

An experimental ecotoxicological model and its application to the behavioral study of inorganic mercury (HgCl₂) in the environment

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Abstract. The aim of this research was to study the accumulation and transfer process of a contaminant (mercury) within an ecosystem. The study was led with a laboratory-conceived ecotoxicological model including two basic factors: biotic and abiotic, and was performed with inorganic mercury (HgCl₂). The chosen method was an interactive model (experimental ecosystem) with three compartments: water, natural sediment and air. The influence of pH and temperature on the behavior of mercury in two compartments, water and air (basic model) were first studied, and then the sediment and fish (*Scardinius erythrophthalmus*) were included. The mercury determination in various mediums was carried out with a laboratory prototype based on the principle of cold vapor flameless spectrometric atomic absorption developed by Livardjani et al. [1]. It was conceived in order to make direct analyses in the air and the water, as well as in the sediment and fish after mineralization. The measurement and mineralization conditions were optimized so as to obtain higher sensitivity and accuracy (90 ng per m³ of air, 15 pg in absolute weight). In an acid medium (pH < 5), mercury remained stable in the water, but its concentration decreased as pH and temperature increased. However, in the presence of sediment (pH 7.2), all the mercury disappeared, and was fixed onto the sediment. In the fish, a maximum level of mercury accumulation was reached after 10 days of exposure, and did not vary thereafter, no matter how long the exposure time lasted.

Key words. Mercury – modeling – ecotoxicology – air – water – sediment – fish.

Introduction

Because of its toxicity and its use and accumulation in human beings, the dispersion of mercury in the environment raises several problems. However, the levels of mercury contamination and more specifically air contamination are not easy to analyze, and this may account for its inaccuracy. As regards analyses of heavy metals (at least other than lead), France is well behind most other industrialized countries. Indeed, in 1994, the “number of heavy metal samples analyzed per year in the air for 10 000 inhabitants” was only “0.5” in France whereas it was “3.2” in Great Britain, “6” in Germany and “13.5” and “16” respectively in California and Canada [2]. European authorities have become aware of this gap since mercury, with cadmium, nickel and arsenic, is now on the “list of other atmospheric pollutants to be considered” in Annex I of the recommendation by the Council of Europe (19/01/1995 version) [2].

In this study, this difficulty was overcome thanks to the apparatus that was designed for direct and extemporaneous mercury measurements both in the air and the water [1].

The aim was to study the evolution of mercury in the air when the only source of contamination is water. Therefore, an experimental ecotoxicological model was designed to observe the influence of biotic (fish, sediment) and abiotic (pH, temperature) factors on the air contamination.

Material and methods

Material

Several prototypes were developed in our laboratory [1]. They were all based on the principle of cold vapor atomic absorption and permit the measurement of mercury amount in air, liquids and in different biological and environmental samples, like blood, urine, ... [1,3–5]. The different physical and chemical parameters likely to improve the detection limit were studied. The chosen prototype, with a 30 cm-long and a 2.2 cm-diameter measuring cell, shows a detection limit of 90 ng/m³ in the air and 15 pg in absolute weight [6]. A specific software for sampling and continuous measurement (from 1 h to 30 days) in the air was developed for our prototype.

Atmospheric mercury as well as inorganic forms of mercury in the water were directly measured with the above described prototype [6]. For organic forms, mineralization was carried out according to the nature of the sample. The mineralization parameters were optimized to reduce loss by volatilization. To do this, we referred to our previous works [3–5,7].

Reagent

Merck reagents containing a minimum of mercury are recommended [5] and satisfying results were obtained.

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Therefore the following reagents were used for our analyses:

- sulfuric acid 95 – 97% (1.84) max 0.0000005% Hg for analysis,
- nitric acid 65% (1.40) max 0.0000005% Hg for analysis,
- potassium permanganate max 0.000005% Hg for analysis,
- oxalic acid max 0.000002% Hg for analysis,
- stannous chloride dihydrate max 0.000001% Hg for analysis.

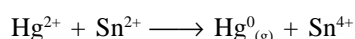
Methods

Sediments mineralization

Sediments are considered a good contamination indicator [8–10]. Sediment analysis is therefore necessary to assess the waste effects on the aquatic environment.

The rate of moisture in the sediment to be analyzed was measured in each of the samples taken separately. Each sample was analyzed three times ($n = 3$). Since the sediment is a complex matrix, a higher temperature and a longer time were required to obtain complete mineralization [7]. The samples were placed into an acid medium ($\text{HNO}_3 + \text{H}_2\text{SO}_4$, 6:3 v/v) in a Pyrex flask during 24 h at room temperature, and then mineralized with a reflux cooling system at boiling temperature (about 110 °C) for 24 h. Oxidation was obtained by adding 5 mL KMnO_4 (0.44 M) to the mineralized product. A solution of oxalic acid (0.5 M) was used for the reduction of permanganate until decoloration [7].

Mercury was reduced to Hg^0 by stannous chloride (5 mL of a 10% solution in double-distilled water) according to the following reaction:



This stannous chloride solution was purged with an argon current, as recommended by Heimburger et al. [5]. This method was compared with the BCR certified values and was in good agreement [7].

Fish mineralization

The mineralization parameters were optimized on aliquot fractions (500 mg) of fish mixtures (*Scardinius erythrophthalmus*). The best results were obtained using a sulfonitric mixture ($\text{HNO}_3 + \text{H}_2\text{SO}_4$, 6:4 v/v). This operation carried out in a reflux cooling system, lasted 1 h at 60 °C. Then, 3 mL of a KMnO_4 solution (0.44 M) were added to oxydate mercury. The excess permanganate was eliminated by adding about 2 mL of oxalic acid (0.5 M). The method was checked against the standard addition method. 95% of the mercury was recovered.

The determination of mercury values in the water was carried out by direct injection of the samples without mineralization.

Development of an ecotoxicological model

Figure 1 shows the model that has been conceived for this study. It is a closed container (200 liter capacity) equipped

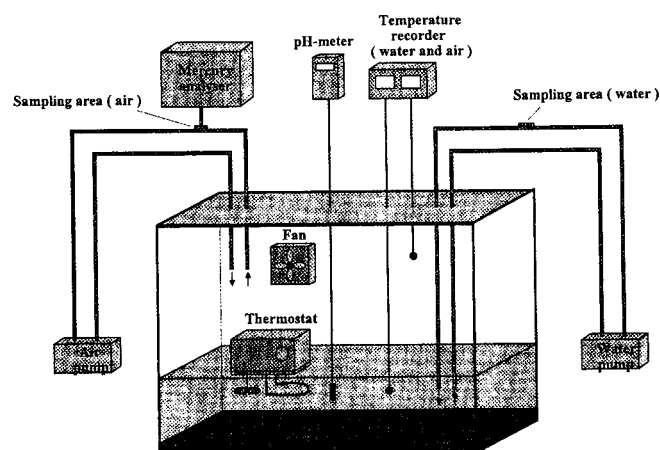


Fig. 1. Scheme of the model.

with a thermostat, pH-meter, pumps, agitator and a mercury analyzer (Fig. 1). The source of Hg was a HgCl_2 solution.

The first experiences were carried out with a basic model (100 L water/100 L air) during which pH and temperature effects on mercury concentrations were followed in the water and in the air of the container.

Then, the system was complicated by adding sediment and then fish.

Sediment compartment: Sediment was taken from a local gravel pit (Wittisheim, France). Its geochemical and granulometric characteristics were determined by the Institut de Géologie de Strasbourg (Strasbourg I University). It was mainly made of Quartz (SiO_2) (40%) and limestone (40%), and the remaining 20% were made of white Mica. The size of the sediment particles was about 0.05 mm. Organic matters as pieces of wood, plants, insects and shells could be observed. The total carbon content was determined with the Carbon Sulfur Analyzer CS 125 (LECO): it was 14%. The natural level of total mercury was 52.7 ± 19 ng Hg/g dry weight.

Fish: *Scardinius erythrophthalmus* were used. They are of the cyprinide family and live in the beds of quiet rivers, ponds and lakes.

For our study, farmed fish were used, aged 4 to 5 months, with an average weight of 13.312 ± 0.041 g and a size of 12.4 ± 0.5 cm. They had not been fed during the 30 days of the experiment so as to avoid the risk of food contamination. The study group had undergone the same treatment. To guarantee consistent results, a set of fish was used. For each analysis, 3 fish were sacrificed.

Results

Temperature influence

The initial Hg concentration in the water was 0.1 mg/L. pH was kept constant (pH: 7.2) and the mercury concentration was analyzed every 15 minutes in the water and in the air of the system, at various temperatures (24, 28 and 30 °C) and over a 48 h period (Fig. 2).

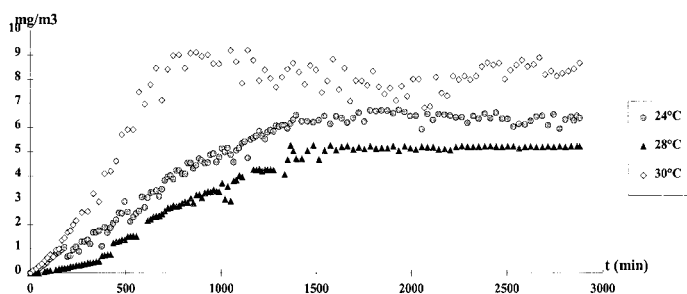


Fig. 2. Hg evolution in the air at various temperatures.

The slope of the curve increased with the temperature and the system reached equilibrium after about 1000 min (16 h). However, at 24 °C, the Hg concentration in the air was higher than at 28 °C.

pH influence

The same experiment was carried out in the same system (same water and air volumes, same initial Hg concentration in the water, same water and air samplings), at 24 °C ± 1, with pH as a variable. Several analyses were carried out: the same temperature and time were kept for all of them (24 °C, for 48 hours) and only pH varied. It was modulated using solutions of hydrochloric acid or sodium hydroxide. The Hg concentration in the air of each system in the 48th hour (after equilibrium was reached) is reported in figure 3.

The results show that mercury remained stable in the water in an acid medium (pH < 5) and that the air was not contaminated (Fig. 3). With pH > 5 however, mercury was released into the atmosphere of our model and reached a fixed value equilibrium after about 1000 min (16 h) (Fig. 3).

The same series of experiments (pH as a variable, for 48 h) were repeated at 28 °C and at 30 °C. Similar results were observed.

Sediment influence

The influence of the sediment was studied by placing 8.384 kg (dry weight) of natural sediment into our study

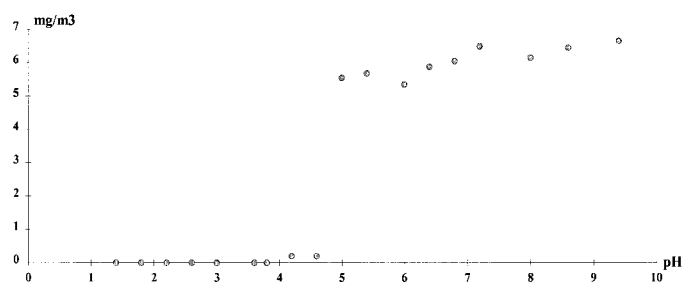


Fig. 3. Hg variation in the air as a function of pH.

system. The natural level of total mercury (determined after sulfonitric mineralization) of this sediment was 52.7 ± 19 ng Hg/g dry weight.

The concentration and time were kept the same (pH = 7.2, $T = 24 \text{ °C} \pm 1 \text{ °C}$). The initial Hg concentration in the water was 0.050 mg/L.

Thirty minutes after the sediment had been added, no trace of Hg was found, either in the water or in the air of the container. However, 620 ± 29 ng Hg/g dry weight were concentrated in the sediment, which means 5.2 mg of Hg in the whole sediment. This shows that all the HgCl_2 added was very quickly fixed by the sediment which immobilizes Hg permanently.

This experiment was sequentially repeated with different amounts of mercury added into the water, depending on temperature and pH. Identical results were found.

The system was monitored over a twelve-month period during which no mercury was detected in the air and the water (Tab. I).

Influence of fish

Fish (*Scardinius erythrophthalmus*) were added to the previous system on the tenth day.

The analyses showed (Fig. 4) that in the course of time, while no mercury was present in the air and the water of the container, its concentration increased significantly in the

Table I. Mercury concentration in the sediment.

N°	Hg added in water (mg)	Theoretical* amount of Hg in the sediment (mg)	Hg amount found in total sediment (mg)	Hg concentration in sediment (ng/g)
Blank	0	—	0.442 ± 0.16	52.7 ± 19
1	5.06	5.502	5.196 ± 0.240	619.7 ± 29
			5.255 ± 0.369	626.78 ± 44
			5.672 ± 0.226	676.5 ± 27
			7.096 ± 0.134	845.8 ± 16
2	2.36	7.862	7.380 ± 0.168	880.2 ± 20
			10.611 ± 0.495	1265.7 ± 59
3	2.47	10.332	10.252 ± 0.369	1222.8 ± 44

*Considering that all the mercury was fixed on the sediment (natural Hg + added Hg in water). Results given in dry weight.

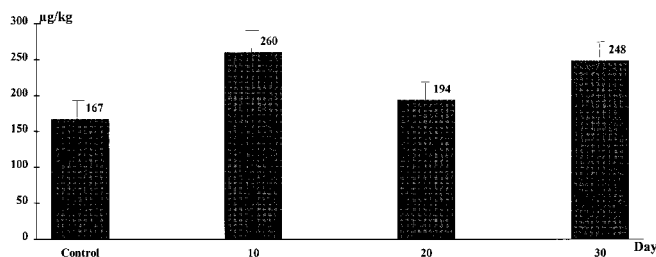


Fig. 4. Influence of time on Hg concentration in fish (water and sediment).

fish. Indeed, on the thirtieth day, the concentration of mercury had reached 248 ± 35 ng/g in the fish, nearly a 48% increase compared to the control fish (167 ± 35 ng/g).

In a parallel study carried out without the sediment but with the water adjusted to 0.107 mg Hg/L (Tab. II), the fish died within 11 days and a high mercury concentration was observed (1.449 mg/kg). Absence of mercury in the water and its high concentration in fish are confirmed by other authors [14,15]. It can be stated that the ratio of mercury concentration in fish and in the water increases as a function of time.

Conclusion

In our system, mercury remains stable in the water in an acid medium but it evaporates and is released into the air as the pH and the temperature of the water increase.

However, when the sediment is added, all the mercury fixes into it and disappears from the water and the air. This occurs very rapidly and the mercury seems to remain permanently fixed onto the sediment. Mercury accumulates in fish and reaches a maximal level after 10 days and does not vary significantly thereafter.

These results confirm that water is a bad exposure indicator, which was also observed by Schuhmacher et al. [16] in their study of the evolution of the effect of temperature, pH and bioproduction on Hg concentration in sediments, water, molluscs and algae of the delta of the Ebro river [16]. Indeed, in case of chronic pollution, analysis of other indicators such as sediment, aquatic plants, fish, etc. is more appropriate.

We noted that mercury accumulation in the sediment can be long-lasting, and penetrate the food chain via the fish.

Our experimental model is extremely convenient for thorough studies of mercury behavior in the environment. They can be much more easily carried out thanks to the development of our automatic apparatus for direct measurements of mercury in the air.

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Table II. Hg concentration in fish as a function of time (without sediment).

Day	Water concentration (mg/L)	Fish concentration (mg/kg)	Ratio $[Hg]_P/[Hg]_E$
1	0.107	0.261 ± 0.03	2.5
1	0.107	0.296 ± 0.04	2.76
1	0.107	0.147 ± 0.02	1.37
2	0.0534	0.372 ± 0.05	6.97
2	0.0534	0.320 ± 0.04	5.99
10	0.0009	1.122 ± 0.02	1246.67
10	0.0009	1.088 ± 0.02	1208.89
11	0	0.996 ± 0.04	
Minamata [15]	0.0001	50	500000
Tartaruglazine River (Brazil) [14]	0.00003	1.225	500000
Agano River (Japan) [15]	0.0001	40	400000