Ecotoxic evaluation of mortar leachate using the amphibian larvae (*Xenopus laevis*)

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Abstract:

Nowadays, many projects aim to reuse by-products (e.g. sewage sludge ash, sediments of dredging, MSWI…) in cement-based materials. However, some questions remain concerning the environmental impacts of these wastes in this kind of application. The cement-based materials in buildings or roads are subjected to leaching/draining by rainwater or subterranean water. Consequently, draining waters may constitute an important reservoir for contaminants, such as water extractible elements, in the aquatic environment. So these elements, which are potentially pollutant, could be in contact with living organisms of the ecosystems.

The regulations in the field of waste materials are mainly based on the chemical composition of the waste, or on the quantity of leached elements and use standard tests. However the quantity of elements that are potentially leachable does not necessarily reflect the ecotoxic behaviour of the waste in the environment. The impact of a leachate depends on many factors such as: the intrinsic toxicity of the elements, speciation of metals, the nature of the medium, and physical and chemical interactions leading to the modification of the bioavailability of the pollutants.

In order to assess the ecotoxic potential of by-products used in cement-based materials, measurements were made to evaluate the effects of acute toxicity and the possible effects of toxicity on the genome (genotoxicity) of larvae of amphibians exposed to various concentrations of mortar leachates. This work is related to the evaluation of the environmental impact of meat and bone meal ashes in the cement matrix using the standardized ISO 21427 amphibian micronucleus assay on *Xenopus laevis* larvae (ISO, 2006).

1- Introduction

Several scientific studies report valorization of by-products in cement-based materials. The uses of these by-products involve an evaluation of their impact on the environmental properties of cement-based materials. These studies and standard thresholds [1999/31/EC, 2003/33/EC, 40CFR261.2, WHO94, 1998/83/EC] only concern the quantities of leached elements that are potentially pollutant. However, it is widely accepted that a knowledge of the quantity of pollutant leached is not sufficient to inform on the ecotoxic potential of materials. In fact, it is not certain that all pollutants are detected and synergic and antagonist phenomena are often unpredictable.

The environmental management of cement-based materials is an important economic and environmental problem. The regulations relative to their management are insufficient, as regards the precise evaluation of the future evolution and possible effects on ecosystems of these contaminated matrices. There is not a clear consensus concerning the strategy for assessing the ecological hazard of these kinds of materials. The need for such a risk assessment has already been emphasized [Sanchez *et al.*, 2003 ; Van der Sloot, 2002]. However, toxicity assessments of complex matrices like cement-based materials generally do not take account of genotoxic endpoints. The interaction of genotoxic compounds with DNA initially causes structural changes in the DNA molecule. Unrepaired changes can potentiate other cell lesions and thus lead to tumour formation [Malins *et al.*, 1990]. The effects of pollutants on DNA integrity have been reported in aquatic animals and, among these, amphibians have proved to be sensitive models [Gauthier, 1996]. They have been used to study the genotoxic potential of pure substances, physical agents, drinking and surface waters, industrial and domestic effluents, leachates of soil and sediments, sludge and industrial wastes [Mouchet *et al.*, 2006], different materials such as meat and bone meal bottom ash [Mouchet *et al.*, 2007] and carbon nanotubes [Mouchet *et al.*, 2008]. The assays concern micronucleus induction in erythrocytes, using the normalized micronucleus test MNT [ISO, 2006]. Formation of micronuclei is a consequence of chromosome fragmentation and/or dysfunction of the mitotic system. Thus, clastogenic compounds and spindle poisons both lead to an increase in the number of micronucleated cells.

The leaching procedure approximates natural contamination of the aquatic compartment and mimics transfer of micro-pollutants from the solid to the aquatic compartment. Its ecotoxicological relevance has already been highlighted in previous research on contaminated soils [Bekaert, 1999].

This paper presents a study of the environmental impact of plain mortar and mortar containing by-products (meat and bone meal ashes). Environmental properties of mortar are evaluated via a leaching test and eco(geno)toxic assay (on Xenope larvae).

2- Materials and methods

The binder was a standard Portland cement, CEM I 52.5R, as specified in European Standard NF EN 197-1. The **compositions of the two mortar mixtures** are presented in table 1. The reference mortar (Ref) was composed of cement and quartz sand. The mortar with MBM-BA contained cement and MBM bottom ash in replacement of quartz sand. Both mortars had the same sand grading. The mixtures were cast in 4x4x16 cm moulds and sealed in plastic bags in a temperature controlled room at 20ºC.

Quantity (g)	Cement	Sand	MBM-BA	Water
Ref	350	1400	-	231
MBM-BA	350	-	1400	404

Table 1. Composition of reference and MBM-BA mortar

Meat and bone meal bottom ash came from an incineration plant equipped with a rotary furnace (12 m long) [Coutand, 2008]. This incinerator has a capacity of 2 tons/hour with an operating temperature of 1000°C. The incinerator was fed with 95% of meat and bone meal from pork production, the remaining 5% being composed of other waste such as plastic bags or sewage sludge.

The leaching tests were carried out at 28 days of age: NF P X31-211 on monolithic material in water (3x3x8 cm pieces).

Elemental analyses were performed by atomic adsorption spectroscopy (AAS) with flame atomization (Perkin Elmer, 2100) and ICP-MS (Perkin-Elmer Elan 6100).

Ecotoxicological investigations were performed using the standardized (ISO 21427) amphibian micronucleus test (MNT) on *Xenopus laevis* larvae. Larvae were exposed in groups of 15 - 20 animals (100 mL/larva) to either the control medium (negative and positive controls) or the test medium containing mortar leachate (Ref or MBM-BA) at 3.125, 6.35, 12.5, 25, 50 and 100 %, where 100 % corresponds to the leachate without water dilution. The negative control (NC) was the reconstituted water alone ((294 mg/L $CaCl_22H_2O$, 123.25 mg/L $MgSO_47H_2O$, 64.75 mg/L $NaHCO_3$, 5.75 mg/L KCl). The positive control (PC) was cyclophosphamide (a well known mutagenic agent) in reconstituted water at 20 mg/L. In the standard ISO 21427, the pH needs to be adjusted to around pH 7 (± 1). In the present work, mortar leachate (Ref and MBM-BA) was tested at its natural pH (without adjustment) and at pH 7 (adjusted with chloride acid)

3- Leaching tests

This paper presents only the result of the monolithic leaching test NF P31-211. However, other leaching tests were performed on reference and MBM-BA [Coutand, 2007] under various conditions [EN 12-457, TCLP], carbonated and not carbonated, at 90 days. In this paper, the leaching test presented is the most unfavourable for MBM-BA.

The leaching results can be compared to thresholds when they exist (for instance TCLP limits, quality of water, and waste acceptance criteria for landfill and municipal solid waste incineration (MSWI) bottom ash to be reused in road construction in France). But, to the authors' knowledge, no thresholds are available for the valorisation of by-products in cement-based materials. So the leaching results are compared to a reference material generally used in current applications, i.e. the reference mortar for this study.

Figure 1 compares the quantity of elements leached from reference and MBM-BA mortars (µg/L). The main elements leached were calcium, sodium, potassium and aluminium. The quantity of leached alkalis was higher for MBM-BA than for the reference because of the initial high content of these elements in the bottom ash. Although phosphorus is the main component of meat and bone meal ash, this element was not present in large quantities in the leachate. This result confirms the low solubility of phosphates, which are the main component of ashes, in cement-based materials.

All potentially pollutant elements (except vanadium and selenium) were found in higher quantities in the leachate of mortar containing MBM-BA. This effect could probably be explained by the presence of these elements in by-products and by the higher porosity of MBM-BA mortar compared to reference mortar [Coutand, 2007].

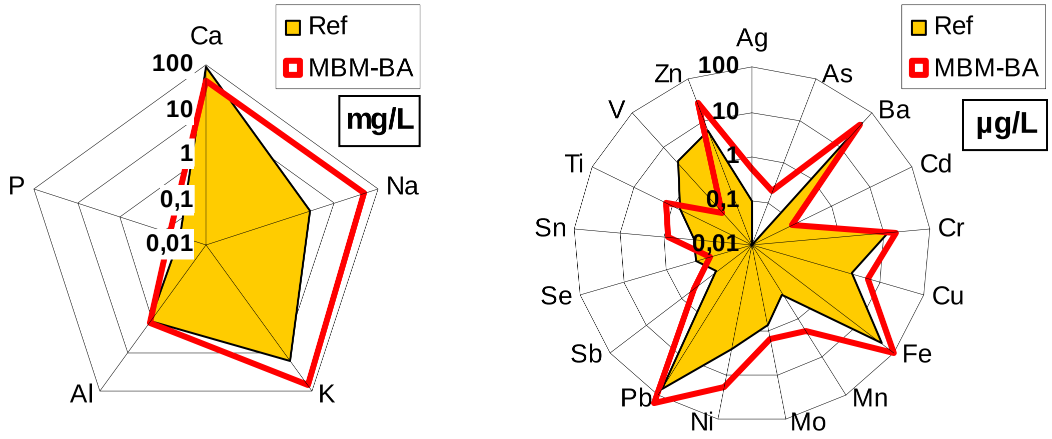

Figure 1. Quantity of elements leached from reference and MBM-BA mortars during leaching test on monolithic materials NF P31-211 (mg/L and µg/L).

However, the sole comparison of leachates can hardly allow us to conclude on the environmental impact of MBM used in cement-based materials, for two principal reasons:

- Heterogeneity of chemical compositions and variability in physical properties of mortars lead to large confidence intervals [Coutand, 2007]. So the differences observed between the concentrations of leached elements are not always statistically significant.
- A knowledge of the quantities of leached elements does not give information about the toxicity and/or genotoxicity of the material on living organisms.

These two reasons explain why other tests were carried out using eco(geno)toxicity assays, on amphibian larvae in our case.

4- Toxicity and genotoxicity investigation in amphibian larvae

Table 2 presents the results of acute toxicity (mortality) in *Xenopus* larvae exposed to various concentrations of leachates (reference and MBM-BA) at two pH conditions: natural (pH around 11.5) and adjusted to 7. pH was readjusted to evaluate the potential impact of a basic pH value since amphibian larvae need a pH ranging 6 and 8 to live, as recommended in the standard amphibian micronucleus test (MNT).

All concentrations above 50% induced toxicity in *Xenopus* larvae but the dilution of aqueous samples reduced the toxicity of both mortar leachates since the concentrations below 12.5% led to an absence of acute toxicity. The behaviour of larvae in 25% leachates was noteworthy: this concentration induced toxicity for all leachates, except for the one obtained from MBM-BA mortar with adjusted pH.
The adjustment of pH led to a diminution of lethality on larvae. Three reasons could explain this result:

- the decrease of aluminium, phosphorus, chromium, iron, nickel, lead, copper, tin and titanium contents in the solution with the diminution of pH.
- the increase of calcium content in the solution with the decrease of pH; calcium is known to reduce some harmful effects of metals [Mouchet, 2003; Sneijs, 2005].
- the high pH is not favourable to the life of *Xenopus* larvae. Some authors [Brezonik, 1990; Pritchard, 1990] note the importance of pH on metal speciation and so on their ecotoxic impact.

Conditions			**Concentrations**					
Reference	**NC**	**PC**	**100%**	**50%**	**25%**	**12.5 %**	**6.25 %**	**3.125%**
Natural pH	░	░	100%	100%	73%	░	░	░
Adjusted pH	░	░	100%	100%	60%	░	░	░
MBM-BA								
Natural pH	░	░	100%	100%	80%	░	░	░
Adjusted pH	░	░	100%	47%	░	░	░	░
░ Absence of acute toxicity			■ Acute toxicity , % percentage of mortality of larvae					

Table 2. Acute toxicity in Xenopus larvae exposed to different concentrations of various leachates (reference and MBM-BA). Concentrations (3.125, 6.25, 12.5, 25 %.) are expressed in percentage of the raw leachate. Positive control (PC), negative control (NC).

Genotoxicity assessments in larvae exposed to the reference and MBM-BA leachates are presented in Figure 2. At natural pH, genotoxicity was observed in larvae exposed to concentrations of 12.5 % and 6.25% for reference mortar and MBM-BA mortar respectively. In the case of larvae exposed to a solution with adjusted pH, no genotoxicity was observed for either mortar for concentrations up to 12.5%

These results show that adjustment of pH led to a reduction in the number of micronucleated erythrocytes per thousand (MNE ‰) in larvae. The three reasons exposed previously can probably explain this result.

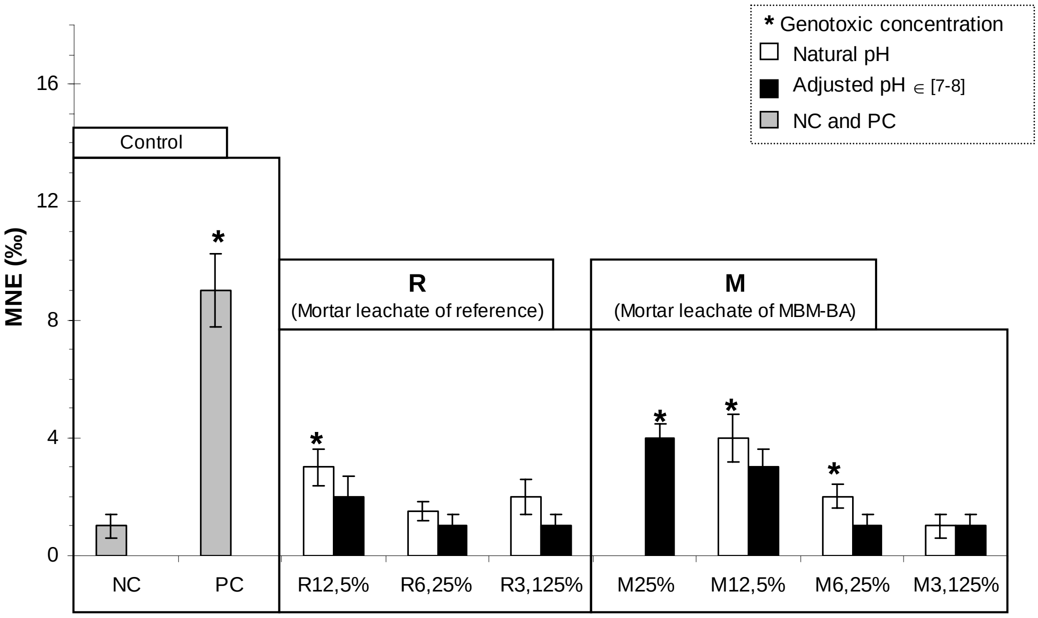

*Figure 2. Median value of micronucleated erythrocytes per thousand (MNE °/oo) after 12 days of exposure of Xenopus larvae to different concentrations of various leachates (reference and MBM-BA): positive control (PC), negative control (NC). Concentrations (3.125, 6.25, 12.5, 25 %) are expressed in percentage of the raw leachate. * indicates that genotoxicity is significant compared to the NC condition (MNE °/oo is expressed by the value of the median and the 95% confidence limits).*

Genotoxicity may depend on the chemical composition of the leachates, which contained heavy metals (Figure 1). It is well known that metals can induce genotoxicity [Mouchet *et al.*, 2006] via production of reactive oxygen species (ROS). Heavy metals can bind to phosphates and a wide variety of organic molecules, including DNA base residues, and can lead to mutations by altering primary and secondary structures of the DNA [Wong, 1988]. Genotoxic properties of Cu, Cd and Hg, have also been demonstrated on X. laevis and P. waltl [Mouchet, 2002] and similar results were obtained with Cr and Fe on P. waltl [Godet *et al.*, 1996]. These results are presented in Table 3.

In our tests, the chromium, zinc, iron, lead, copper and cadmium contents of leachates were below all concentrations inducing genotoxic effects (Table 3). In fact, mortar leachates were composed of many non-isolated metals, and the coexistence of many metals could generate synergic and/or antagonist effects. So, it is difficult to associate the effect of mortar leachates observed on larvae with the presence of one element or group of elements.

Biological model	Reference	Chemical element	Concentrations with effects (mg/L)		Concentrations without genotoxic effect (mg/L)
			Toxic	Genotoxic	
Pleurodeles larvae	Godet, 1996	Cr^{3+}			0.1 – 1 – 5 – 10
		Cr^{6+}		0.1 – 1	0.01 – 0.5 – 5 – 10
		Zn^{2+}		0.1	0.01 – 1 – 10
		Fe^{3+}		4.5	0.5 – 1.5 – 13.5
Xenopus larvae	Mouchet *et al.*, 2007	Pb^{2+}	30 – 50 – 100	1 – 10	0.01 – 0.1
	Mouchet, 2002	Cu^{2+}	1.6 – 0.8 – 0.4		0.005 – 0.025 – 0.075
	Coutand, 2008	Cd^{2+}	5 – 10 – 50	0.002 – 0.2 – 2	

Table 3. Concentrations of metals inducing toxic and genotoxic effects on aquatic organisms.

5- Conclusion

The results of the XP 31-211 leaching test show that MBM bottom ash in mortar leads to an increase of leaching of some potentially pollutant elements such as lead, zinc, arsenic, copper, iron and lead. Toxic and genotoxic investigations on *Xenopus* larvae, using leachate with natural pH, show that the toxic effect is similar for both mortar leachates and that the reference leachate is less genotoxic than MBM-BA leachate. Concerning leachates with adjusted pH, the genotoxic effect is similar for both mortar leachates and the reference mortar leachate is more toxic than MBM-BA leachate.

These results show the importance of pH on the ecotoxic impact of mortar leachate. However, these investigations did not allow us to conclude on the element or groups of elements responsible for the toxic and/or genotoxic impact of leachates. In fact, the chemical compositions of leachates are complex, so various synergic and antagonist effects exist between the chemical elements.

Eco(geno)toxic assays are of great interest for our understanding of the environmental impact of mortar leachates. These kinds of test are already used in the environmental characterisation of waste. It could be interesting to study other organisms (e.g. bacteria, plants, etc.) with various kinds of contact (e.g. volatile particles …) and at different scales (e.g. laboratory essay, microcosms …).

6- References

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