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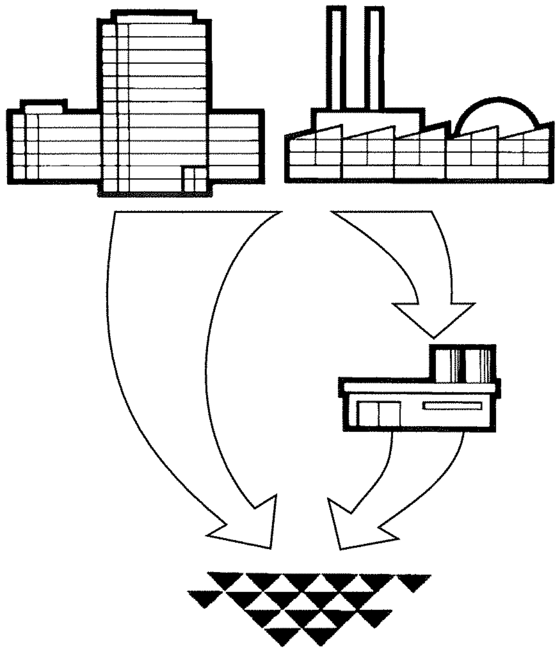
Ecotoxicological characterization and risk assessment of industrial wastewaters



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Abstract

Procedures for performing ecotoxicological characterization of industrial wastewaters and for the use of information from ecotoxicological tests in risk assessment and management of industrial pollution are described. This approach is recommended as a cost-efficient strategy for management and control of industrial water pollution.

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1. Industriavløpsvann	1. Industrial waster water
2. Toksisitet	2. Toxicity
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Ecotoxicological characterization and risk assessment of industrial wastewaters

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Preface

A water pollution abatement programme was launched in 1991 as part of a bilateral agreement between Norway and the Czech Republic on co-operation in the field of environmental protection. The use of ecotoxicological tests in protection and improvement of water ecosystems was selected as subject for one of the projects in the program. This project was carried out in co-operation between the Water Research Institute, Ostrava and the Norwegian Institute for Water Research with assistance from Podovi Odry, Ostrava.

A screening and preliminary environmental risk assessment of industrial discharges was carried out in 1992-1993 and has been reported in a previous report (Källqvist & Soldán 1993). Based on the results, some industries were selected for additional ecotoxicological characterization of effluents. This final report contains recommendations for the use of ecotoxicological test methods for risk assessment and characterization of industrial wastewaters with examples from studies of selected industries in the Odra river catchment.

Oslo, 07.01 1998

Torsten Källqvist

Contents

Summary

Ecotoxicological tests are useful supplementary tools to chemical analysis for characterization of industrial wastewaters. A stepwise procedure including identification of problem discharges, effluent characterization, risk assessment of wastewater discharge, identification of toxic components, and pollution control measures is described. This approach is recommended as a cost-effective strategy for management and control of industrial water pollution.

1. Introduction

Discharge of industrial wastewater constitutes major point sources of toxic pollutants to the aquatic environment. Efficient management of industrial wastewater pollution requires techniques for characterization of the wastewater as regards their potential to cause environmental effects. Due to the complexity and variability in wastewater composition, characterization based on chemical identification and quantification of potentially toxic components is usually not possible. Furthermore, even if the chemical composition of a wastewater was fully described, prediction of environmental effects would be complicated by lack of information on the ecotoxicological properties of many components as well as their combined effects.

Ecotoxicological testing may be applied both on single compounds and complex mixtures and thus provides useful information on the ecotoxicological properties of industrial wastewaters. A combination of chemical and ecotoxicological characterization is therefore a cost effective approach for management of industrial effluents.

Formalised procedures involving ecotoxicological testing for risk assessment and regulation of industrial discharges have been adopted by several countries including USA (EPA 1984, 1991), Sweden (Statens Naturvårdsverk 1990) and Denmark (Pedersen et al. 1994). This document gives a brief introduction to the field of ecotoxicological wastewater characterization and some proposals as to how information obtained from toxicity tests can be used in risk assessment and wastewater management.

2. Ecotoxicological test methods

The potential of a chemical to cause environmental damage is first of all dependent on its toxicity. On the organism level, the toxic effect may be acute, which means that the response (e.g. lethality) occurs after a short exposure, or chronic, which means that effects on e.g. growth or reproduction occur after exposure over a substantial part of the organism's life cycle. When an ecosystem is exposed to toxic chemicals at a concentration above the limit for acute or chronic effects, the structure and function of the ecosystem may be impaired.

In addition to toxicity, also properties which influence the fate and distribution of the compound in the environment are essential for assessment of environmental impact. These properties include physical properties such as solubility and volatility as well as the potential for biodegradation and bioaccumulation.

Test designed to give information on the toxicity, biodegradation and bioaccumulation of chemicals are known as ecotoxicological tests. Protocols for such test has been developed and standardised by international bodies like OECD and ISO. The main use of the tests have been in characterization of single chemicals, e.g. in connection with notification, environmental labelling and risk assessment, but the test methods may also be adapted for testing of complex chemical mixtures and waste waters.

2.1 Toxicity

Test procedures have been developed for assessment of toxic effects of chemicals or mixtures on a large number of aquatic organisms (OECD 1995). Because the sensitivity may vary substantially between species and groups of organisms, tests with different organisms are usually required to obtain an estimate of a chemical's toxic potential. Most test schemes for risk assessment therefore prescribe the use of a battery of test organisms for toxicity assessment. On the basic level of toxicity characterization, tests with algae, crustacean and fish are usually included. These organisms represent three main food chain levels in aquatic ecosystems; producers (algae), primary consumers (crustacean) and secondary consumers (fish).

The general principle for the toxicity tests is that test organisms are exposed in a series of increasing concentrations of the test compound or wastewater, and the response is observed within a specified time period (see fig. 1). The observed responses are compared to the response of organisms held in the same medium without the influence of the test substance (control). The result is normally presented as a concentration/response plot showing the response as a function of concentration. From this plot the concentration causing 50 % response can be derived. When the response is lethality, this concentration is denoted LC_{50}. For other type of the responses EC_{50} (effect concentration) is applied.

The principles of the most commonly used test categories are briefly described below.

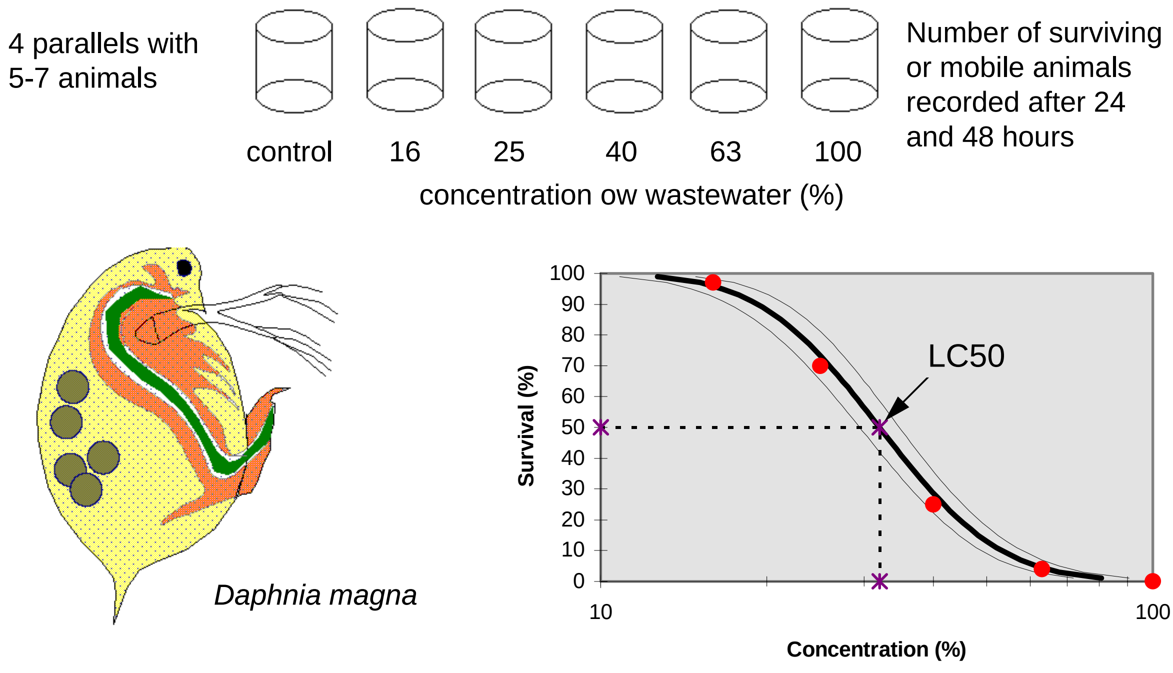

Fig. 1. The principles of toxicity tetsting of wastewater examplified by an acute test with the crustacean *Daphnia magna*

2.1.1 Algal growth inhibition test

Protocols for toxicity tests with fresh water microalgae have been developed by OECD (Guideline 201, 1984) and ISO (8692). These are identical in all essential aspects. Recommended species are the green algae *Selenastrum capricornutum* or *Scenedesmus subspicatus*, although the OECD Guideline allows use of alternative species.

A concentration series of the test substance in the growth medium is inoculated with test algae and incubated under defined conditions. The cell density in the cultures is measured at 24 hour intervals for three days. The EC_{50} for effect on growth of the test alga is estimated from the concentration/response curve.

In algal tests of wastewaters a growth stimulating effect is often observed at low concentrations of wastewater. Testing of turbid or strongly coloured effluents is complicated by light absorption in the wastewater which reduces the light available for the algal photosynthesis. Filtration may be required to reduce turbidity although it should be noted that this manipulation may remove toxic components adsorbed to particulate material.

2.1.2 Daphnia immobilisation test

Protocols for acute toxicity tests with the freshwater copepod *Daphnia magna* are found in ISO 6341 and OECD Guideline 202 (1984). Juvenile animals are exposed in a concentration series of the test substance in a defined medium. The number of immobilised animals are recorded after 24 and 48 hours. The EC_{50} for effect on immobilisation is calculated.

When testing wastewater with a high content of biodegradable organic material, oxygen concentration may drop below the recommended lower limit in the test protocol. In such cases use of test containers with a high surface/volume ratio, e.g. petri dishes may be required.

2.1.3 Fish acute toxicity test

Protocols for acute toxicity tests with fresh water fish can be found in ISO 7346 and OECD Guideline 203 (1984). These protocols differ in length of exposure period and specifications on test species and dilution water. The test can be carried out as a static test, i.e. without renewal of the solutions, semistatic, with daily renewal or continuous in a flow through system. In the case of waste waters, continuous flow is normally not applicable, unless the test can be carried out on the site. Lethality of fish is recorded daily for 48 (ISO) or 96 (OECD) hours and the LC_{50} value is derived from the response-curve.

When testing wastewater with a high content of biodegradable organic material, the oxygen concentration may drop below the recommended lower limit in the test protocol. To reduce this problem, aeration of the tanks should be considered, but it should be noted that volatile toxic compounds may be stripped of by this treatment.

2.1.4 Daphnia magna reproduction test

A protocol for the *Daphnia magna* reproduction test has been developed by OECD (Guideline 211, 1997). The reproduction test is a test of chronic toxicity. Young, female *D. magna* are held individually or in groups in containers with different concentrations of the test substance. The animals are transferred to freshly prepared solutions every 2-3 days and fed with fresh green algae. The number of offspring produced is recorded for 21 days. From the results, the EC_{50} for effect on reproduction and the NOEC (No observable effect concentration) are calculated. NOEC is defined as the highest concentration tested, where reproduction is not significantly different from the control.

When performing the *Daphnia* reproduction test on industrial wastewater, enough portions of the sample should be frozen for preparation of new test solutions during the test.

2.2 Biodegradation

Biological degradation of organic chemicals occurs mainly through the action of heterotrophic micro-organisms (bacteria and fungi), which use organic matter as a source of energy. The degradability of chemicals is dependent on the molecular structure and some configurations are very resistant to attack by the enzymes involved in biological degradation. Such chemicals (e.g. polychlorinated biphenyls, PCB and polycyclic aromatic hydrocarbons, PAH) may be persistent in the environment with the potential to cause long lasting harm.

Test methods for biodegradability can be classified in different categories depending on their purpose and the type of information they provide. The OECD guideline distinguishes between tests for ready biodegradability, tests for inherent biodegradability and simulation tests. The category employed as the first step in characterization of biodegradability of a chemical is the test for ready biodegradability. Those chemicals that are not fulfilling specified criteria for ready biodegradability may be further tested for inherent degradability to disclose whether they may be degraded under more favourable conditions or if they should be considered as persistent. Simulation test are designed to provide information on the rate of degradation in various environments e.g. biological waste water treatment plants, lakes or rivers.

Although biodegradation tests are mainly applicable to characterization of single chemicals they may also provide useful information on the property of wastewaters, when used in combination with toxicity tests and specific chemical analysis. For wastewater characterization, tests for ready biodegradability are usually the most relevant.

2.2.1 Test for ready biodegradability

Several alternative methods have been developed for assessing ready biodegradability of chemicals. (OECD Guidelines 301 a-f). Common for all is that the test substance is added to a defined mineral medium which is inoculated with a mixed micro-organism community from a waste water plant. Test for ready biodegradability are normally run for 28 days. The degradation is recorded as reduction of dissolved organic carbon, production of carbon dioxide or consumption of dissolved oxygen. For testing of complex mixtures, specific analysis of various components may also be applied. The effect of degradation on toxicity may be investigated by repeating a toxicity test (e.g. with *Daphnia*) on the sample after degradation.

When testing the biodegradation potential in wastewaters, care should be taken to avoid inhibition of degradation caused by toxic components. This may require dilution of the wastewater before testing. Dilution may also be required to keep the amount of organic carbon within the range recommended in the test protocol.

2.3 Bioaccumulation

The tendency of some chemicals to accumulate in living organisms is of importance for their distribution and potential to cause adverse effects in the environment. Although the bioaccumulation of toxic substances will ultimately be manifested as a toxic effect at some level in the ecosystem, such effects may not necessarily be disclosed by simple short term toxicity tests. The potential for bioaccumulation needs therefore to be addressed specifically in ecotoxicological characterization of chemicals.

Tests for bioaccumulation in aquatic organisms are usually carried out by exposing fish to sublethal concentrations of the chemical for a sufficient length of time to achieve steady state. Samples of fish and water are analysed for the specific chemical and the bioconcentration factor (BCF) calculated as the ratio between concentrations of the chemical in fish and water respectively at steady state.

The process of bioaccumulation can be approximated as a partitioning between water and a lipid phase in the biological material. As a result the bioconcentration factor is correlated to the lipophilicity e.g. as expressed by its octanol/water partitioning coefficient (P_{ow}). Measurement of P_{ow} is therefore often used as a substitute to the resource demanding bioaccumulation tests in characterization of chemicals. (OECD Guidelines 107, 117). For classification purposes, chemicals with log $P_{ow} > 3$ are considered to have potential for bioaccumulation.

Since bioaccumulation is a character that applies to single components and not to the wastewater it will generally require a chemical specific approach. This means that the bioaccumulation potential of all known constituents of the wastewater should be assessed. For unknown components separation of lipophilic fractions of the wastewater using chromatographic techniques (TLC, HPLC) followed by gas chromatographic analysis may give indications on the presence of potentially bioaccumulative components (Renberg et al. 1985, Hynning 1996).

3. Sampling of wastewater

General guidance on the sampling of industrial wastewaters is given in ISO/DIS 5667-16

Industrial wastewaters often show large variation in volume and composition, and the ecotoxicological characteristics of the wastewaters will vary correspondingly. Large variations can be expected especially when the production is batchwise or when different processes are alternating in the production. Industries with continuous processes generally generate wastewater of more uniform quality. Retention basins and treatment plants will have the effect to reduce variations.

Before sampling for an ecotoxicological characterization is planned, an assessment of the qualitative and quantitative variations in the discharge should be made. The sampling strategy has to be adapted to the wastewater variations and the purpose of the study. Normally a time-averaged or flow-proportional sample, e.g. taken over 24 hours, is appropriate. The retention time in the wastewater system can be used as a guidance for deciding the averaging time for sampling.

If the wastewater variation is large, e.g. in industries with batchwise production with several processes, it may not be possible to base the characterisation on one integrated sample. If the averaging time has to be several days to cover all variations in wastewater quality, there is a risk that peaks in discharge of toxic components, which may cause acute toxic effects in the receiving water are diluted to the extent that they are not reflected by the toxicity tests. In this case characterization should be performed on several samples.

Industrial wastewater may contain unstable components. Fore that reason the sample should be tested as soon as possible after collection. To reduce qualitative changes during transport and storage, the sample should be cooled to a temperature between 2 and 4 °C. If the testing can not be started within 24 hours after the sample has been taken, freezing of the sample should be considered. Further advice on storage and pre-treatment of industrial wastewater samples are given in ISO/DIS 5667-16

4. Wastewater characterization and risk assessment

Wastewater characterization for pollution control and management will normally be carried out as a stepwise procedure, where the need for further assessments is considered after each step. (See fig. 2). The use and interpretation of ecotoxicological test data at each step is discussed below.

4.1 Step 1 - Identification of potentially harmful wastewaters

The first step in wastewater characterization is to identify potentially harmful wastewaters that require further testing and assessment. This step includes collecting relevant background information on the wastewater and conducting screening tests for toxic effects. Based on the information obtained, a preliminary risk assessment is performed and the need for a more detailed study is considered.

4.1.1 Basic information on wastewater

All available information on the industrial process and the wastewater is collected including:

- Raw materials
- Chemical processes and formation of by-products
- Use of water and production of wastewater
- Periodicity in processes
- Variations in wastewater volume
- Variations in wastewater quality
- Treatment of wastewater
- Discharge arrangements
- Chemical data on wastewater composition

4.1.2 Toxicity screening

A screening toxicity test e.g. with algae, *Daphnia* or bacteria (Microtox) is used to give a first estimate of effluent toxicity.

4.1.3 Preliminary risk assessment

The preliminary risk assessment is based on the screening toxicity test and the estimated degree of dilution in the receiving water. Since only limited information on toxicity is available at this stage, no risk calculations can be made, but if the EC_{50} value in the screening test is lower than 100% (i.e. the toxic response is above 50% in the undiluted sample), the need for further characterization must be considered. Other factors that should be considered are the wastewater dilution in the receiving water, and known or suspected content of harmful substances in the waste water.

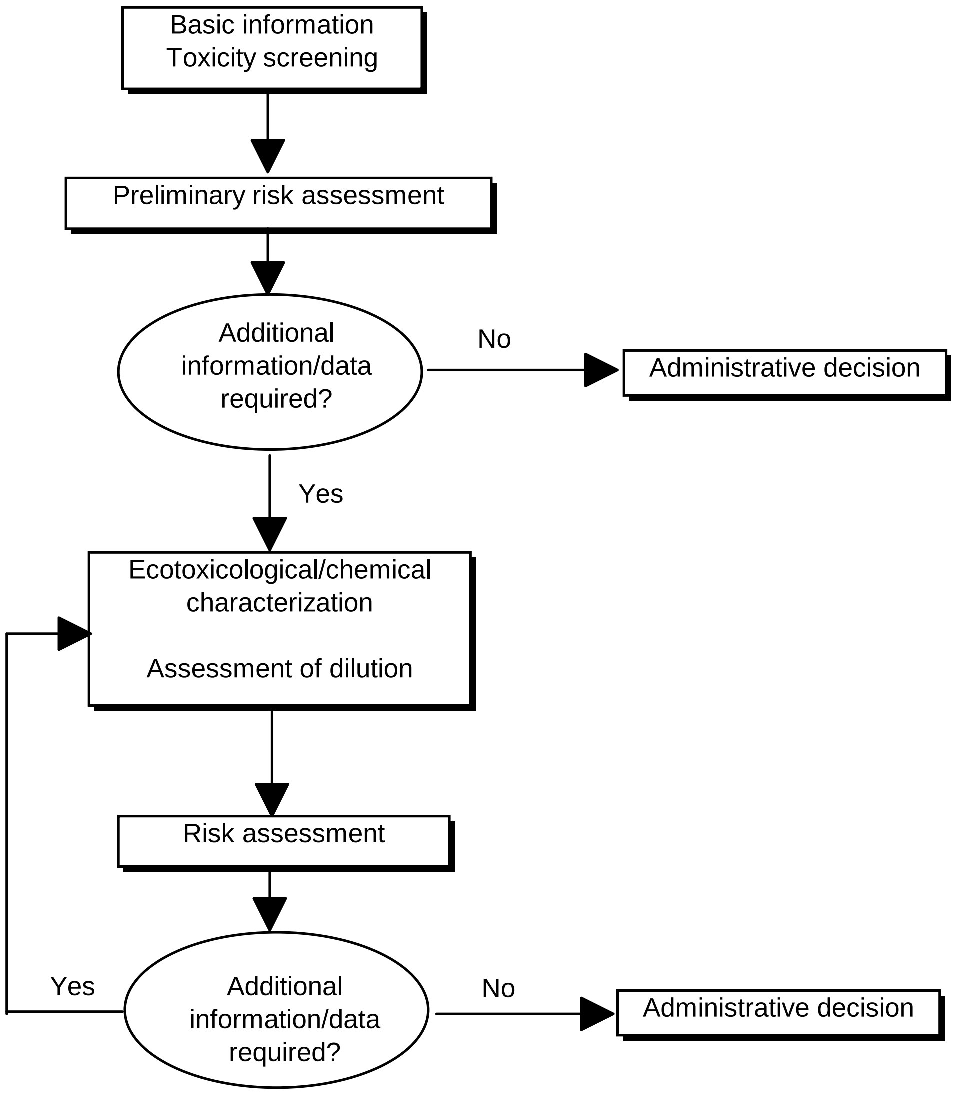

Fig. 2. Scheme of stepwise characterization/risk assessment of wastewaters

4.2 Step 2 - Characterization and risk assessment

When the preliminary risk assessment indicates that the wastewater may have unacceptable effects, further characterization should be initiated, including ecotoxicological testing and chemical analysis. The dilution of wastewater in the receiving water is also estimated in more detail.

4.2.1 Toxicity testing

The toxicity of the wastewater is investigated with algae, *Daphnia* and fish as test organisms.

4.2.2 Chemical characterization

The chemical characterization should include basic parameters such as pH, electrolytical conductivity, chemical oxygen demand, biochemical oxygen demand and total organic carbon. Additional specific analysis of any known toxic components in the wastewater should be included, depending on the nature of the wastewater.

4.2.3 Assessment of dilution in receiving water

The concentration of wastewater in the receiving water is estimated from the volume of wastewater and the hydraulic conditions in the receiving water. The initial dilution is defined as the average dilution in the cross section of the waste water plume when the waste water has lost so much momentum that its velocity relative to the receiving water is zero. The initial dilution will depend on the velocity of the waste and receiving waters, the design of the discharge pipe and density differences between the two water masses.

In the case of discharge to a river, the wastewater plume is spreading in a fan-like fashion and the concentration of wastewater in the plume gradually decreases until the wastewater is diluted in the whole river flow (see fig. 3). At this point the theoretical wastewater concentration is Qw/Qr, where Qw and Qr are the flow rates of waste water and receiving water respectively (e.g. as l/s). The time required to reach the point of maximum dilution is depending on the flow conditions and the depth and width of the river. Rapid and turbulent flow causes more efficient mixing than slow, laminar flow.

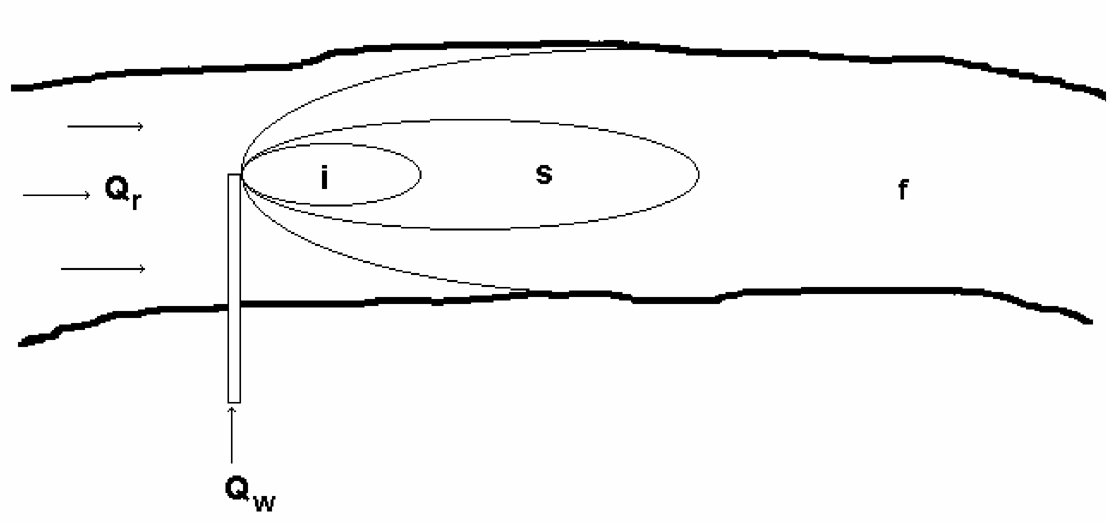

Fig. 3. Schematic view of dilution of wastewater in a river. i = initial dilution zone, s = secondary dilution zone and f = final dilution zone.

Models for calculation of dilution of wastewater in rivers are described in OECD (1989). Another approach is to measure the dilution by analysing a conservative component in the wastewater in sections of the river downstream of the discharge point. If no suitable component for such measurements can be found, a tracer (e.g. rhodamine) may be added to the wastewater.

4.2.4 Risk assessment

The principle for the risk assessment is that the predicted environmental concentration (PEC) is compared with the predicted no effect concentration (PNEC). Calculation of PEC/PNEC ratios are performed for acute and chronic effects.

The minimum criteria for protection of the rivers should be that:

- No acute toxic effects will occur after initial dilution of the wastewater

- No chronic toxicity will occur after complete dilution of the wastewater in the receiving water at minimum flow (e.g. 95 percentile, which is the flow that is exceeded 95% of the time).

This implies that acute toxic effects may be accepted in the initial mixing zone (zone i in fig. 3) and chronic effects are accepted in a restricted zone of the river (zone s). Chronic effects may extend into zone f when river water flow is less than the minimum flow (95 percentile).

If the wastewater is discharged to a lake, the extension of the zone where chronic effects are accepted will have to be decided, based on local conditions.

Calculation of PEC

The PEC used for calculation of acute toxicity risk is the concentration after initial dilution (PEC_i). If no information of initial dilution is available, PEC_i should be assumed to be 20%. It should be noted, though, that PEC_i can never be lower than the concentration obtained after complete dilution in the receiving water.

The PEC used for calculation of chronic toxicity risk is the concentration at full dilution of the wastewater in river water at minimum flow ($Q_{r(min)}$, 95% percentile) (PEC_f). For wastewater, the average flow is used in the calculation: $PEC_f = Q_w/(Q_{r(min)}+Q_w)$

Calculation of PNEC

The PNEC is calculated from the information of toxicity obtained from the toxicity tests or, in the case when single toxic components have been identified, on available information on their toxicity.

It is obvious that the PNEC will be lower than the EC_{50} values found in the (three) short term toxicity tests. First of all the "no-effect" concentration has to be estimated from the 50%-effect concentration. Also the risk that not tested species may be more sensitive than those included in the toxicity tests has to be taken into account, Finally, addition of several sources of uncertainty involved when predicting effects in the real environment from laboratory tests must be considered.

Application factors to derive chronic PNEC for single substances from toxicity tests have been proposed by OECD (1992) (Table 1). In the case of complex mixtures there is evidence that interspecies variations in sensitivity are lower than for single substances, and the application factors can be lower. The Danish EPA has proposed the following application factors for wastewaters (Pedersen et al. 1994) (Table 2):

Table 1. Application factors for calculation of PNEC from data obtained in toxicity tests of chemicals. (Adapted from OECD 1992)

Available information	Application factor
Lowest acute $L(E)C_{50}$ derived from a set of one or two aquatic species	1000
Lowest acute $L(E)C_{50}$ derived from a set of data at least consisting of algae, crustaceans and fish	100
Lowest NOEC value for chronic toxicity derived from a set of data at least consisting of algae, crustaceans and fish	10

Table 1. Application factors for calculation of PNEC from data obtained in toxicity tests of wastewaters.

Available information	Application factor ($PNEC_{acute}$)	Application factor ($PNEC_{chronic}$)
Lowest $L(E)_{50}$ for acute toxicity	100	200
Lowest $L(E)C_{50}$ of tests with at least one algae, one crustacean and one fish species	10	20
Lowest $L(E)C_{50}$ for acute toxicity of more than 5 species representing different groups	5	10
Lowest NOEC found in chronic toxicity tests with at least one algae, one crustacean and one fish species	-	5

Proposed criteria for triggering further action are listed in table 2.

Table 2. Proposed criteria for further action based on risk assessment

PEC/PNEC (acute or chronic)	Actions
<0.1	No further actions
0.1 - 1	Additional characterization / risk assessment (Step 3)
>1	Pollution abatement, additional characterization / risk assessment

In addition to PEC/PNEC ratios also other information on possible harmful constituents in the wastewater, which are not reflected by the toxicity tests can call for further actions. If bioaccumulating, toxic substances are present, pollution control actions may be required even if the PEC/PNEC ratios are less than 0.1.

4.2.5 Calculation of toxicity emission factor

The total toxic load imposed by the waste water can be expressed as the toxicity emission factor, TEF. TEF values are useful for comparing the total toxic load from different industries, e.g. to assess the relative contribution of different pollution sources to the overall toxic load in a receiving water.

For calculation of TEF, the EC_{50}-value is first converted to toxical units (TU):

$TU = 1/EC_{50}$

The TEF value is obtained by multiplication of TU with the waste water flow (Q_w, l/s)

$TEF = TU*Q_w$

4.3 Step 3, extended characterization and refined risk assessment

When PEC/PNEC ratios for acute or chronic effects are in the range 0.1 - 1, further information should be collected to increase the confidence of the risk assessment. The additional studies may include:

- Test for chronic toxicity e.g. on crustacean and/or fish
- Additional chemical characterization
- Biodegradation/toxicity study to assess persistence of toxic components
- Refinement of dilution calculation (e.g. dilution models)

5. Ecotoxicological testing for wastewater toxicity reduction

When the ecotoxicological risk assessment has shown that the risk for toxic effects in the recipient in unacceptable, measures to reduce the toxic discharge have to be implemented. Since the aim of the measures is reduction of toxicity, ecotoxicological tests may play an important role in the process of finding the optimal solutions.

5.1 Identification of toxic substreams

In industries where wastewater is generated from several different processes, toxicity tests are useful for identification of the most important sources of toxic constituents. Which test(s) to use should be decided from the results of the wastewater characterization. Often simple screening tests may be sufficient for this purpose. Calculation of TEF values allows assessment of the contribution of each substream to the total effluent toxicity.

5.2 Characterization and identification of toxic component(s)

Identification of toxic components in complex industrial wastewaters by chemical means is usually very complicated. An approach using chemical fractionation in combination with toxicity tests may be an efficient approach to describe the physical/chemical characteristics of the toxicant and to identify which class of compounds the toxicant belongs to. Such information makes the final chemical identification more easy. The effect of various manipulations performed in the procedure on the effluent toxicity may also provide useful information for the design of wastewater treatment processes.

A scheme for performing toxicant characterization has been developed by US. EPA (1991), Burkhard & Ankley 1989. The scheme involves several treatments of the wastewater sample that will remove or inactivate different classes of toxic chemicals. After each treatment, the toxicity is compared with that of the original sample. By this technique, the toxicity may be tracked to a certain class of chemical compounds. Several of the manipulations are carried out in acid and basic environments as well as at the original pH of the wastewater in order to detect anionic or cationic toxicants. After the manipulation, the pH is adjusted back to the original pH before toxicity testing. By changing the pH, the ratio of ionized to un-ionized species in solution for a chemical is changed significantly. The ionized and un-ionized species have different physical and chemical properties as well as toxicity.

An example of an effluent manipulation scheme for toxicity evaluation is shown in figure 3. The manipulations involved are described below. A blank sample should be used along with the wastewater through all manipulations in order to disclose any artefacts caused by the treatments.

5.2.1 Oxidant reduction.

In the oxidant reduction step, a reducing agent, sodium thiosulphate is added to reduce oxidants. A concentration series of the wastewater is prepared and treated with 10 mg/l of $Na_2S_2O_3$/l. Test organisms are introduced to the samples one hour after treatment. Reduced toxicity as compared to the baseline toxicity test indicates presence of toxic oxidants (e.g. chlorine or bromine).

5.2.2 EDTA chelation

EDTA (ethylenediaminetetraacetic acid) is an organic chelating agent that preferentially binds with divalent cationic metals. When a metal is bound to the EDTA the toxicity of the metal is generally reduced. A concentration series of the wastewater is prepared and treated with 1-5 mg/l of EDTA. Test organisms are introduced to the samples three hours after treatment. Reduced toxicity as compared to the baseline toxicity test indicates presence of toxic divalent metals. (See Appendix A)

5.2.3 Aeration

Air from an oil-free air pump is bubbled through the wastewater sample in a glass cylinder for one hour. Reduced toxicity as compared to the baseline toxicity test indicates the presence of volatile toxicants (e.g. H_2S or volatile hydrocarbons). Removal of toxicity by aeration at high pH may indicate presence of ammonia.

5.2.4 Filtration

In the filtration step, the wastewater sample is passed through a glass fibre filter with pore size of approx. 0.5 µm. Organic membrane filters are not recommended since they may adsorb dissolved organic contaminants. Reduced toxicity as compared to the baseline toxicity test indicates that toxic components are adsorbed to particles.

5.2.5 C_{18} solid-phase separation

In the manipulation, reverse phase liquid chromatography is applied to extract non-ionic organic toxicants from the wastewater sample. The sample is passed through a disposable C_{18} column and the post-column effluent tested for toxicity. Absence of toxicity after treatment suggests that organic toxicants were active in the original sample. Elution of the column with methanol can be used to return toxicants to aqueous solution to confirm toxicity. Alternatively, XAD resin can be added to the wastewater sample to adsorb organics as described in Appendix B.

5.2.6 Graduated pH procedure

In the graduated pH test, the pH of the wastewater is adjusted within a physiologically tolerable range (i.e. pH 6, 7 and 8) before toxicity testing. (A suitable range has to be checked for the test species used). Generally, the non-ionized form of a toxicant is able to cross biological membranes more readily than the ionized form and thus is more toxic. The test may indicate presence of ammonia (toxicity increases at high pH) or ionizable organics such as chlorophenols (toxicity decreases at high pH).

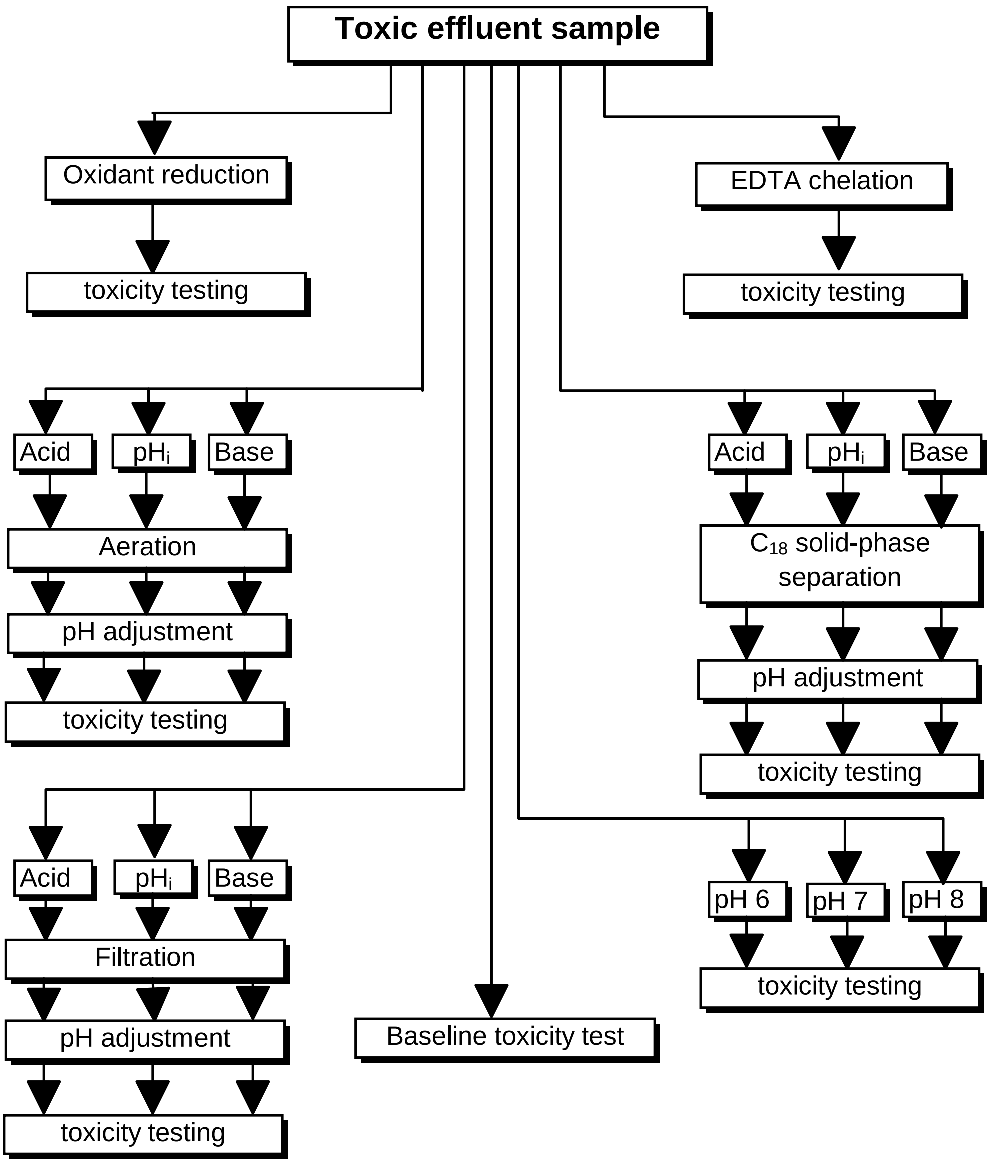

Fig. 3. Characterization and identification of toxic components through fractionation

5.3 Testing effects of wastewater treatment processes

When technical solutions to reduce pollution have been found, it may be tested whether these are effective in removing toxic components. Changes in industrial processes or various designs for waste water treatment may be tested on pilot scale, and the effects on waste water toxicity checked using screening toxicity test.

6. Effect -related discharge permits

Discharge permits for industrial waste water are normally based on quantities or concentrations of specific components. The use of ecotoxicological tests gives the option also to include effect-related criteria in discharge permits. The criteria may be derived from the risk assessment and should be based on toxicity tests with the most sensitive of the standard test organisms used in characterization. If the aim is to achieve that no acute toxicity occurs after initial dilution of the wastewater, the effect-related criterion can be calculated from the $L(E)C_{50}$ of the most sensitive species, application factor (table 1) and concentration of wastewater after initial dilution (PEC_i). If, for example, if the PEC_i = 10 %, and *Daphnia* is the most sensitive species, the effect-related criteria to include in the discharge permit will be that *Daphnia* LC_{50} > 10x10 % or > 100%, which means that the immobilization of *Daphnia magna* shall be less than 50% in the undiluted wastewater.

7. Monitoring

Ecotoxicological testing can be used for monitoring waste water discharges and receiving waters. Selection of test methods for wastewater monitoring should be based on previous characterization and preferably include the most sensitive of the standard test species. A regular monitoring will disclose changes in wastewater composition that may require execution of a new characterization / risk assessment study. In the case that effect-related criteria are included in the discharge permit, the toxicity monitoring program will show if the criteria are exceeded.

Monitoring of receiving water should be carried out to check that the in situ toxic effects do not exceed the predictions made in risk assessment. Toxicity testing may be included in the monitoring as a supplement to biological field surveys. Samples for acute and chronic tests can be taken from different locations downstream of the wastewater discharge, and a sample from an upstream location included as reference.

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Appendix A.

Identification of toxic components in wastewater

1. Nova Hut

Identification of toxic components in wastewater from Nova Hut

During the preliminary toxicity screening of wastewater in the Odra river basin (Källqvist & Soldan 1993), the discharge from Nova Hut to Lucina river was identified as one of the major potential sources of toxic pollutants.

The wastewater stream from Nova Hut which is discharged to Lucina River was sampled on 17.03.94 for toxicity screening. Toxicity tests with the alga *Selenastrum capricornutum* showed successively increasing growth inhibition at wastewater concentrations above 1 %. The EC_{50} for growth rate inhibition was 3.6 %. (See fig. 1). The calculated dilution rate of the wastewater stream in Lucina River is 18% at average flow (Källqvist & Soldan 1993). Thus, the wastewater discharge most certainly will have an impact on the algal vegetation in Lucina River. Since Nova Hut is a metallurgical industry, it was expected that the toxicity is caused by metals in the wastewater. However, a rather high chemical oxygen demand found in a previous study (24 mg/l) indicates that organic pollutants may also be present.

In order to investigate if metals were the main toxic components in the wastewater, a toxicity test with *Selenastrum capricornutum* at a concentration of 10% wastewater with and without the addition of the chelator EDTA (5 mg $Na_2EDTA \bullet 2\ H_2O$/l). The result is shown in fig. 2. In the sample without EDTA the growth was almost completely inhibited, but when EDTA was present the growth curve was almost identical with the control culture without wastewater. The result shows that the toxicity of the wastewater is significantly reduced by addition of a chelator, which strongly indicates that the toxicity is caused primarily by metals in the wastewater.

Chemical analysis of the wastewater sample confirms that the concentration of zinc is very high (2.5 mg/l). This corresponds to 100µg/l at the EC_{50}. This zinc concentration i sufficient to explain the observed toxicity.

Table 1. Content of heavy metals in wastewater from Nova Hut 17.03.94.

Cd (µg/l)	Cr (µg/l)	Cu (µg/l)	Ni (µg/l)	Fe (µg/l)	Zn mg/l)
4.19	0.8	1.7	<5	0.85	2.5

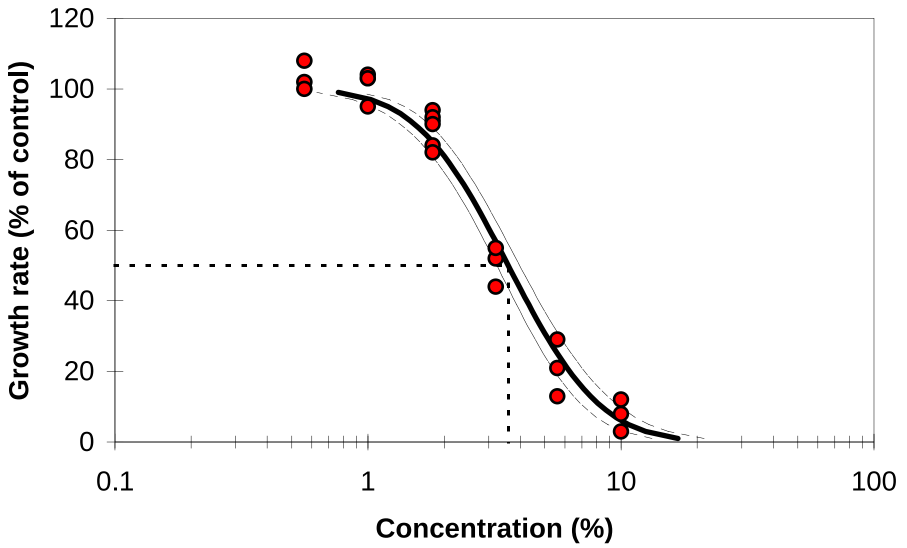

Fig. 1. Effect of wastewater from Nova Hut on the growth rate of *Selenastrum capricornutum*.

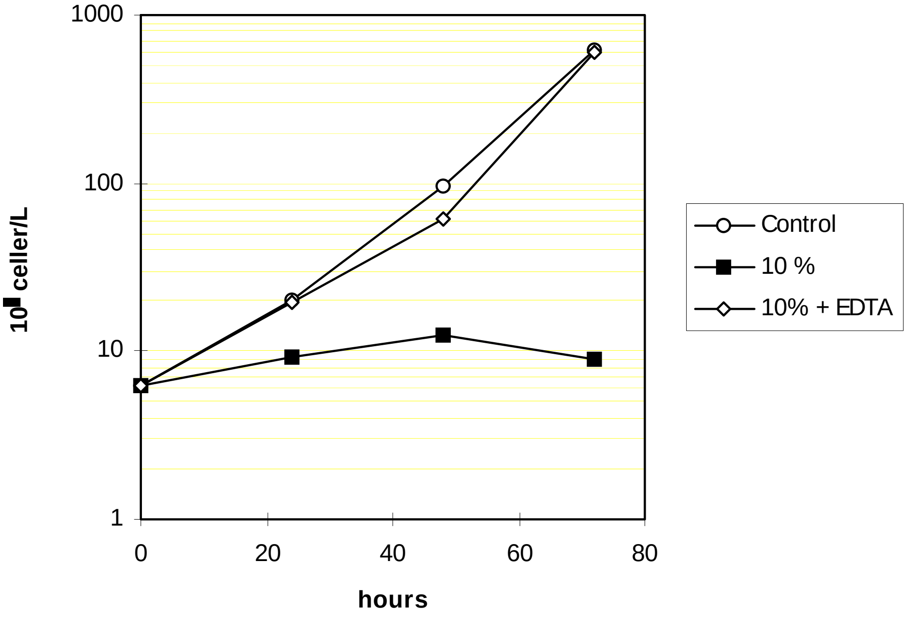

Fig. 2. Growth of test algae (*Selenastrum capricornutum*) in 10% wastewater from Nova Hut with and without addition of EDTA.

Appendix B.

Identification of toxic components in wastewater

2. Ferak Raškovice and Bochemie

Paper presented at H.I. International Conference Odra 97 - Wastewater Treatment and Pollution Control in the Odra river Basin.

RNDr. Premysl Soldán

DETECTION OF THE MOST TOXIC POLLUTANT OF WASTE WATER

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Summary

The area of the Odra River Basin belongs to the most affected industrial regions in the Czech republic with a double density of settlement in comparison with national average. More than 700 point sources of waste waters after various levels of treatment are situated in the basin area spreading over 6 264 km^2 . The most significant sources produce 268 700 000 m^3 of waste waters annually (1995). Due to value of complex deterioration of biological parameters in the Odra River Basin there is an urgent need to set an abatement strategy programme based on ecotoxicological characterisation and risk assessment of industrial waste waters.

The sound abatement strategy has to be based on identification of substance or property which is the most responsible one for the waste water toxic impact. This identification is not possible by standard toxicity testing. Our laboratory have suggested a method of detection of the most toxic pollutant of waste water. This approach is based on US EPA method. Presented method is more simple to be widely applicable in the routine praxis. Our results are documented by the two selected examples.

Introduction

The area of the Odra River Basin belongs to the most affected industrial regions in the Czech Republic with a double density of settlement in comparison with national average. More than 700 point sources of waste waters after various levels of treatment are situated in the basin area spreading over 6 264 km^2. The most significant sources produce 268 700 000 m^3 of waste waters annually (1995). Due to value of complex deterioration of biological parameters in the Odra River Basin there is an urgent need to set an abatement strategy programme based on ecotoxicological characterisation and risk assessment of industrial waste waters.

Problem description

Waste water represents a mixture of substances which can have very different influence on aquatic organisms. The main parameter which is generally evaluated for every waste water in the Czech Republic is BOD_5 , which gives information about possible impact of organics load on selfpurification processes in the stream. Value of BOD_5 may be misrepresent if waste water contains substances which cause the inhibition of life processes of microorganisms. In this case the value is lower. Obtained result can lead to false conclusion that waste water contains less biologically degradable substances (it is very necessary to consider ratio of COD and BOD_5).

Data of BOD_5 are completed by results of chemical analyses of content of different substances -pollutants. These results can be very exact but the analyses of all substances which can be present in waste water and may influence the aquatic biota is time and financially expensive and in majority of cases even impossible. In addition, exact results can be used only for very rough estimation of toxic impact of waste water on organisms of stream recipient since the substances in mixture can add, potentiate, in several cases reduce their individual effects (CHRISTENSEN, 1984).

Adverse effects of waste water can be evaluated from results of toxicity tests. Exploitation of toxicity tests for assessment of waste waters was discussed by a lot of authors. The OECD has edited a monograph on this topic in 1987. Also Czech authors has relatively very early discussed this problem PYTLÍK (1934), ZELINKA (1954), MARVAN a ZELINKA (1964).

For setting the abatement strategy it is very necessary to detect as precise as possible the source of problems. But the reaction of organism on sample in toxicity test is complex not specific. Detection of a main waste water property, which is responsible for its adverse effect from the tests performed according the standard methods is very questionable. In the framework study financed by the Ministry of Environment of the Czech Republic we have intended on finding of the most appropriate approach, which can make this detection possible (SOLDÁN, LEONTIDIS 1996).

Used method

We have suggested approach, which is based on US E.P.A. method (1991), but is adapted to satisfy the needs of usual routine use. Principle of suggested approach is that firstly the sample of waste water is tested for its acute toxicity by standard tests with different organisms. Than the waste water is treated by different agents to inactivate certain waste water property, which can cause the adverse effect of waste water and the acute toxicity of treated sample is evaluated again with test on the most sensitive organism for tested waste water. Finally the results are compared. The cases of reduced toxicity indicate impact of inactivated property on the total toxicity of waste water.

Selected potentially adverse properties of waste water:

a) High content of suspended solids

Some toxic substances can be adsorbed on surface of suspended solids particles. These substances can act due to ingestion of them by test copepods. Suspended solids can acts also by mechanic way (reduction of light intensity necessary for algae growth, barrier for free exchange of gases due to adhesion on the surface of breathing epitels).

Possible inactivation by filtration.

b) Extreme values of pH

pH optimum for the majority of water organisms is between value of 5 to 8,5. Extreme values of both sides of the pH spectrum can have a serious adverse impact on them.

Possible inactivation by neutralisation. It can be done using necessary volume of 3M solution of HCl or 1M solution of NaOH. Arising precipitate should be filtered before repeated testing..

c) High content of metals

Metals are well know for their toxicity to water organisms. The most important parameters which influence the toxicity of metals in water are concentration of Ca and Mg, acid neutralization capacity, pH value (in acid conditions the metals are more toxic due to level of dissociation of metal compounds) and also presence of complex (chelate)-creating substances which can inactivate metals by bonding them in complexes (chelates).

Possible inactivation by complexation. It can be done by adding Chelaton 3 (Na_2 EDTA = $C_2H_{14}O_8N_2$ $Na._2..2$ H_2O) in concentration of 1mg/l (more can be toxic) into all tested solutions of sample and also into the control (to prove no adverse effects of chelaton). Level of dissociation of chelaton and by the same way its bonding capacity to metals is strongly dependent on pH value, it goes up with rising alkalinity of medium. Maximum acceptable value of pH is 8,5 (due to possible adverse effects of extreme alkalinity - see paragraph b). Samples slightly acid should be neutralised before adding chelaton).

d) High content of oxidants

Some oxidants like chlorine or peroxides can be toxic.

Possible inactivation by adding sodium thiosulfate. Na_2 S_2 O_3 is add in the concentration of 10 mg/l (more can be toxic) into the test solutions and control.

e) Adverse impact of organics

A lot of organic substances is toxic.

Possible inactivation is removal of organics from the solution by absorption on absorbents. For 2 litres of sample prepare 4 ml of equal mixture (2+2) XAD resins 2 and 4. The mixture should be free from possible contaminants. Therefore wash prepared mixture before use by methanol (at least 15 minutes) and subsequently by deionized or destiled water. After washing add the mixture into the sample and shake for 24 hours. Than remove resins by a sieve and provide toxicity tests with the sample.

f) Adverse effects of volatile substances

Volatile substances could be toxic during the period they are present in the solution.

Possible inactivation by bubbling by nitrogen or any other inert gas. Bubble for 60 minutes and make sure that before the start of toxicity test there is a required value of dissolved oxygen in tested solution.

g) Adverse impact of degradable substances

Toxicity of waste water can be caused by relatively easy degradable substances. These substances can be degraded in waste water by spontaneous physical, chemical and also by biological processes. This impact can be detected when subsequential tests of waste water toxicity are performed after period of 7 days of sample storage in temperature from 15 to 20^{o} C with aeration.

Examples of use

The examples was selected to show the mechanism of identification of the most toxic component of waste water.

A) The case of the Ferak Works

Producer: Ferak Raškovice

Products: Ni-Cd accumulators

Type of waste water treatment: neutralisation, sedimentation

Results of routine chemical analyses of waste water: pH neutral, time stable, increased concentrations of Cd (170 μg/l), Pb (185 μg/l), Ni (760 μg/l) and **As (982 μg/l).**

Toxicity evaluation:

Acute toxicity tests of untreated waste water showed the highest toxicity to Daphnia magna. This organism was selected for further testing of waste water after treatment intended on the inactivation of specific waste water properties. Results are expressed in table 1 and picture 1.

Table 1: Toxicity of waste water of Ferak Works to Daphnia magna

Used reagent	48 IC 50 (ml/l)	inactivated impact of .
no treatment	83,4	-
XAD resins	83,9	organics
sodium thiosulfate	88,3	oxidants
NA_2EDTA	**105,8**	**metals**

Discussion of results:

From the presented results we can state, that toxicity is mainly caused by **metals.** This in agreement with results of chemical analyses of waste water.

B) The case of the BOCHEMIA Works

Producer: BOCHEMIA Bohumín

Products: desinfection agents, Ni-Cd accumulator material, fungicides, herbicides, pure and special inorganics ($ZnCl_2$, ZnO, $HgCl_2$, HgI_2 , $HgNH_2Cl$ and other more than 200 different salts), pure and special gases and gas mixtures, milled sulphur and other

Type of waste water treatment: neutralisation, sedimentation

Results of routine chemical analyses of waste water: pH neutral, time stable, very toxic concentration of **Zn (13 mg/l).**

Toxicity evaluation:

Acute toxicity tests of „untreated“ waste water again showed the highest toxicity to Daphnia magna. This organism was selected for further testing of waste water after treatment intended on the inactivation of specific waste water properties. Results are expressed in table 2 and picture 2.

Table 2: Toxicity of waste water of the Bochemia Works to Daphnia magna

Used reagent	48 IC 50 (ml/l)	inactivated impact of .
no treatment	221, 4	-
XAD resins	**420, 5**	**organics**
sodium thiosulfate	222, 2	oxidants
NA_2EDTA	280, 8	metals

Discussion of results:

Chemical analysis of waste water showed highly acute toxic concentration of zinc, organic substances routinely analysed in waste water were not detected in consequentially high concentrations. This finding can raise conclusion that toxicity is caused by mentioned metal, but from the results of acute toxicity tests is clearly visible, that the toxicity is mainly caused by **organics.** In case of the Bochemia Works that metal is probably partly inactivated (in different compounds). Toxicity of waste water is probably caused by special substances originating during production in the Bochemia Works. This substances were missed by routine chemical analysis of waste water. To solve the problem of toxicity of waste water, there is a need to find the problem substances, to detect their content in waste water, to find the technological step of their origin and their way to waste water.

Conclusisons

We have suggested approach which is very useful for detection of the main property of waste water responsible for their adverse effect. From mentioned examples is clear that application of this approach enabled more accurate specification of the source of problem of concrete waste waters.

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