



COSMOS-rice technology abrogates the biotoxic effects of municipal solid waste incinerator residues[☆]



Michela Guarienti^{a,1}, Sdenka Moscoso Cardozo^{a,1}, Laura Borgese^b, Gloria Rodrigo Lira^c,
Laura E. Depero^{b,**}, Elza Bontempi^b, Marco Presta^{a,*}

^a Zebrafish Facility, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy

^b INSTM and Chemistry for Technologies Laboratory, University of Brescia, Via Branze, 38, 25123 Brescia, Italy

^c Unidad de Vigilancia Ambiental y Genotoxicología, Instituto de Biología Molecular y Biotecnología, Universidad Mayor de San Andrés, La Paz, Bolivia

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ABSTRACT

Fly ashes generated by municipal solid waste incinerator (MSWI) are classified as hazardous waste and usually landfilled. For the sustainable reuse of these materials is necessary to reduce the resulting impact on human health and environment. The COSMOS-rice technology has been recently proposed for the treatment of fly ashes mixed with rice husk ash, to obtain a low-cost composite material with significant performances. Here, aquatic biotoxicity assays, including daphnidae and zebrafish embryo-based tests, were used to assess the biosafety efficacy of this technology. Exposure to lixiviated MSWI fly ash caused dose-dependent biotoxic effects on daphnidae and zebrafish embryos with alterations of embryonic development, teratogenous defects and apoptotic events. On the contrary, no biotoxic effects were observed in daphnidae and zebrafish embryos exposed to lixiviated COSMOS-rice material. Accordingly, whole-mount in situ hybridization analysis of the expression of various tissue-specific genes in zebrafish embryos provided genetic evidence about the ability of COSMOS-rice stabilization process to minimize the biotoxic effects of MSWI fly ash. These results demonstrate at the biological level that the newly developed COSMOS-rice technology is an efficient and cost-effective method to process MSWI fly ash, producing a biologically safe and reusable material.

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1. Introduction

Thousands of millions of tons of municipal solid waste (MSW) are produced every year. The increasingly stringent limits imposed on atmospheric emissions from waste incineration have produced a significant change from the gaseous emissions to the solid residues of the process. Thus, fly ashes warrant significant environmental concern. Indeed, the amount of fly ash produced by an MSW incinerator (MSWI) is in the order of 1–3% of the waste input mass

on a wet basis and contains high levels of heavy metals, with Zn and Pb having the highest content (approximately 19–41 g/kg and 7.4–19 g/kg, respectively; Zacco et al., 2014). MSWI ashes are usually landfilled or reused as secondary raw material. Thus, recycling and reusing represent the only sustainable future vision for waste ash management. To this aim, the Colloidal Silica Medium to Obtain Safe inert (COSMOS) technology has been developed to stabilize MSWI residues (Bontempi et al., 2010) and to produce inert safe material (Guarienti et al., 2014). Still, the cost of the process, mainly due to the addition of colloidal silica, represents a critical drawback of the COSMOS process.

Rice husk is the external protecting covering of rice grain. It is used for the production of thermal energy because of its high calorific value. Rice husk ash (RHA), the byproduct of the combustion, is extremely rich in silica (Benassi et al., 2015a) and can be employed in several fields. In the last decade, a large number of activities were devoted to transform rice husk ash into commercially valuable products, such as catalyst carriers, production of pure silica, silica gels, geopolymers, sand filled polymers, fillers for cement and adsorbent (Kumar et al., 2015; Soltani et al., 2015).

Abbreviations: COSMOS, colloidal silica medium to obtain safe inert; MSWI, municipal solid waste incinerator; MSW, municipal solid waste; RHA, rice husk ash; FGD, flue gas desulfurization; TCLP, toxicity characteristic leaching procedure; LP, leaching procedure; TXRF, total-reflection X-ray fluorescence; FET test, fish embryo toxicity test; GMS, general morphological defect score; GTS, general teratogenic score; hpf, hours post-fertilization; WISH, whole-mount in situ hybridization.

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* Corresponding author.

** Corresponding author.

E-mail address: marco.presta@unibs.it (M. Presta).

¹ The two authors contributed equally to this work.

Recently, the COSMOS method has been improved significantly by the substitution of the colloidal silica with RHA, leading to the development of the COSMOS-rice technology (Bosio et al., 2013, 2014) [website: <http://cosmos-rice.csmt.eu>]. The composites obtained with the new filler were tested in different polymeric matrices and possible products have been already realized (Besco et al., 2014). Thus, evaluation of their biotoxicity remains the mandatory final step for the exploitation of this new technology.

The Toxicity Characteristic Leaching Procedure (TCLP) to evaluate the toxicity of fly ash and the effectiveness of stabilization processes (EPA, 1992) is designed to determine the mobility of both organic and inorganic substances present in wastes. Similar protocols have been standardized and proposed also more recently, such the one described in EN 12457 (CEN, 2002). However, the actual biotoxicity of fly ashes cannot be evaluated by chemical analysis only, since the presence of different metals may cause relevant synergic biotoxic effects (European Commission, 1991, 2008; Stiernström et al., 2011).

Several biological tests have been developed to assess the biotoxic effects of waste materials before and after stabilization processes. Even though bacteria-based tests and *in vitro* animal methods have been developed (Huang et al., 2008; Piersma, 2004, 2006), *in vivo* biotoxicity tests performed with non mammalian species may allow a better understanding of the biological effects of hazardous substances and may guarantee the proper management of fly ashes (Chen and Lin, 2006; Kaneko, 1996; Scholz et al., 2008; Stiernström et al., 2011; Wang et al., 2008).

The small daphnidae crustaceans (Persoone et al., 2009), commonly named water fleas, are one of the major component of zooplankton and are highly sensitive to chemicals and freshwater pollutants, especially heavy metals (Martínez-Jerónimo et al., 2008; Rodgher et al., 2010). Thus, acute daphnidae toxicity tests represent standard assays to assess the biotoxicity of chemicals and to monitor industrial effluents and waste water (ISO, 2012; OECD, 2004).

Among non-mammalian vertebrates, the teleost zebrafish (*Danio rerio*) is becoming one of the most frequently used animal model in the *in vivo* bio-toxicological tests (Dai et al., 2014; Hill et al., 2005; Peterson and Macrae, 2012) due to its high sensitivity to toxic compounds during embryonic development (Peterson et al., 1993). Bioassays with zebrafish embryos have been standardized at an international level and recommended in conventional eco-toxicity testing strategies (DIN, 2001; ISO, 2007; OECD, 1992a, 1992b, 1998, 2013). Of note, fish embryo toxicity (FET) tests and adult fish acute toxicity tests provide comparable quality data (Embry et al., 2010; Martins et al., 2007). Moreover, when compared to adults, zebrafish embryos represent a complex vertebrate organism in active development and differentiation (Lammer et al., 2009; Nagel, 2011). Thus, the FET test allows the evaluation of possible biotoxic sublethal and teratogenous effects of chemicals, together with their effects on embryo survival. Thus, the FET test may represent a valuable tool to assess the biotoxicity of single substances (Martins et al., 2007; Lammer et al., 2009) as well as of waste materials (Stiernström et al., 2011).

Recently, the zebrafish embryo-based FET test demonstrated the efficacy of the COSMOS technology to stabilize heavy metals in MSWI fly ashes and to produce an inert material (Guarienti et al., 2014). In the present work, daphnidae acute toxicity assays and the zebrafish embryo-based FET test were used to assess the efficacy of the COSMOS-rice technology to stabilize biotoxic MSWI fly ash.

The results demonstrate that COSMOS-rice technology represents a sustainable procedure to obtain biologically safe high performance materials based on an efficient recycling of different wastes.

2. Materials and methods

2.1. Fly ash samples

MSWI fly ash was collected at a waste-to-energy plant from Slovakia. This ash was chosen because of the high amount of leachable Pb and Zn (Bosio et al., 2014). A2A company (situated in northern Italy) provided the flue gas desulfurization (FGD) residues and the coal fly ashes, while RHA was provided by Curtiriso company (Italy). X-ray diffraction (XRD) data showed that MSWI fly ash contains CaClOH, NaCl, KCl, CaCO₃ and CaSO₄. FGD residue contains CaSO₃*0.5H₂O and Ca(OH)₂. XRD pattern of coal fly ash showed that the only crystalline phases are mullite and quartz (Bontempi et al., 2010; Bosio et al., 2014; Benassi et al., 2015b).

2.2. COSMOS-rice process

The stabilized COSMOS-rice sample was prepared by adding 17 g of RHA and 300 g of water to the mixture of 200 g of three powders. These powders were MSWI fly ash, FGD residues and coal fly ash at 65, 20 and 15 wt% respectively. During mixing (30 min), the temperature was maintained at 100 °C to promote a more efficient silica extraction from RHA when compared to the same procedure performed at lower temperatures (Bosio et al., 2014). Next, water was evaporated at room temperature for 12 days. The stabilized material was washed with a solid to water 1/10 ratio and the soluble salts were retrieved by crystallization (Bontempi et al., 2010). The resulting powder was used for the chemical and biological tests. The powder sample had a grain size smaller than 100 μm (with particle size distribution D50 equal to 10 μm). XRD patterns of stabilized material after washing showed the presence of the following crystalline phases: quartz and cristobalite (SiO₂), calcium sulfate hydrate (CaSO₄*0.5H₂O), calcite and vaterite (CaCO₃) (Struis et al., 2013).

2.3. Leaching procedure

A leaching procedure (LP), i.e. a solid sample extraction method to simulate leaching in a landfill and to evaluate the toxicity of wastes, was applied to assess the mobility of heavy metals from MSWI fly ash, in particular Pb and Zn, before and after the COSMOS-rice stabilization process. LP was performed according to the protocol described in EN 12457 (CEN, 2002), the liquid to solid ratio was equal to 10 and samples were powdered and sieved to obtain particle sizes <100 μm. Deionized water produced by Elix 10 Water Purification System (Merck Millipore, Vimodrone, Italy) was used to obtain the required dilution.

The elemental analysis of the solutions was performed by total-reflection X-ray fluorescence (TXRF) technique.

2.4. Total X-ray fluorescence analysis

TXRF specimen was prepared drying a drop of the solution to be analyzed (about 10 μL) on quartz substrates. TXRF analyses were performed by the Bruker TXRF system S2 Picofox (air cooled, Mo tube, Silicon-Drift Detector), with operating values of 50 kV and 750 μA and using an acquisition time of 600 s. For the quantification procedure, an internal standard was used (1.0 mg/L of gallium). Three water specimens were prepared and analyzed for each solution and the concentration values were averaged (Borgese et al., 2009).

2.5. Biotoxicity test chemicals

Saturated filtered solutions from MSWI fly ash, its stabilized

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