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Toxic pressure in surface water
A pilot of new monitoring techniques

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Abstract

In a pilot study, alternative monitoring methods for toxic pressure in surface water were tested. One relies on *calculated* toxic pressure of an analyzed cocktail of metals in a surface water sample, the other is based on *measuring* toxicity of an unknown cocktail of organic micropollutants extracted from a water sample.
In surface water samples, taken from different locations in the Netherlands, the dissolved concentration of sixteen elements was analyzed. Ecotoxicity data were collected in order to calculate the combined toxic pressure of the analyzed metals as a single number. From the same sample sites also large volumes of water were taken in order to extract and concentrate the unknown cocktail of organic micropollutants. The acute toxicity of these concentrates for seven different micro-bioassays was determined. The results were used to estimate the toxic pressure due to the unknown cocktail of organic micropollutants in the original water sample.
Both monitoring methods are indispensable for monitoring toxic pressure. The results demonstrate that toxic pressure in the River Rhine is significantly lower than in the Rivers Meuse and Scheldt.

Preface

We thank Hannie Maas and her colleagues of the National Institute of Inland Water Management and Wastewater Treatment (RIZA) for their contribution to test the method for measuring the toxic pressure of organic micropollutants in surface water.

Contents

Samenvatting

Monitoring van toxische stoffen in oppervlaktewater is doorgaans gebaseerd op het systematisch meten van de concentratie van een aantal verbindingen. De selectie van deze stoffen wordt ingegeven door eventueel schadelijke eigenschappen die aan deze stoffen worden toegedicht en door gegevens over productie, gebruik en emissie. Deze aanpak heeft nadelen: 1) het aantal te meten stoffen in de complexe toxische cocktail van organische micro-verontreinigingen (omv's) is noodzakelijkerwijs beperkt, waardoor schadelijke stoffen aan de aandacht kunnen ontsnappen, 2) van veel stoffen ontbreken toxiciteitgegevens waardoor uit de gemeten concentraties niet eenvoudig schade aan het aquatische ecosysteem kan worden afgeleid en 3) het is nog niet goed mogelijk om het gecombineerde effect van een mengsel van stoffen te kwantificeren.

In dit rapport worden twee methoden gepresenteerd die het mogelijk maken om toxische druk te monitoren en daarom als aanvulling kunnen dienen op de gangbare chemische monitoring. In een samenwerkingsverband tussen het RIVM (ECO en LAC) en het RIZA werd een pilot uitgevoerd.

Al een aantal jaren loopt een onderzoeksprogramma waarin onder de naam "pT" ("toxische potentie") een methode wordt ontwikkeld waarmee de toxische druk van het complexe mengsel in oppervlaktewater rechtstreeks gemeten kan worden, zonder de individuele verbindingen te kennen. Daartoe worden de organische micro-verontreinigingen zo goed mogelijk uit het watermonster geïsoleerd en op een zodanige wijze geconcentreerd dat toxiciteitsmetingen uitgevoerd kunnen worden met diverse aquatische organismen. Hieruit wordt een toxische druk omv's afgeleid (zie figuur).

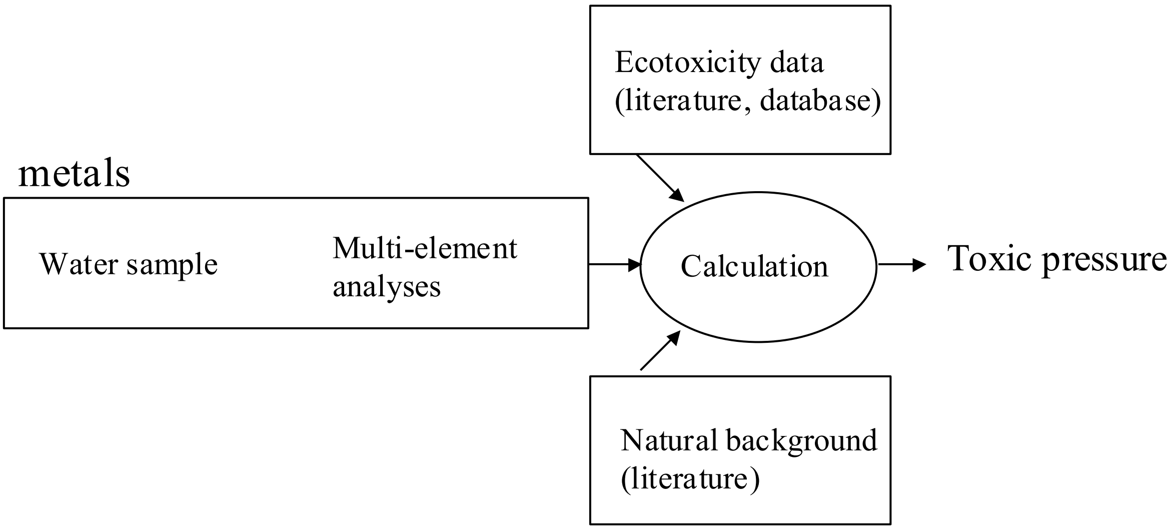
De pT-methode is ongeschikt voor metalen in water. Voor deze categorie stoffen werd een methode ontwikkeld, gebaseerd op multi-elementanalyse en toxiciteitsgegevens uit de literatuur. Hiermee werd de toxische druk van 16 metalen berekend vanuit gemeten concentraties in een watermonster. Per metaal zijn ecotoxicologische data verzameld om de Potentieel Aangetaste Fractie (PAF) van soorten uit te kunnen rekenen, gerelateerd aan de gemeten opgeloste concentratie van het betreffende metaal waaraan alle soorten worden blootgesteld. De gecombineerde toxiciteit van alle metalen werd eveneens als PAF uitgedrukt.

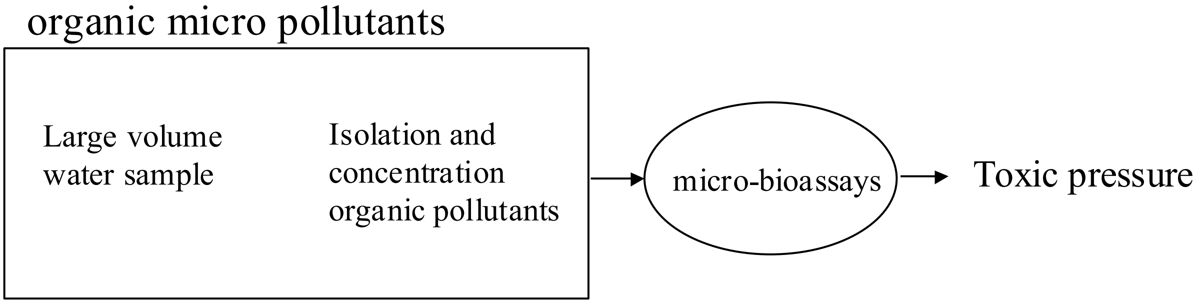
Op acht lokaties werden watermonsters genomen voor multi-element analyse m.b.v. Inductively Coupled Plasma- Mass Spectrometry. Het bleek dat in het monster van de Rijn bij Lobith een metalendruk heerst die de natuurlijke achtergrond benadert.

Van de acht monsterlokaties werden, in samenwerking met het RIZA, vijf locaties geselecteerd voor onderzoek naar de toxiche druk door omv's. Wederom bleek dat in de Rijn bij Lobith nagenoeg geen toxische druk aanwezig is. Ofschoon meer gegevens (in de tijd) nodig zijn, lijkt dit onderzoek uit te wijzen dat de toxische druk van metalen en organische microverontreinigingen significant lager is dan in de Maas en de Schelde.

De toxische metalendruk in de acht watermonsters varieert tussen 10 % (achtergrond) en 60 %, uitgedrukt als “berekende PAF”. Hoewel dit waarschijnlijk een overschatting is vanwege onvoldoende gegevens over (lagere) beschikbaarheid, kan geconcludeerd worden dat deze methode onmisbaar is voor het monitoren van toxische druk. De “gemeten PAF” voor omv’s blijkt in de vijf monsters te variëren tussen 0 % (blanco) en 4,4 % (Schelde), waarbij moet worden opgemerkt dat dit een onderschatting is omdat bij de extractie van omv’s verlies optreedt.
Deze twee technieken zijn interessant voor het monitoringnetwerk oppervlaktewater, aangezien het de mogelijkheid biedt om toxische druk in oppervlaktewater te scheiden van andere milieustressoren.

Schematische weergave van de twee procedures, toegepast in de pilot toxische druk in oppervlaktewater monsters van verschillende (niet)Rijkswateren.





Summary

Monitoring organic chemicals in surface water is usually based upon systematically measuring the concentration of a number of substances. The attributed harmful properties and data on production, use and emission determine the selection of these chemicals. This approach has certain disadvantages: 1) the number of chemicals to be measured in the complex cocktail of organic micropollutants is necessarily limited, 2) toxicity data of many chemicals is lacking, impeding the assessment of damage to the aquatic ecosystem and 3) it is not feasible to quantify the combined effects of mixtures of chemicals. In this report two methods are presented which enable monitoring toxic pressure and as such may be seen as a supplement to conventional chemical monitoring. A joint pilot study was conducted by RIVM (Laboratory for Ecotoxicology and Laboratory for Inorganic Chemistry) and RIZA (the National Institute of Inland Water Management and Wastewater Treatment).

Since several years, research is ongoing, referred to as “pT” (the “toxic potency” approach) to develop a method for measuring toxic stress from complex mixtures, without identifying the individual chemicals. Organic micropollutants are as good as possible extracted and isolated from a water sample and concentrated in such a fashion that toxicity experiments can be conducted with various aquatic test organisms. From the obtained results a certain level of toxic pressure can be evaluated (see figure page 6).

The pT method is not suitable for metals in water. For this category of toxic substances another method was developed, based on multi-element analysis and the availability of a multitude of toxicity data. The toxic pressure of 16 metals could be calculated from measured concentrations in a water sample. For each metal ecotoxicity data have been collected in order to be able to calculate the Potentially Affected Fraction (PAF) of species, related to the measured dissolved concentration of the metal to which all species in water are exposed. The combined toxicity of all metals was also expressed as an affected fraction of species.
On eight sites, water samples were collected for multi-element analysis with Inductively Coupled Plasma - Mass Spectrometry. Apparently, the toxic metal pressure in the sample of the River Rhine was close to the natural background.

In collaboration with RIZA, five out of the former eight sample sites were chosen for investigation of toxic pressure due to the presence of organic micropollutants. It appeared again that in the River Rhine virtually no toxic pressure could be measured. Although more data (in time) is needed, this pilot seems to indicate that toxic pressure from metals and organic chemicals in the River Rhine is significantly lower than in the Rivers Muese and Scheldt.
The toxic metal pressure in the eight water samples varies between 10 % (background) and 60 %, expressed as “calculated PAF”. This is probably an overestimation, because information on possibly reduced bio-availability was not available, however the conclusion could be drawn that the method is mandatory for monitoring toxic pressure. The “measured PAF” (organic chemicals) varied between 0 % (blank) and 4.4 % (Scheldt), however, this is an underestimation due to the loss of substances during the extraction procedure.
The combination of the two techniques is interesting for monitoring surface water because it offers the possibility to separate toxic pressure from other environmental stressors.

1. Introduction

As methods for direct monitoring toxic pressure are lacking, monitoring chemical concentrations of toxic substances in surface water is usually done as a substitute. For a good interpretation it is essential that sufficient ecotoxicity data of each measured compound be known. However, there are serious limitations on chemical monitoring. For example, Hendriks et al. (1994) reported that a major part of the toxic compounds remains unidentified in the Rhine delta. There are more than 100,000 organic chemicals known in the European Inventory of Existing Chemical Substances (EINECS) and obviously, some chemicals may escape our notice. Moreover, it is not feasible to take all joint effects into account.
Here we present a pilot study on two methods for monitoring toxic pressure in surface water, as an alternative for conventional chemical monitoring.

Since several years, research is ongoing in the Laboratory for Ecotoxicology to develop a method for measuring local ecotoxicity and the evaluation of risk, without identifying the individual chemicals. Environmental water samples are taken into the laboratory to evaluate the toxicity with bioassays, without any qualitative or quantitative chemical analysis on organic micropollutants. The method, referred to as the “toxic potency” approach (De Zwart et al., 1996), is focused on the unknown cocktail of toxic organic micro-pollutants in surface water. The aim is to evaluate the toxic potency as “the fraction of generic species, chronically exposed from a complex mixture above the no-observed effect level” (Roghair et al., 1997). In this context, toxic potency of a water sample has been named the *measured* Potentially Affected Fraction (PAF), indicating that bioassays are applied to investigate the toxicity of concentrated water samples.
The experimental procedure consists of extracting and isolating organic compounds from a large volume of water sample in order to prepare an aqueous concentrate of organic chemicals that is compatible to microbiotests. Recently, the extraction method has been improved to span a wider range of physico-chemical properties in an attempt to cover the majority of organic micropollutants (Struijs et al, 1998; Struijs & Van de Kamp, 2000).

This extraction technique is not suitable for measuring toxic effects of metals. One of the questions to be answered is how the total toxic pressure is underestimated if the metals are neglected. One single method, covering both organic chemicals and metals, while making use of bioassays, is probably not feasible. In the framework of monitoring such a procedure is not expected to soon meet the requirements of simplicity and cost-efficiency.
The number of metals to take into account, however, is relatively small and often ecotoxicity is known and if not, missing toxicity data can be obtained from a limited amount of experimental work. The last decade, a multi-element analytical technique, ICP-MS, has been improved and has become useful for monitoring the appropriate array of metals in surface water. In this report a procedure is proposed to utilize the ICP-MS technique in combination with geochemical and ecotoxicity data that have become available.

In collaboration with the Institute of Inland Water Management and Wastewater Treatment (RIZA), five water samples were tested on the combined toxicity of the unknown cocktail of organic micro-pollutants.
From the same locations and from three additional sites, samples were taken for chemical analyses of 16 metals. Ecotoxicity data are collected from several sources to convert each concentration into an effect on the aquatic ecosystem. For each sample, the effects of all metals were combined to one number, representing potential toxic pressure due to dissolved concentrations of these 16 metals.

2. Calculated and measured toxic pressure

Toxic metals and organic micropollutants differ considerably in nature, origin, composition and the availability of toxicity data. Both categories would also differ in feasibility to extract and concentrate the chemicals to prepare a sample that is compatible to micro-bioassays. For practical reasons we have chosen for different strategies to monitor their contribution to the total toxic pressure on surface water.

The number of toxic metals is limited and toxicity data are available. Because several metals have a record of water pollution, toxic properties have been investigated in quite a number of ecotoxicity tests, often with a variety of aquatic species. Relevant data can be retrieved from large ecotoxicity databases, such as AQUIRE (USEPA, 1984). With sufficient ecotoxicity data available, metal concentrations could be utilized to calculate toxic pressure on the water compartment. However, natural background levels, bioavailability and metal essentiality may complicate a meaningful conversion to a certain effect.
In principle, such a procedure would also be suitable for measured concentrations of organic micropollutants. In addition, however, a method would be required to account for the cocktail of unidentified organic chemicals. For example, toxicity data for pesticides and detergents is usually available, but these sub-groups comprise only a small number of chemicals when compared to the 100,000 to 150,000 EINECS substances. Toxic pressure calculated from analyzed organic micropollutants is not further addressed here (see Posthuma et al., 2000).

In this pilot we combine measured concentrations of metals with known ecotoxicological properties in order to *calculate* toxic effects, denoted as *PAFcalculated* in this report. In order to be able to account for the unknown cocktail of organic chemicals, an extraction procedure is combined with direct toxicity measurements. Without specifying organic compounds individually, aqueous extracts are prepared for direct measurements of toxic effects with bioassays (*PAFbioassays*).

2.1 Toxic pressure from of a known mixture: *PAFcalculated*

2.1.1 Species Sensitivity Distribution of a single metal

The set of No Observed Effect Concentrations (NOECs) of a certain metal can be represented by a log-logistic distribution (e.g. see Aldenberg & Slob, 1993). The probability density plot, known as the Species Sensitivity Distribution (SSD) has a maximum at α, being the ^{10}log of the geometric mean of all NOEC values. The width of the distribution of this set of NOECs is given by β, approaching 0.55 times the standard deviation of log NOEC. The Potentially Affected Fraction (Posthuma et al., 2000) of species, exposed to a concentration higher than a certain concentration C is given directly by the cumulative function of the logistic probability density function:

$$PAF = \frac{1}{1+\exp\left(\frac{\alpha - \log C}{\beta}\right)} \qquad 1)$$

In practice, only from a limited number of NOECs the required species sensitivity distribution is assessed. The NOEC values usually pertain to nominal concentrations of a test substance, which is added to the test media. The concentration measured in a water sample is the dissolved concentration, operationally defined in this study as the total concentration in the water column after 0.45 μ filtration. Without further knowledge on parameters influencing the physico-chemical speciation of the metal, the dissolved concentration as defined above is considered 100% bio-available. PAF is calculated both for the measured dissolved concentration (PAF_{total}) and the similarly defined dissolved natural background concentration (PAF_{bg}).

2.1.2 The fraction of species affected by a known metal mixture

Different metals may have different modes of toxic action and therefore the combined effect of n metals - msPAF (multiple substance) - can not be derived from concentration addition. Instead, msPAF is calculated from effect addition and the combined toxic metal pressure for n metals is calculated according to Posthuma et al. (2000):

$$PAFcalculated = msPAF = 1 - \prod_{i=1}^{n} (1 - PAF_{total,i}) \qquad 2)$$

2.2 Measured toxic pressure: *PAFbioassays*

The experimental procedure to measure msPAF for organic micropollutants is visualized in Figure 1. It is attempted to extract all toxic organic compounds from the aqueous phase in order to prepare a thousand-fold concentrated water sample. This limited volume of concentrated water sample is the aqueous material for further testing. Instead of a certain concentration of a specific chemical applied to a test medium, a *concentration factor* (CF) of a water sample is applied. Dilution series are prepared to expose a certain test organism from the unknown cocktail in decreasing strengths. The parameter of interest is the concentration factor by which the original water sample has to be concentrated (or diluted) to cause 50% acute effect, ECF_{50}(acute). This is the analogue of EC_{50}(acute) of an individual chemical in a conventional acute toxicity test, a result with the dimension of concentration, for example mg/L. If sufficient ECF_{50}(acute) values are available for species varying in morphology, again the log-logistic species sensitivity model can be applied. Both the location parameter and the scale parameter refer to the distribution of acute ECF_{50} and are denoted as α^*(acute) and β*(acute). Chronic No Observed Effect Concentration *Factors* (NOECFs) are required to compute α^* and β^* from which PAFbioassays is derived. As it is not feasible to conduct chronic tests with a small volume of concentrated water sample, the extrapolation by De Zwart et al. (2000) is used:

$$\alpha^*(chronic) = \alpha^*(acute) - 1$$

$$\beta^*(chronic) = \beta^*(acute) \qquad 3)$$

The parameters α*(chronic) and β*(chronic), derived from acute toxicity data, are employed to calculate the cumulative NOECF distribution function. Equation 1 can be applied, but now as a function of log CF in stead of log C. To the original water sample a concentration factor, CF, equal to one is assigned. Inserting log CF = 0 and the parameters α*(chronic) and β*(chronic) in the equation for the cumulative SSD, yields the fraction of generic species that is potentially affected by chronic exposure from an unknown mixture of organic chemicals in the original surface water sample:

$$PAFbioassays = \frac{1}{1+\exp\left(\dfrac{\alpha^*(chronic)}{\beta^*(chronic)}\right)} \qquad 4)$$

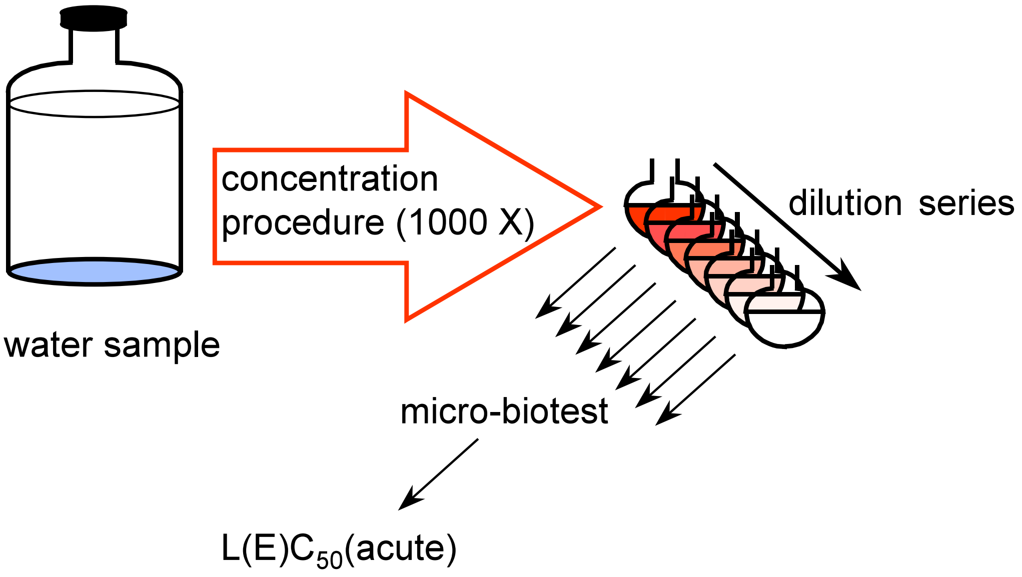


Figure 1 The experimental approach to obtain acute toxicity data related to an unknown cocktail of organic micropollutants in a surface water sample.

3. Material and methods

3.1 Metals

3.1.1 Selected elements

Thirteen metals, two metalloids (B and As) and one nonmetal (Se) were selected for this pilot study. In a short form they are referred to in this study as “sixteen metals”. Each element can be characterized according to analytical chemical, geochemical and ecotoxicological data, given in Tables 1 and 2.

3.1.2 Sampling

Surface water samples were collected by the end of November, 1998, at the following eight sites in the Netherlands (see Fig 2):

1. **Eijsden** (River Meuse), 1 Dec 1998
2. **Lobith** (River Rhine), 26 Nov 1998
3. **Maassluis** (Canal Nieuwe Waterweg/River Rhine delta), 26 Nov 1998
4. **Schaar van Ouden Doel** (River Scheldt), 26 Nov 1998
5. **Noordwijk** (Noordzijderpolder), 20 Nov 1998
6. Nauwernasche Braak at **Assendelft** (a small stream), 30 Nov 1998
7. “De Zoomwijckvijver” (An artificial pond located in the center of the small town **Oud-Beijerland**, dug over 10 years ago), 26 Nov 1998
8. River “Aa” at **Rosmalen** (a small stream), 27 Nov 1998

3.1.3 Sample handling

All glassware used for handling the samples was on forehand washed with a diluted nitric acid solution at pH 2. Five liters of water were sampled by simply dipping a glass bottle in the surface water. The bottles were rinsed at least two times with the surface water to be sampled, before the actual sample was taken. The samples were cooled at 5 °C and transported to the laboratory.

The samples were filtered in the laboratory over a 0.45 µm pore size acetate filter. The filtrate was divided over two polyethylene bottles. One bottle of 100 ml was acidified with concentrated nitric acid to set the pH to 1, and used for metal analyses. The second bottle was used for the determination of anions, pH and DOC.

3.1.4 Multi-element analysis

Cations were analyzed by ICP-MS (Hewlett Packard). As far as possible, two isotopes were quantified. The concentration of one of the isotopes in the sample was reported whereas the other isotope was used for verification purposes. Element concentrations were quantified on the basis of an external calibration solution, using NIST 1643D certified reference material for quality control. Recoveries were in-between 80% and 120% of the certified value. Silver was an exception as the recovery was only about 75%. This is probably due to sorption to the tubes and to the tubing used.

3.2 Organic chemicals

3.2.1 Sampling

For measuring the toxicity of the unknown mixture of organic contaminants, 100 L samples were taken in the same season at the locations 1.) to 5.) (Figure 2).

3.2.2 The extraction/concentration procedure

The method based on XAD-4/8 and acetone, according to Struijs et al. (1998), was used for extracting and concentrating the organic micropollutants. The standard operating procedures, SOP ECO/303/00 (1996) and SOP ECO/310/00 (1997) were improved by controlling the process of drying the XAD resins. In order to verify that the loss of water meets the criteria, the XAD resins were weighed before elution with acetone.

3.2.3 The bio-assays

The following micro-biotests were applied to concentrates of the surface water samples: Thamnotoxkit F (*Thamnocephalus platyrus*)[1], Rotoxkit F (*Brachyonus calyciflorus*)[1], Microtox (*Vibrio fischieri*)[2] and Daphnia IQ (*Daphnia magna*)[1]. These toxkits have been applied in the project “Geographic Representation of Ecotoxicological Effects of Substances” (Roghair et al., 1997).

The recently developed micro-biotest PAM (Pulse Amplitude Modulation)[2] is an algal photosynthesis inhibition test with *Selenastrum capricornutum* (Van Beusekom, 1999).

A standard *Daphnia magna* test[1] was applied to five organisms per 10 mL concentrated water sample, in four replicates. Daphnids with age less than 24 hr were exposed during 24 and 48 hr before the number of immobile organisms was counted. From this data, the concentration factor at which 50% of the organisms are immobile is derived (ECF_{50}).

A mayfly (*Ephoron virgo*) test[3], described by Greve et al. (1999), was conducted with 15 organisms per 20 mL in triplicate. Larvae of the mayfly were exposed to different concentrates during 96 hr before lethality is observed to determine LCF_{50}.

3.2.4 Data handling

The mean, standard deviation of log transformed measured $(L)EF_{50}$ values were calculated in order to derive the (chronic) parameters α^* and β^*. Subsequently, PAFbioassays and the confidence limits were calculated according to Roghair et al. (1997).

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[1] Test performed by RIZA

[2] Test performed by RIVM

[3] Test performed by the University of Amsterdam

4. Results

4.1 Metals

The SSD parameters, α and β (Table 1), were obtained from:

1. a report containing Maximum Permissible Concentrations (MPCs) and Negligible Concentrations (NCs) for 17 metals (Crommentijn et al. 1997), in the framework of the project "Setting Integrated Environmental Quality Objectives";
2. a home-made database with SSD parameters. This database has been constructed by converting, rearranging and ranking information from a large database containing a wide variety of ecotoxicity data (AQUIRE, USEPA1984).

Initially, natural background concentrations in freshwater of all metals but boron and silver were taken from Crommentuijn et al. (1997). For boron and silver values by Van de Plassche (1999) were used. Recent data on seven metals (Van de Berg & Zwolsman, 2000) was preferred over the natural background levels reported by Crommentuijn (1997).

For 12 metals the detection limit is below the natural background concentration. Four metals, however, seem to have a natural background that can not yet be measured with the analytical chemical technique employed in this study.

Table 1. Chosen elements, detection limits (μg/L), reported natural background concentrations (in μg/L dissolved, operationally defined) and characteristics of the species sensitivity distribution for sixteen metals; α and β pertain to μg/L.

Element	Detection Limits	Natural background		SSD parameters		
			Lit. ref.	α	β	Lit. ref.
Beryllium (Be)	0.01	0.02	1)	0.94	0.59	1)
Boron (B)	15	40	2)	5.64	0.39	4)
Vanadium (V)	0.2	0.82	1)	3.81	0.24	4)
Chromium (Cr)	0.15	0.4	3)	2.40	0.50	1)
Nickel (Ni)	0.4	1.2	3)	2.70	0.83	1)
Copper (Cu)	0.4	0.7	3)	1.30	0.41	1)
Zinc (Zn)	0.5	0.8	3)	1.90	0.38	1)
Arsenic (As)	0.5	0.77	1)	2.90	0.54	1)
Selenium (Se)	0.02	0.04	1)	2.40	0.55	1)
Molybdenum (Mo)	0.2	1.4	1)	5.84	0.52	4)
Silver (Ag)	0.2	0.04	2)	1.45	0.45	4)
Cadmium (Cd)	0.003	0.002	3)	1.20	0.56	1)
Tin (Sn)	0.1	0.0002	1)	3.30	0.66	4)
Antimony (Sb)	0.12	0.29	1)	4.88	0.77	4)
Mercury (Hg)	0.1	0.001	3)	0.80	0.49	1)
Lead (Pb)	0.06	0.07	3)	2.20	0.39	1)

1) Crommentuijn et al. (1997); 2) Van de Plassche et al. (1999); 3) Van de Berg & Zwolsman (2000); 4) AQUIRE (USEPA, 1984)

PAF (detection limit), PAF_{bg} and PAF_{total} for each metal are given in Table 2. In the 8 water samples, msPAF ranges between 0.1 (background) and 0.6. However, these values seem to be dominated by a few metals only. The major part of this range, from 0.1 to 0.5, is attributed to the metals zinc, copper and nickel.

Table 2. PAF (detection limit), PAF_{bg} and PAF_{total} for measured metal concentrations in Dutch water samples (see Appendix 2).

Element	Detection limit	Back ground	Eijsden	Lobith	Schaar v. OD	Maassluis	Noordwijk	Assendelft	O-Beijerland	Rosmalen
Be	0.0068	0.011	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02
B	0.0000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V	0.0000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr	0.0016	0.004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Ni	0.0234	0.041	0.06	0.05	0.08	0.05	0.04	0.08	0.06	0.11
Cu	0.0157	0.028	0.08	0.08	0.08	0.07	0.02	0.13	0.02	0.27
Zn	0.0030	0.005	0.17	0.04	0.09	0.03	0.00	0.06	0.00	0.35
As	0.0027	0.004	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.01
Se	0.0006	0.001	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Mo	0.0000	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ag	0.0084	0.000	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cd	0.0013	0.001	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02
Sn	0.0015	0.000	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Sb	0.0005	0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	0.0248	0.000	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Pb	0.0002	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
MsPAF	**0.087**	**0.09**	**0.30-0.33**	**0.17-0.20**	**0.26-0.29**	**0.17-0.20**	**0.10-0.13**	**0.26-0.29**	**0.11-0.14**	**0.59-0.62**

4.2 Organic chemicals

Acute $E(L)CF_{50}$ data for seven different micro-biotests are given in Table 3, from which the parameters α^*(acute) and β^*(acute) are derived (not tabulated). SSD parameters, pertaining to chronic NOECFs, the mean value of PAFbioassays and the confidence limits are given in Table 4.

Table 3. Acute $E(L)CF_{50}$ data of Spa mineral water (blank) and five surface water samples.

Sample	Ephoron V	Daphnia M	Daphnia IQ	Thamnotox F	Rotox F	Microtox	Algae PAM
Blank	460	300	375	440	267	325	250
Eijsden	29	36	55	99	113	62	15
Lobith	69	54	118	73	128	47	48
Maassluis	68	34	133	101	240	42	37
Schaar v OD	117	34	100	58	172	55	8
Noordwijk	78	44	193	51	134	25	84

Table 4. SSD parameters derived from log transformed $E(L)CF_{50}$ data, PAFbioassays and confidence limits of Spa blauw (Blank) and five surface water samples.

Sample	α^*(chronic)	β^*(chronic)	PAF bioassays (%)	CL 5-95 (%)
Blank	1.528	0.057	0.0	0 - 0.00
Eijsden	0.682	0.170	1.8	0 - 16.7
Lobith	0.852	0.098	0.0	0 - 0.8
Maassluis	0.866	0.176	0.7	0 - 10.4
Schaar v OD	0.749	0.243	4.4	0.4 -25.6
Noordwijk	0.854	0.166	0.6	0 - 9.1

The location parameter α^*(chronic) for Lobith, Maassluis and Noordwijk is in the range between 0.85 and 0.87 and PAFbioassays for these sample sites is below one percent (see Figure 2). Of these three sites, Lobith is particular because the width of the distribution, β^*, is relatively small, but not as small as mineral water (the blank), which has the lowest β^*. Both the blank and Lobith have very low PAFbioassays with a high certainty. The toxic pressure in the Lobith sample would be almost as low as mineral water.

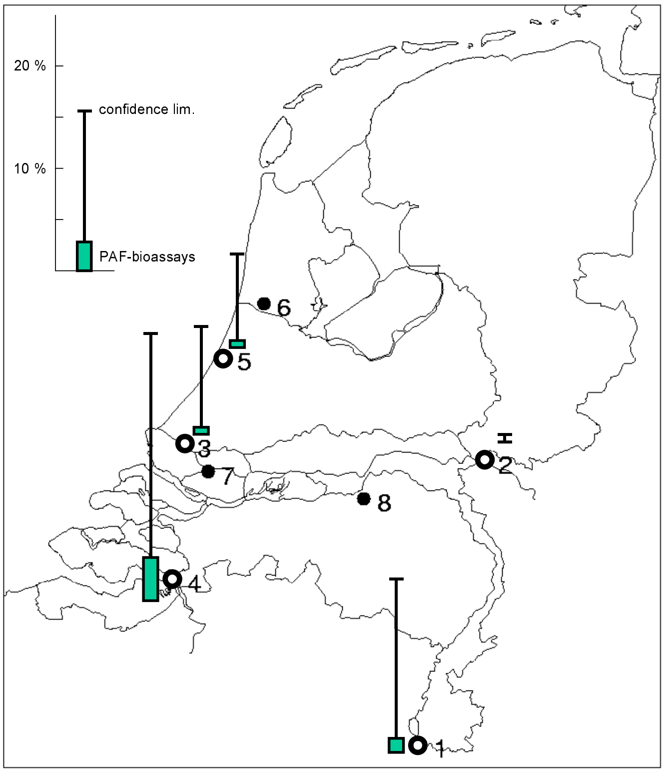

Figure 2 Sample locations and PAFbioassays results. **1** River Meuse; **2** River Rhine; **3** Canal Nieuwe Waterweg/River Rhine delta; **4** River Scheldt; **5** Noordwijk (pond); **6** Nauwernasche Braak (a small stream); **7** De Zoomwijckvijver (artificial pond); **8** River Aa (a small stream)

5. Discussion, conclusions and recommendations

We have tested two procedures, which are applicable to monitor toxic pressure in surface water. Real surface water samples, subjected to both methods, demonstrate varying levels of toxic pressure. Toxicity of metals is expressed by converting an array of 16 concentrations, measured in a water sample, into a single number. This number could be compared to the background toxic pressure, similarly calculated from the natural background concentrations, which were found in various scientific papers and reports. The toxicity, due to the presence of the unknown cocktail of organic micro-pollutants is physically extracted and subsequently measured by means of several (micro)bio-tests. In addition to five surface water samples, mineral water was also tested as a reference.

The *calculated* toxic pressure in surface water seems considerably higher than the *measured* toxic pressure.

Conclusion 1: Monitoring instruments for toxic pressure in surface water should include a procedure for toxic metals.

The question is raised if a very laborious procedure for organic pollutants in surface water is really necessary when toxic pressure is only a few percent, compared to tens of percent for toxic metals. In this chapter we put forward several arguments why organic chemicals should not be neglected.

5.1 Multi-element analysis and calculated PAF

Simplicity of sampling, treatment of the water samples and analysis characterize the practical aspects of the procedure. This approach seems suitable for incorporation in monitoring networks. With the current knowledge on ecotoxicity, the lower limit of the method[4] is 8.7 %, which is partly due to the elements Ag and Hg (0.8 and 2.5 %, respectively). For these metals the detection limit is higher than the background concentration. Lowering the detection limits for silver (0.2 μg/L) by a factor 5 and for mercury (0.1 μg/L) by a factor 10 would result in a lower limit of the method equal to 6 %. With more sensitive analytical techniques, a mercury level around or lower than 0.01 μg/L is commonly found in the River Rhine since 1990. Therefore, it is likely that msPAF for Lobith is 2.4 % lower. For the other elements, PAF is approximately equal or below the natural background.

Conclusion 2: optimization of the analytical procedure has not a high priority.

There is a difference in bio-availability between metals in aquatic toxicity tests, from which NOECs are derived, and in surface water samples. If the bio-available fraction of metals in surface water is lower than in standardized toxicity tests, where NOECs are usually related to concentrations added to the test medium, the method would overestimate toxic pressure of metals. If the pH in sampled surface water is considerably lower than in standardized toxicity tests, the opposite may be true. A procedure to account for differences in bio-availability, based upon easily accessible surface water parameters such as pH, DOC (dissolved organic

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[4] Operationally defined as msPAF calculated from detection limits.

carbon) and concentrations of nutrients, is still lacking. Until now, we have considered the dissolved concentration, operationally defined as measured in the water column after filtration over 0.45 µ, as totally effective for aquatic organisms or "100 % available". For most elements in Dutch surface waters such a correction does not seem necessary, because only for copper, zinc and nickel, such an availability correction would influence the final result.

Conclusion 3: a calculation rule, based on parameters that are common in monitoring activities, may be useful to account for the bio-available fraction of Cu, Zn and Ni, dissolved in surface water.

Good insight into the natural background concentrations in fresh surface water is a condition for appraising the risk of toxic metals. For that purpose we reported geochemical data for the Rhine catchment area, derived from several studies (Crommentuijn et al., 1997; Van de Plassche et al., 1999; Van de Berg & Zwolsman, 2000), but we tacitly considered them a reference also for the rivers Meuse and Scheldt.

In the early 70's, levels of zinc, copper, mercury and cadmium exceeded recently derived water quality standards (Crommentuijn et al., 1997), sometimes by more than a factor of ten. The discharge of these metals has decreased, resulting in a decline of toxic pressure from metals as plotted in Figure 3. In 1973, the contribution of zinc and copper to PAF_{total} was 83 and 47 %, respectively, for Meuse/Eijsden, while 57 and 35 % for Rhine/Lobith. Interestingly, for cadmium and mercury these percentages were lower, but with 18 and 7 % (Meuse) and 11 and 8 % (Rhine) still exceeding water quality standards. Figure 3 suggests that the toxic pressure due to dissolved metals in the River Rhine is approaching the natural background. The application of a more sensitive analytical method for mercury and silver would have resulted in a value for calculated PAF as low as 17 %. Thus the distance to the toxic pressure due to the natural background is rather small and almost entirely determined by elevated concentrations of the essential elements Zn and Cu.

For the River Meuse, however, the decline in toxic metal pressure seems to have ceased since 1990 (Fig 3) and is at a plateau substantially higher than the natural background of the River Rhine. It is not clear if the natural backgrounds in the River Rhine also apply to the Rivers Meuse and Scheldt are, but probably the concentrations of Ni, Cu and especially Zn are partly of anthropogenic origin.

The water sample taken at Rosmalen was polluted with toxic metals at a level comparable to the rivers Rhine and Meuse during the 70's.

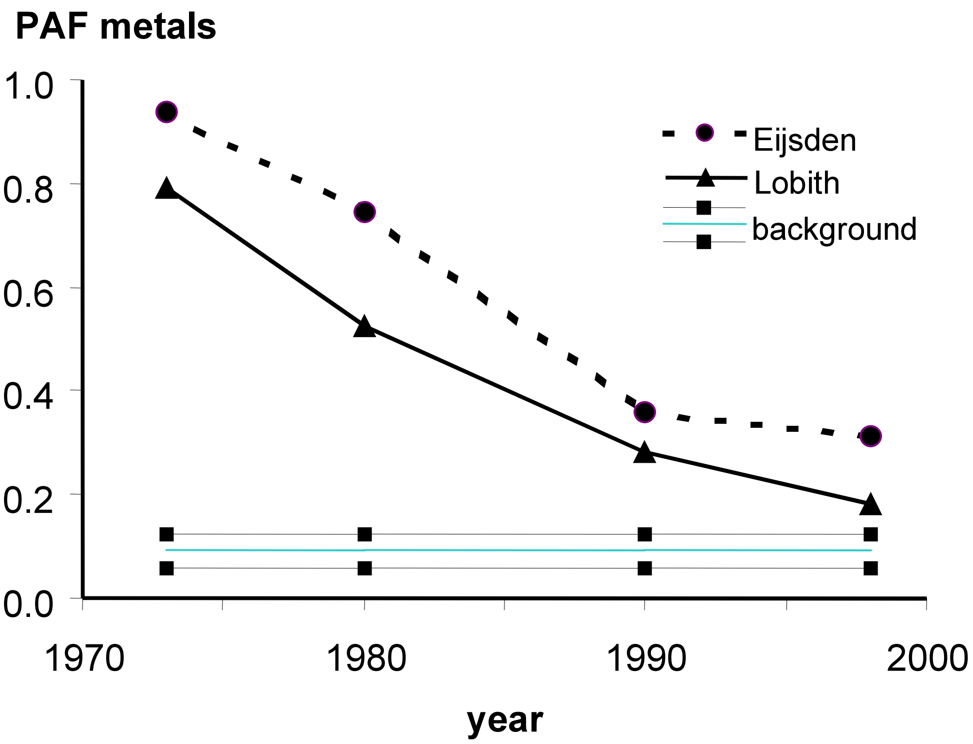

Figure 3 Toxic metal stress on river water. The background is calculated from natural background concentrations with 50 % margins. PAF_{total} *in 1973, 1980 and 1990 is computed from data on copper, zinc, cadmium, mercury and lead.*[5]

5.2 The unknown cocktail of organic contaminants

The procedure for direct measuring the toxicity in a water sample is laborious in view of monitoring. Large water samples have to be collected and stored before the extraction/concentration procedure in the laboratory is conducted. Hundred of litres of surface water were required to apply seven toxkits. The good result obtained with mineral water (PAFbioassays = 0.0 %) gives confidence that during the extraction procedure with XAD and acetone, as well as during storage of the acetone concentrates and - at a later stage - the removal of acetone to prepare concentrated water samples, no toxic substances were introduced. However, the manipulations needed before the actual toxicity is tested, are expensive and time consuming.

It is certain that toxic pressure is underestimated because the average recovery is probably not higher than 50 % (Struijs et al., 1998). Nevertheless, the results of this pilot also demonstrate that the method is a tool to discriminate levels of pollution by organic chemicals. The method would have given more pronounced differences if also surface water samples were taken from more contaminated areas.

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[5] From in Annual reports of the Coordinating Committee for the Monitoring of Radioactivity and Xenobiotic Substances.

Recommendation 1: investigate the toxic pressure due to organic compounds in the rivers Rhine, Meuse, Scheldt and some other, more polluted sites (elsewhere in Europe or in effluents).

The highest toxic pressure due to organic micropollutants (4.4 %) was found in the River Scheldt. This is close to the value of 3.8 %, reported earlier at the same location (Roghair et al., 1997), which is the combined result of bimonthly samples taken over the year 1996. The lowest toxicity was found in Rhine/Lobith water, less than 1 % with 95 % certainty, which is also in good agreement with 1996 (0.0 %). The sample of the River Meuse/Eijsden in this pilot has a higher mean value (1.8 %) than when obtained from the combined bimonthly samples in 1996: 0.6 %. Samples of Maassluis and Noordwijk have a low toxicity: an average of less than 1 % and a CF 95 not higher than 10 %.

5.3 Pollution order Rhine, Meuse and Scheldt

If we take into account the 16 metals and the extracted cocktail of organic micropollutants, the toxic pressure in the three major rivers decreases in the order of Scheldt, Meuse and Rhine. How polluted is the river Scheldt and how clean is the river Rhine? The toxic pressure from 16 metals on the water sample of Schaar van Oude Doel is 20 % higher than the background, if natural background concentrations of the river Rhine were applicable to the river Scheldt. Although there is some uncertainty if this assumption is valid, emission data suggest that elevated metal concentrations be of anthropogenic origin.

The potentially affected fraction of species, chronically exposed to the unknown cocktail of extractable organic substances in the Scheldt sample has a 95 % confidence interval between 0.4 % and 25.6 %, with a mean value of 4.4 %. Corresponding percentages for Lobith are as low as 0.0 % and 0.8 %, with a mean value of less than 0.1 %.

Conclusion 4: there is a significant difference in toxic pressure due to organic chemicals between the rivers Scheldt and Rhine.

The underestimation of toxic pressure when measured as PAFbioassays justifies this conclusion. As mentioned before, the method does not cover all organic chemicals to a high extent as for some substances the recovery is only limited due to volatilization or ionization. The following exercise on reduced recovery may illustrate this. A loss of 50 % for all toxic chemicals is roughly simulated by dividing all values of acute E(L)F50 data in Table 3 by a factor of two. As a consequence, SSD parameters, PAFbioassays and confidence limits would differ from the values in Table 4. Results for the blank and the three major rivers would have been obtained as in Table 5.

Table 5. SSD parameters, PAFbioassays and confidence limits of a simulation exercise. These results would be obtained if E (L) CF50 data of Table 3 were divided by a factor two.

Sample	α^*(chronic)	β^*(chronic)	PAF bioassays (%)	CL 5-95 (%)
Blank	1.23	0.057	0.0	0 - 0.00
Eijsden	0.38	0.170	9.6	2.0 - 36.1
Lobith	0.55	0.098	0.4	0 - 7.0
Schaar v OD	0.45	0.243	13.7	3.9 - 41.9

This simulation convinces that mineral water is a suitable blank sample: the upper CL 95 value is still negligible. Apparently, the presence of toxic substances in the concentrated water sample is not the result of contamination during the extraction procedure but has its origin in the water sample itself. Although for Lobith the upper CL 95 value has increased up to 7 %, the mean (PAFbioassays) is still very low: 0.4 %. The contrast with Schaar van OD and Eijsden has increased remarkably with PAFbioassays now being higher than 10 % and CL 95 more than 40 %. Therefore, it is likely that the Rivers Scheldt and Muese suffer from toxic pressure of organic chemicals.
Conclusion 5: The procedure for organic micropollutants, although very laborious and expensive, is essential for monitoring toxic pressure in surface water. Moreover, the procedure enables to isolate toxic pressure from other environmental stressors.

Several organic chemicals in rivers are chemically monitored. This raises the question if PAFcalculated (organic micropollutants), more or less in analogy to PAFcalculated (metals) would be useful. Moreover, how would that compare to PAFbioassays?
Recommendation 2: Investigate PAFcalculated (organic micropollutants) as a third monitoring tool for toxic pressure, taking into account concentration additivity for chemicals with an equal mode of action and effect additivity for (groups of) chemicals with a different mode of action.

Results of this pilot suggest that the two methods are useful supplements to chemical monitoring. They may help to understand why in some aquatic ecosystems the biodiversity is improving and why in some other river systems such an improvement fails to occur.
Conclusion 6: In addition to systematic chemical monitoring there is a need for monitoring toxic pressure that provides authorities with data of more ecological relevance. The methods presented here may be useful to obtain that information.

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Appendix 1 Mailing list

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Appendix 2 Dissolved concentrations of 16 metals

Table A. Measured dissolved concentrations (in µg/L, 0.45 µ) of 16 metals in 8 fresh water samples. Bold are concentrations that are equal to the detection limit and higher than the natural background concentration.

	Eijsden	Lobith	Schaar v. OD	Maassluis	Noordwijk	Assendelft	O-Beijerland	Rosmalen
Be	0.030	0.010	<0.010	0.010	0.010	0.020	<0.010	0.060
B	54.630	80.440	596.400	236.300	114.500	300.300	139.500	69.190
V	0.980	0.941	2.937	0.940	2.872	2.591	0.544	4.399
Cr	0.540	0.185	0.150	<0.150	0.356	0.420	0.477	1.372
Ni	2.466	1.473	4.791	1.777	1.426	4.199	2.983	9.809
Cu	1.882	1.943	2.108	1.746	0.465	3.192	0.407	7.744
Zn	19.350	4.867	10.310	4.220	0.516	6.696	<0.500	46.290
As	0.767	0.981	3.407	1.187	5.683	2.947	2.236	1.622
Se	0.080	<0.020	0.983	<0.020	<0.020	0.020	0.030	<0.020
Mo	1.420	1.568	5.256	1.855	0.967	2.083	0.516	1.323
Ag	**0.200**	**0.200**	**0.200**	**0.200**	**0.200**	**0.200**	**0.200**	**0.200**
Cd	0.043	0.019	0.023	0.043	**0.003**	0.027	0.052	0.077
Sn	0.313	**0.100**	1.163	0.704	**0.100**	0.494	0.138	**0.100**
Sb	0.122	0.285	1.429	0.312	0.303	0.351	0.144	0.696
Hg	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**
Pb	0.371	0.136	0.185	0.180	0.076	0.969	0.060	1.370