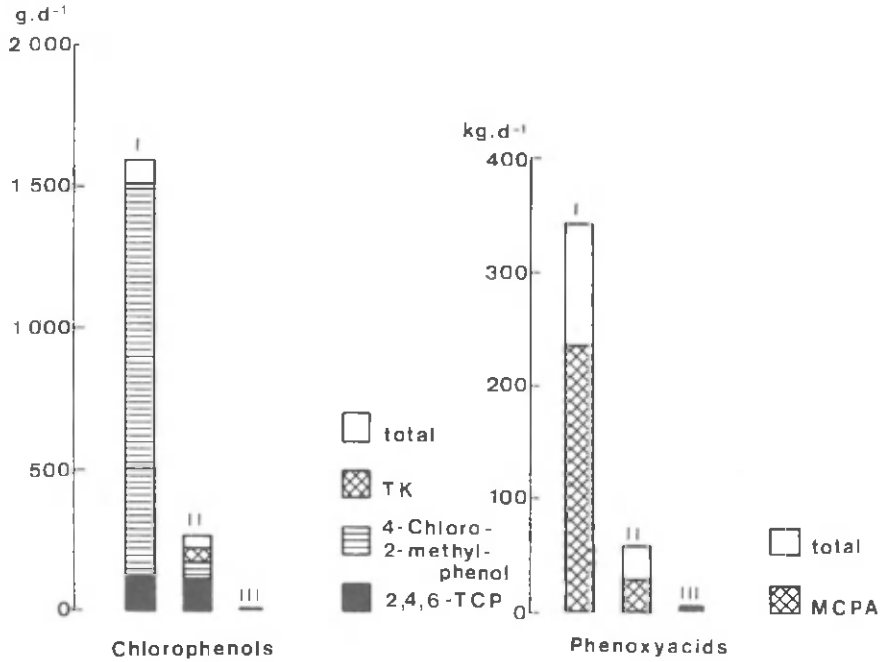

Fig. 1. Daily discharged amounts of phenoxyacids and chlorophenols. The figure shows the daily discharged amounts from the herbicide manufacturing plant (i), the secondary effluent (ii), and the secondary effluent taken in a period of no discharge from the herbicide plant (iii).

RESULTS AND DISCUSSION

CHEMICAL ANALYSIS

The results of the chemical analysis of the wastewater samples are presented in Fig. 1. The daily discharged amounts were obtained by multiplying the concentration of the chlorophenols and phenoxyacids in the flow-proportional 3-weeks-samples with the daily average amount of water discharged over the same period.

CHEMICAL FATE

No significant biodegradation in seawater at 4 and 15 °C was observed for MCPA, MCPP, and 2,4-DP when these were the only supplied carbon source (Table II), or as components in the complex wastewater (Table III).

Both 4-chloro-2-methylphenol and 2,4,6-trichlorophenol were biodegraded for more than 90 % at 4 and 15 °C when supplied as the only carbon source in a concentration of 0.1 $mg.l^{-1}$ of specific compounds. No degradation was observed in a concentration of approximately 14 $mg.l^{-1}$ DOC (Table II). The missing biodegradability at the high concentration might be explained by a toxic effect of this concentration on the microorganisms in the inoculum (Table IV).

When 4-chloro-2-methylphenol was supplied to the test system as components of the wastewater, the compound was biodegraded for more than 90 % at 15 °C while only a minor decomposition (20 % after 56 days) of 2,4,6 -trichlorophenol of the wastewater was observed (Table III).

Two other chlorophenols in the wastewater, namely 2,4- and 2,6-dichlorophenol were both degraded for more than 90 % in 56 days at 15 °C, while 4,6-dichloro-2-methylphenol was not biodegraded (Table III). In another study De Kreuk and Hanstveit (1981) found 2,6-dichlorophenol and 2,4,6-trichlorophenol to be biodegradable in seawater.

The chemical fate of the five major components is summarized in Table IV giving the significance of the results to the receiving seawater.

TOXIC EFFECTS

The toxic effects of the wastewater samples are summarized in Table V. Microalgae proved to be the most sensitive organisms to the wastewater from the herbicide manufacturing plant (i). The photosynthesis of natural phytoplankton, the growth of the cultured algae Phaeodactylum tricornutum, and mortality and reproduction of the crustacean Nitocra spinipes showed almost the same sensitivity towards sample (i). Guppies were almost insensitive to the wastewater, and turbot larvae showed to be only slightly more sensitive than adult fish. No sublethal effects on fish larvae during long exposure periods were observed. The least sensitive test organisms were heterotrophic microorganisms.

Table IV. Evaluation of the significance of the chemical fate processes in a marine environment of pH 8 and a temperature of 15 °C

Chemical compound	Adsorption to sediment	Adsorption constant Kp	Bioaccumulation	Bioconcentration factor	Evaporation	Biological degradation (seawater)	Degradation rate	Photo-Chemical degradation
2,4-DP	-[a]	1×10^{-2}	-	0.31	-	-	0	-
MCPA	-	3×10^{-4}	-	0.02	-	-	0	-
MCPP	-	1×10	-	0.31	-	-	0	-
4-chloro-methylphenol	+	7.9	+	79	-	+	$0.01 - 0.13/d^{-1}$	-
2,4,6-trichlorophenol	+/-	0.4	+	6.3	-	+	$0.01 - 0.15/d^{-1}$	-

[a]Not significant

Table V. Toxicity of wastewater samples. The significance effect level is calculated as LC(EC)10 or EC20. (LC = lethal concentration, EC = effect concentration). The level of significance in each test is fixed according to the standard deviation of control experiments in this and other investigations

Test organisms	Test parameter	Test duration	Significant effect levels: Effect[b]	Wastewater samples (ml.l^{-1}) (i)	(ii)	(iii)
Heterotrophic microorganisms	Oxygen consumption	3 h	EC20	850	> 800	> 800
Natural phytoplankton	Photosynthesis (^{14}C-uptake)	6 h	EC20	21	n.a.[a]	40
Crustacean (Nitocra spinipes)	Mortality	96 h	LC10	29	> 300	> 300
Guppy (Poecilia reticulata)	Mortality	96 h	LC10	> 300	> 300	> 300
Algae (Phaeodactylum tricornutum)	Growth (cell density)	7 d	EC10	30	> 100	> 100
Crustacean (Nitocra spinipes)	Reproduction	12 d	EC20	23	> 300	> 300
Fish larvae: turbot (Scophthalmus maximus)	Mortality	4 w	LC10	100	> 100	> 100
	Growth (weight)	4 w	EC10	100	100	100
Eelgrass (Zostera marina)	Growth of leaves	6 w	EC10	> 2	> 100	> 300

[a]Not analyzed
[b]The significant effect level is calculated as LC10, EC10, or EC20. The level of significance in each test is chosen according to the standard deviation of control experiments

Tests on the effluent from the sewage treatment plant (ii) showed the reduction in toxicity caused by the dilution of the wastewater (i) and the degradation of phenoxyacids and chlorophenol in the treatment plant.

Tests on the effluent in periods of no discharge from the herbicide plant (iii) showed that, except for the photosynthesis of natural phytoplankton, no toxic effects could be detected. The effect on phytoplankton of sample (iii) could not be explained by the presence of phenoxyacids or chlorophenols in the wastewater but no further attempt was made to explain the observation.

Many studies have shown that toxic effects of mixtures could be predicted by concentration addition (Sikka et al., 1976 ; Könemann, 1981).

The present study showed that the toxic effect of the wastewater from the herbicide plant (i) and effluent from the sewage treatment plant (ii), could be explained by the concentration of phenoxyacids present while chlorophenols did not at all contribute to the effect of the wastewater.

FIELD INVESTIGATIONS

The field investigations with transplanted eelgrass and eel positioned near the wastewater outlet during 6 weeks and on a reference locality showed no significant difference in leaf production of the eelgrass, nor in taste of the eel (tested in an organoleptic triangle test) or in chlorophenol content (detection limit 0.5 $ng.g^{-1}$ eel muscle and skin).

CONCLUSION

This study shows that methods to evaluate biological effects on the marine environment - including biodegradation studies in seawater, biotests on marine organisms and field investigations - are available and can be used to characterize a complex wastewater.

By using a characterization procedure combining chemical analysis, chemical fate evaluations, and laboratory and field investigations, phenoxyacids proved not to be significantly eliminated from the marine environment, while no adsorption onto the sediment or bioaccumulation could

be expected. The concentration of phenoxyacids in the industrial effluent studies explains the acute and chronic toxic effects of the wastewater on marine test organisms of which microalgae showed to be most sensitive.

The chlorophenols 2,4,6-trichlorophenol and 4-chloro-2-methylphenol are biodegraded in seawater and some adsorption onto the sediment and bioaccumulation of these substances is to be expected. The chlorophenols did not - due to the low concentrations present - contribute to the toxic effects of the wastewater.

In situ investigations with transplanted eelgrass positioned on a reference locality and near the wastewater outlet showed no difference in leaf production. Likewise, in situ studies on off-flavour and bioaccumulation with eel placed in cages near the wastewater outlet and at a reference station, showed no difference in taste nor in chlorophenol content.

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