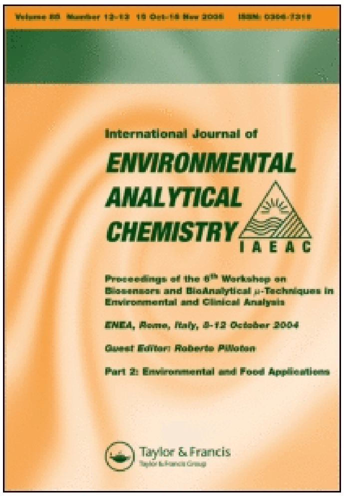

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I. Heninger[a]; M. Potin-gautier[a]; M. Astruc[a]; D. Snidaro[b]; V. Vignier[b]; J. Manem[b]
[a] Laboratoire de Chimie Analytique Université de Pau et des Pays de l'Adour, Pau, France [b] C.I.R.S.E.E. Lyonnaise des Eaux, 38, rue du President Wilson, Le Pecq, France

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SELENIUM IN SEWAGE SLUDGE; GENERAL ASPECTS AND ANALYTICAL CHALLENGE

I. HENINGER[a,*], M. POTIN-GAUTIER[a], M. ASTRUC[a], D. SNIDARO[b], V. VIGNIER[b] and J. MANEM[b]

[a]Laboratoire de Chimie Analytique Université de Pau et des Pays de l'Adour, 64000 Pau France; [b]C.I.R.S.E.E. Lyonnaise des Eaux, 38, rue du President Wilson, 78230 Le Pecq, France

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Selenium is both essential and toxic for living organisms (including human beings) in a relatively narrow range and at trace level. Its presence in sewage sludge can be positive in the case of land application when concentrations are relatively low, but can also lead to a long-term hazard to plants, water resources, animals, and human beings.

We have then developed a method for the wet digestion of sewage sludge and the determination of their total selenium concentration by DPCSV and ETAAS. Quality assurance was made by the analysis of two BCR certified sewage sludge reference materials (CRM 145R and CRM 007) and tested on a natural sample from a representative French sewage treatment plant.

However, knowledge of speciation is necessary when speaking of the toxicological risk represented by an element. Different techniques of soft extraction, applied to CRM 007 and respecting species distribution, are discussed.

Keywords: Selenium; sewage sludge; digestion; extraction; speciation

INTRODUCTION

The great effort undertaken for about twenty years to improve the quality of surface waters has led to the setting of numerous waste water treatment plants, generating an increasing amount of sludge. Waste water and sludge treatment processes represent an important point in the hydrological cycle at which the disposal of substantial quantities of trace elements to the environment must be regulated. Since the law on waste recovery and disposal in 1975 to the european guideline about wastes in 1991, the priority has been given to waste recovery and recycling[1]. The most common methods of sludge disposal are agricultural land

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* Corresponding author. Fax: + 33-59.029377.

application, land reclamation, landfilling, ocean dumping or incineration[2,3]. With increasing pressure to ban all sludge dumping at sea and the prohibitive costs of landfilling and incineration, there is a great tendency to dispose of sludge on land (40% in 1988 to 55% in 1992)[3].

Although numerous studies have demonstrated the intrinsic value of sludge for soil amendment, through nitrogen, phosphorus and homogeneous organic matter content[4-6], evidence has been accumulated in recent years that numerous environmental problems can arise because of the presence in sludges of high contents of trace elements (potentially toxic to plants and to human beings and liable to be concentrated along the food chain) among which selenium is particularly interesting[7-9].

The case of selenium is complex as it is also an essential element for living organisms (including human beings)[10]. The amendment with sewage sludge is sometimes used to increase the selenium content in crops[11], and afterwards in cattle, when there is a proven lack of this element in a given place. Nevertheless the border between essentiality and toxicity is relatively narrow and expressed at trace level. It is then really important to survey the selenium concentrations encountered in sewage sludge, especially as guidelines and regulations concerning these data will probably be strengthened.

GENERAL ASPECTS

Se distribution and rate of transfer between the different environmental compartments are different from one to another and vary according to the species concerned (organic and inorganic Se(-II), Se(0), Se(IV) and Se(VI))[12] and the physicochemical parameters of the environment.

The main origins of selenium in the environment can be divided in two sections: natural sources and anthropogenic ones. Natural sources come from physical phenomena (particles of Se(0) or Se(-II)) or biochemical ones (gaseous emissions of methylated compounds) and anthropogenic sources of selenium are mainly industrial and in any form[12,13].

According to that possible origins for selenium, it is feasible to have an idea of how this element enters sewage treatment plants and how its presence there can also step in the environmental cycle. Selenium can be brought into sewerage by soils leaching, rain, dust or other airbone particles. In domestic sewage, selenium is encountered mostly because of the use of some domestic product such as cosmetics, dandruff shampoos, paints as well as tap water, but in fact residential, commercial and even agricultural sources are relatively minor compared with those from industrial activity. Sewage Se content will then vary with location, time of sampling, spectrum of nearby industries and their changes in production rates and periodic relocation[14].

There is in fact very little information on the levels of Se discharged into receiving water and the forms of Se present at that stage. Konrad *et al.*[15] have shown in a survey conducted by the state of Wisconsin (US) in 1974 that about three tons of selenium had been discharged into receiving waters for the whole year.

Concerning the forms of selenium eventually present, we can suppose that when there is an industrial influence any species is liable to be present, but domestic sewage (generally without industrial influence) will probably contain higher portions of organic Se forms (derived from human wastes).

To that point we can consider that approximately 50% of the discharged Se passing through waste water treatment plants are concentrated in sludges[14] by settling and adsorption phenomena or absorption by the biomass[1].

There are also few data in the literature about Se in sewage sludge, but species present are probably also a function of the origin of sewerage and of some transformations likely to occur in that kind of medium.

Sewage sludge of mainly industrial origin will probably contain reduced selenium with little organic or oxidized Se forms. Moreover the anaerobic digestion treatment of sludges may reduce the oxidized forms and degrade any organic species to the elemental state Se(0). The domestic sludge receiving less advanced treatment, will probably contain higher portions of organic Se and oxidized forms[16]. Cappon *et al.*[14,17,18] have observed in anaerobically digested sludges, obtained from four local municipal waste water treatment plants servicing Rochester (USA), that a significant percentage (9.4-39.9%) of the total sludge Se was Se(VI), the remainder being Se(-II) and Se(IV).

It is also well known that some microorganisms have biochemical interactions with selenium and can alter the partitioning of species and their eventual complexation[19-28]. From these studies three main transformations can be considered possible in sewage sludge: reduction, oxidation and biomethylation (volatilization through methylation). Concerning the reduction, a distinction is made between assimilatory reduction (bacterial assimilation) almost unknown[22] and dissimilatory reduction (bacterial respiration) that has already been well studied[22-26].

Although the bacterial respiratory reduction of selenates to elemental selenium has been observed in sewage sludge along with denitrification[22,24]. there are few well documented cases of selenium oxidation[26]. Reduced forms of selenium such as Se(0) or copper selenide have been reported to be oxidized by laboratory bacterial cultures and soils but have never been studied, as far as we know, in waste water treatment plants.

Volatilization through methylation is thought to be a protective mechanism used by microorganisms to avoid selenium toxicity in seleniferous environments. Bacteria or fungi can transform in aerobic conditions SeO_3^{2-}, SeO_4^{2-}, Se(0) and various organoselenium compounds into a less toxic, volatile form, dimethylselenide (DMSe). DMSe seems to be the major metabolite of biomethylation, although other Se compounds such as dimethyldiselenide (DMDSe), dimethyl selenone ($(CH_3)_2SeO_2$), methane selenol (CH_3SeH) and dimethyl selenyl sulfide (CH_3SeSCH_3) may also be produced[26]. In sewage treatment plants two kinds of Se-methylating microorganisms have been isolated: *Candida humolica*[27]and *Penicillium sp.*[20].

Considering all the factors enhancing volatilization[26], biomethylation is more likely to occur in activated sludges or during the aerobic stabilization of sludges. However, though *Reanter et al.*[28] have reported the conversion of inorganic Se to volatile methylated species, these results were only observed in sludges spiked with selenium. It must also be considered that some heavy metals (Mo, Hg, Cr and Pb) or nitrate and nitrite can have an inhibitory effect on the volatilization of selenium[26]. Moreover, selenium is also likely to be bound to the -SH sites of organic matter because of its strong affinity for sulfhydryl groups[14] and there can also be interactions with some heavy metals such as Cd, Hg and Pb to form various seleno-metal-protein complexes.

In 1987, in Western Europe[5]. the concentration of selenium in sewage sludge was generally lower than 5 mg $Se.kg^{-1}$ sludge with a range of 1 to 9 mg $Se.kg^{-1}$ sludge, but in the USA[19] the mean concentration was of 26 mg $Se.kg^{-1}$ sludge with a range of 10 to 180 mg $Se.kg^{-1}$ sludge.

In France selenium concentration in sludges must not exceed 200 mg $Se.kg^{-1}$ dry sludge (maximum level) to be used as manure, the reference level being of 100 mg $Se.kg^{-1}$ dry sludge. Moreover sludge must not be used on soils containing more than 10 mg $Se.kg^{-1}$ dry weight (AFNOR U 44-041 norm[29]). In the present state of knowledge there is no serious risk in the short term concerning the disposal of sludges on land in France, but the accumulation of selenium in soils through the addition of sludge to land can have in the long term an impact on groundwater, animals and human beings[30].

For that reason we have developed a method for the wet digestion of sewage sludge and the determination of their total selenium concentration by Differential Pulse Cathodic Stripping Voltammetry (DPCSV) and ElectroThermal Atomic Absorption Spectrometry (ETAAS). Quality assurance was made by the analysis of two BCR certified sewage sludge reference materials (CRM 145R and CRM 007) and tested on a natural sample from a representative French sewage treatment plant.

However knowledge of speciation is necessary when speaking of the toxicological risk represented by an element. Different techniques of soft extraction, applied to CRM 007 and respecting species distribution, are also discussed.

EXPERIMENTAL

Samples

Sewage sludge from a French treatment plant The sewage treatment plant of Tarbes (south-west of France) has been chosen because of its proximity and representativeness. This municipal sewage treatment plant has a capacity of 40 000 population equivalent and functions in extended aeration with an excellent treatment yield. The water line is composed of a usual pretreatment, an oxidation ditch with four aeration screws, followed by a clarifier before discharge. Sludges are dried by filter pressing before being used at 100% in agriculture. Three liters of activated sludge were sampled in the ditch on May 15th 1995. These samples were settled for 2 h and centrifugated at 4000 $tr.min^{-1}$ for 30 min. The solid phase was then freeze-dried for 48 h, crushed and finally stored at -20°C.

Certified reference materials Samples of sewage sludge were obtained from the Community Bureau of Reference (Brussels, Belgium). As no Reference Material corresponded exactly to the actual French sludge studied (rural origin and low Se concentration) two different ones were selected: CRM 145R (mainly of industrial origin with an indicative concentration of 3.3 mg $Se.kg^{-1}$on a dry weight basis), and CRM 007 (representative of an American residential area with industrial influence and with a certified value of 15 ± 1.8 mg $Se\text{-}kg^{-1}$ on a dry weight basis).

Reagents

Stock standard solution of Se(IV) was prepared with sodium selenite (Na_2SeO_3, $5H_2O$) from MERCK (pro analysis). All the solutions for standard additions and calibration were prepared from this standard solution by successive dilutions.

Reagents used for digestions (HNO_3 65%, HCl 37%, H_2SO_4 96%, H_2O_2 30%), reductions (HCl 37%) and supporting electrolytes (H_2SO_4 96%) were all Suprapure products from MERCK.

A stock solution of palladium (II) was prepared by dissolution of 5.3 mg (Pd) of palladium nitrate (N_2O_6Pd, $2H_2O$), from SIGMA, in 10 ml HNO_3 1%. The palladium modifier solution was then prepared by a ten times dilution with deionized water of the stock solution.

The solution of NaOH 2M for soft extraction was prepared from Suprapure sodium hydroxide from MERCK.

The ammonium phosphate-citric acid buffer for enzymatic extractions was prepared in 100 ml from 2.1 g of citric acid, 1.15 g of mono-ammonic phosphate and 5 ml of ethanol (5% (v/v)) bring to pH = 7.5 by concentrated ammoniac. All the constituents came from PROLABO RP Normapur. The enzymes used were a non specific lipase (type VII) and a non specific protease (Pronase E) from SIGMA. Deionized water (resistance 18 MΩ) was obtained from a MilliRO/MilliQ system (Millipore).

Instrumentation

Two analytical techniques have been chosen for the determination of selenium in these samples: Differential Pulse Cathodic Stripping Voltammetry (DPCSV) and ElectroThermal Atomic Absorption Spectrometry (ETAAS) for confirmation.

DPCSV measurements were made with a P.A.R. 264A, equipment furnished with a P.A.R. 303A hanging mercury drop electrode, a Ag/AgCl saturated KCL reference electrode saturated in AgCl a platinum auxiliary electrode and a P.A.R. 305 magnetic stirrer. Data were displayed on a Kipp and Zonen X-Y recorder.

Analytical conditions previously determined in our laboratory[31] for the measurement of Se had to be optimized for these complex matrices that gave birth to important interferences, Different analytical parameters have been retained and are presented in Table I. Results were then obtained by three standard additions. The instrumentation used for the ETAAS Se determination is composed of a Unicam GF 90 Furnace, associated with a Unicam 939 QZ AA Spectrometer coupled to a FS 90 Furnace Autosampler. Data are treated by the Solaar AA System software. All the mesurements were made with a boosted hollow cathode lamp of Se, a Zeeman-effect background correction and Pd as matrix modifier. Pyrolitic graphite tubes were used exclusively.

Projections have been observed in the furnace, for the basic extracts analysis, with the furnace programme generally used in our laboratory for Se measurement (programme 1). A new programme has then been determined for this kind of sample (programme 2). The furnace programmes are presented in Table II.

TABLE I Optimized parameters for the Se(IV) determination in sewage sludge by DPCSV

Parameters	*CRM 007-Sludge of Tarbes*	*CRM 145R*
Supporting electrolyte	H_2SO_4(0.1 mol.L^{-1})	H_2SO_4(0.1 mol.L^{-1})
Volume of solution in the analysis cell	10 cm^3	10 cm^3
Purge time (N_2)	8 min.	8 min.
Initial potential	E_i = -0.2 V	E_i = -0.35 V
Final potential	E_f = -0.7 V	E_f = -0.85 V
Pulse amplitude	ΔE = 50 mV	ΔE = 50 mV
Scan rate	v = 2 mV.s^{-1}	v = 2 mV.s^{-1}
Deposition time	120 s	220 s
Equilibration time	30 s	30 s
Film dissolution time	100 s	100 s
Potential of the film dissolution peak	E_{peak} = -0.45 V	E_{peak} = -0.55 V

Results were obtained by calibration with five standard solutions, this method giving better results on our matrices than standard additions.

The solutions were then stored at 4°C in closed polyethylene flasks and analyzed within a week.

Samples Pretreatments Before Analysis

For the determination of total selenium in solid samples by the chosen analytical methods, it is necessary to solubilize selenious forms and transform organic species into inorganic ones by digestion. The digested sample can then be directly analyzed by ETAAS but, as digestion assures a major transformation of all the selenium present to Se(VI), it is necessary to carry out a reduction of this sample before analysis by DPCSV, as this technique detects only Se(IV).

TABLE II Furnace parameters for the determination of total Se in sewage sludge by ETAAS

Phase	*Temperature (°C)*	*Graduation (s)*	*slope (°C.Sec^{-1})*
Programme 1: Se in digested solid samples			
1	90	15	0
2	250	5	10
3	950	6	200
4	2300	3	0
5	2500	1	0
Programme 2: Se in digested extracts			
1	90	10	0
2	110	5	1
3	250	5	5
4	850	5	120
5	2300	3	0
6	2500	1	0

When the speciation is concerned it is necessary to carry out a "soft" extraction of Se contained in the sample prior to the analysis. We will present in this paper only the extraction yields obtained with different extractions, so all the extracts were digested (and reduced for DPCSV measurements) before analysis to determine the total amount of selenium contained.

Soft Extraction Enzymatic extraction was achieved on 0.25 g of sewage sludge mixed in a haemolysis tube with 25 mg of pronase and 13 mg of lipase in 5 ml of ammo- nium phosphate-citric acid buffer. The mixture was maintained at 37°C with constant stirring for 16 h.

The other extractions were also achieved on 0.25 g of sewage sludge. 10 ml of the extractant were added to the sludge in a haemolysis tube and submitted to sonication for 3 h. Extracts were separated from solid residues by centrifugation at 4 000 $tr.min^{-1}$ for 30 min.

The extracts and the solid residues were then digested (and reduced for DPCSV measurements) to determine the concentrations of selenium in the different subsamples and, for instance, the percentages of Se extraction and Se loss during the process.

Digestion In the frame of this study an optimization of sludge digestion was realised in open Teflon beakers (previously cleaned with HNO_3 10%) heated on a hot plate with several mixtures of different reagents.

Reduction Optimal efficiency of the reduction to Se(IV) had already been demonstrated in our laboratory[31] to occur with HCl 6 M as reducing agent in a sealed ampoule (to avoid Se losses by volatilization) warmed at 90°C for 45 min.

RESULTS AND DISCUSSION

Determination of the Sludges Digestion Procedure

The choice of the different acid mixtures here studied is the result of a bibliographical study. Few data are encountered about the digestion of sewage sludge for the determination of selenium[32-34]. To broaden this study we also examined papers concerning the digestion of the certified samples retained for that work (for the determination of several other elements)[35-38], and concerning the digestion of soils[39-43], sediments[43-44] and some biological materials[45-47] for the determination of total selenium.

The combinations retained for that study on the basis of the results presented in the literature are: HNO_3 alone[33,34,38,43,47], aqua regia (HNO_3-HCl (1/3) (v/v)[33,35-37,40,42,43], HNO_3-H_2SO_4[32,40,41,44-47] and HNO_3-H_2O_2[39-42,47]. All the combinations including the use of $HClO_4$ were rejected in spite of the efficiency of this reagent, for safety reasons.

The selected procedures were then applied to CRM 145R. Table III contains the description of these digestion procedures, All the samples were then filtered on 0.45 µm before storage. All attacks were run in triplicate and solutions obtained were analysed five times each by DPCSV and ETAAS.

TABLE III Digestion conditions of CRM 145R

Sample		*1st reagent*	*Volume (ml)*	*2nd reagent*	*Volume (ml)*	*Final volume (dilution with water) (ml)*
CRM 145R	①	HNO_3	10	HNO_3	10	50
	②	HNO_3 + HCl	3 + 9	HNO_3 + HCl	3 + 9	50
	③	HNO_3	10	H_2SO_4	10	50
	④	HNO_3	10	H_2O_2	10	50

TABLE IV Results obtained for the determination of total Se in CRM 145R, CRM 007 and the sewage sludge of tarbes

Sample	*Digestion procedure*	*Mean concentration (mg.kg^{-1} dry weight)*		*RSD (%)*	
		DPCSV	*ETAAS*	*DPCSV*	*ETAAS*
CRM 145R	① HNO_3	–	2.46 ± 0.08	–	3
(3.3 mg Se.kg^{-1})	② HNO_3 - HCl	2.47 ± 0.69	2.97 ± 0.69	28	14
	③ HNO_3 - H_2SO_4	2.35 ± 0.37	–	15	–
	④ HNO_3- H_3O_2	3.37 ± 0.30	3.30 ± 0.30	9	9
CRM 007	④ HNO_3 - H_2O_2	15.38 ± 1.20	15.16 ± 0.05	8	<1
(15 mg Se.kg^{-1})					
Sludge of Tarbes	④ HNO_3 -H_2O_2	1.23 ± 0.12	1.32 ± 0.13	10	10

The presence of SO_4^{2-} ions caused interferences for the determination of Se by ETAAS. Moreover we can not take advantage of the fact that the use of H_2SO_4 allows to increase the attack temperature of the medium (increasing the action of other oxidants), as it can be done by microwave oven digestion[32,33,42- 44,46,47].

This method was then rejected. The aqua regia digestion has given poorly reproducible results in both DPCSV and ETAAS and has then also been rejected.

The mixture HNO_3-H_2O_2 led to the best results for CRM 145R analysis, caused few problems in the analysis by DPCSV and ETAAS and was therefore retained for the analysis of CRM 007 for confirmation and for further sludge samples. The results obtained are presented in Table IV.

Extractions

The mobility of selenium and its toxicity to the biosphere are related to its association with various sludge or soil constituents as well as to its total concentration. "Soft" extraction is necessary when the aim of the study is the speciation. Different approaches have been elaborated for the extraction of selenium. One consists of making one extraction, or repeated extractions, with a single reagent (or a mixture of reagents) to try to extract the greatest quantity of selenium. The other solution involves a set of different reagents to extract different categories of selenium. In parallel extractions, these reagents are used on different subsamples and in sequential extractions they are applied successively to the same solid sample. In both cases the extractants used must separate selenium from the matrix without inducing any loss or change in the partitioning of species. The mechanisms involved for each extractant must correspond to processes occurring in nature and are then associated with special fractions of selenium: soluble (soluble species and non-specifically adsorbed Se) exchangeable (specifically adsorbed Se released by anion-exchange), "oxidizable" fraction (Se associated with Fe, Al and Mn oxides, carbonates and hydrolizable organic matter), "mineral" fraction (Se in mineral-lattice structure)[54].

There are few papers concerning the extraction of selenium from sewage sludge[6,16], some more deal with the extractions from soils[39,48-52]. Sequential extractions have been criticized by Gruebel et al.[53] for lack of selectivity and accumulation of errors. It is the reason why a parallel extraction with three types of extractants was chosen for this study: warm water (soluble fraction), ammonium phosphate-citric acid (soluble + exchangeable fraction) and sodium hydroxide (soluble + exchangeable + "oxidizable" fraction). We have also tested an enzymatic extraction procedure which had already been proved, in our laboratory, to be interesting for the attack of biological materials.

All the extractions were first applied to CRM 007 because of its high Se concentration. The results presented in Table V are the mean of five extractions, each sample being analysed five times. The percentages of loss during the extraction are not significant if we consider RSD (Relative Standard Deviation) values. As enzymatic extractions were carried out in an ammonium phosphate-citric acid buffer we can conclude through the results obtained that the action of enzymes is not significant. By substraction of the result obtained by the previous weaker extractant it is possible to evaluate the percentage of Se in each fraction.

The extraction procedure tested on CRM 007 has shown that about 25% (soluble fraction 11% + exchangeable fraction 14%) of the selenium present could be directly available for plant uptake if transfered in that form in soils by water leaching. 39% more of total Se ("oxidizable" fraction), obtained with a stronger attack, are conditionally available in the environment through chemical and microbial mobilization. As matter of fact, lowering of the redox potential and pH of a medium (soil or sludge) may render Fe and Mn oxides soluble and therefore release the associated Se into a soluble or available form. Oxidative weathering can also decompose secondary sulfides, monosulfide minerals, and organic materials whereby Se contained therein would be made available in the environment[48]. These two fractions represent respectively the real and potential impact of Se on the environment.

The 36% of residual Se ("mineral" fraction) are highly resistant as far as their impact on the environment is concerned and will probably represent no danger for the biosphere.

TABLE V Results of Se extraction in CRM 007 by different extraction procedures

Extractant	*Percentage Of extraction (%)*		*RSD (%)*		*Percentage of Se loss during the extraction (%)*	
	[1]	*[2]*	*[1]*	*[2]*	*[1]*	*[2]*
Warm water	11.0 ± 0.9	11.3 ± 2.3	8	20	5	6
Ammonium phosphate citric acid	24.5 ± 0.9	-	4	-	3	
Enzymatic extraction	25.3 ± 1.2	-	5	-	3	
Sodium bydroxide (2 mol.l,-1)	63.5 ± 3.1	64.1 ± 4.0	5	6	2	2

[1] Analysis by DPCSV.
[2] Analysis by ETAAS.

CONCLUSION

Digestion procedures for total Se concentration in sewage sludge were studied using a certified sewage sludge reference material (CRM 145R). The selected procedure (digestion by a mixture of HNO_3 and H_2O_2) was successfully applied to another certified sewage sludge reference material (CRM 007) and to a natural sample from a representative French sewage treatment plant. The result obtained for this sample is much lower than the value in norm AFNOR U 44-041[29] and is supposed to be representative of what can be encountered in France. However, the treatments realised on sludges tend to concentrate Se in the medium. Studies must then be realised on dried sludges just before their application to land. This work is in process actually.

Parallel extractions applied to CRM 007 have shown that 25% of the total Se contained in that sludge can have a real impact on the environment and that 39% more represent a potential danger for the biosphere. The sodium hydroxide extraction procedure (allowing to extract the real and potential available Se) will then be used to determine the different species present in various certified and natural samples.

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