Science of the Total Environment 511 (2015) 331–340

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of fuel zinc content on toxicological responses of particulate matter from pellet combustion *in vitro*

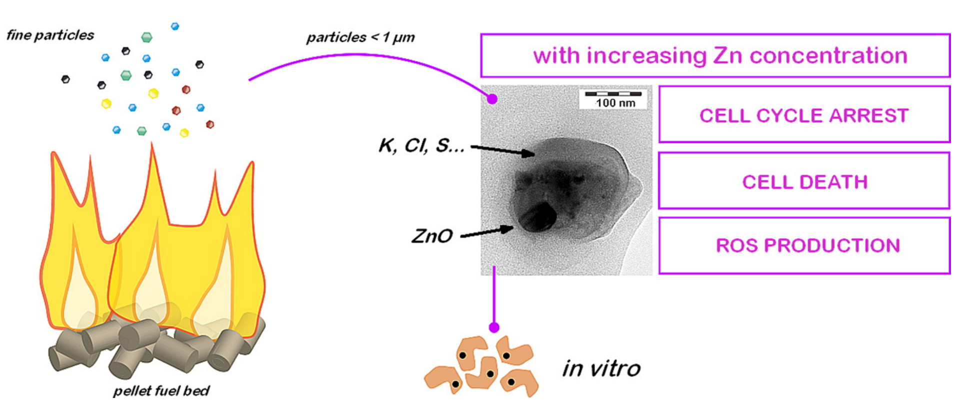
O. Uski [a,b,*], P.I. Jalava [a], M.S. Happo [a], T. Torvela [a], J. Leskinen [a], J. Mäki-Paakkanen [b], J. Tissari [a], O. Sippula [a], H. Lamberg [a], J. Jokiniemi [a,c], M.-R. Hirvonen [a,b]

[a] University of Eastern Finland, Department of Environmental Science, P.O. Box 1627, FI-70211 Kuopio, Finland
[b] National Institute for Health and Welfare, Department of Environmental Health, P.O. Box 95, FI-70701 Kuopio, Finland
[c] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Espoo, Finland

HIGHLIGHTS

- Zinc powder was added into the pure pellet fuel with different concentrations.
- Zinc enriches in PM_1 emissions and enhances particulate toxicological responses.
- Zn-rich samples caused cytotoxicity, ROS generation and cell cycle arrest *in vitro*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 8 December 2014
Accepted 18 December 2014
Available online xxxx

Editor: P. Kassomenos

Keywords:
Wood combustion
Transition metals
Zinc
Health effects
Chemical composition
Particulate matter

ABSTRACT

Significant amounts of transition metals such as zinc, cadmium and copper can become enriched in the fine particle fraction during biomass combustion with Zn being one of the most abundant transition metals in wood combustion. These metals may have an important role in the toxicological properties of particulate matter (PM). Indeed, many epidemiological studies have found associations between mortality and PM Zn content.

The role of Zn toxicity on combustion PM was investigated. Pellets enriched with 170, 480 and 2300 mg Zn/kg of fuel were manufactured. Emission samples were generated using a pellet boiler and the four types of PM samples; native, Zn-low, Zn-medium and Zn-high were collected with an impactor from diluted flue gas. The RAW 264.7 macrophage cell line was exposed for 24 h to different doses (15, 50,150 and 300 µg ml^{-1}) of the emission samples to investigate their ability to cause cytotoxicity, to generate reactive oxygen species (ROS), to altering the cell cycle and to trigger genotoxicity as well as to promote inflammation.

Zn enriched pellets combusted in a pellet boiler produced emission PM containing ZnO. Even the Zn-low sample caused extensive cell cycle arrest and there was massive cell death of RAW 264.7 macrophages at the two highest PM doses. Moreover, only the Zn-enriched emission samples induced a dose dependent ROS response in the

* Corresponding author at: University of Eastern Finland, Department of Environmental Science, P.O. Box 1627, FI-70211 Kuopio, Finland.
E-mail addresses: oskari.uski@uef.fi (O. Uski), pasi.jalava@uef.fi (P.I. Jalava), mikko.happo@uef.fi (M.S. Happo), tiina.torvela@uef.fi (T. Torvela), jani.leskinen@uef.fi (J. Leskinen), jorma.maki-paakkanen@thl.fi (J. Mäki-Paakkanen), jarkko.tissari@uef.fi (J. Tissari), olli.sippula@uef.fi (O. Sippula), heikki.lamberg@uef.fi (H. Lamberg), jorma.jokiniemi@uef.fi (J. Jokiniemi), maija-riitta.hirvonen@uef.fi (M.-R. Hirvonen).

http://dx.doi.org/10.1016/j.scitotenv.2014.12.061

exposed cells. Inflammatory responses were at a low level but macrophage inflammatory protein 2 reached a statistically significant level after exposure of RAW 264.7 macrophages to ZnO containing emission particles.
ZnO content of the samples was associated with significant toxicity in almost all measured endpoints. Thus, ZnO may be a key component producing toxicological responses in the PM emissions from efficient wood combustion. Zn as well as the other transition metals, may contribute a significant amount to the ROS responses evoked by ambient PM.

1. Introduction

Particulate matter (PM) is ubiquitous in urban as well as in rural environments, since it occurs in heterogeneous mixtures which are generated by many different sources such as traffic, industry and energy production. *Via* residential heating biomass combustion is recognized as being one of the most important sources of fine particulate matter (particles < 2.5 μm in aerodynamic diameter, $PM_{2.5}$) not only in developing countries but also in the industrial countries and in urban environments (Boman et al., 2003; Saarikoski et al., 2008; Favez et al., 2009). Several epidemiological studies have revealed associations between daily mortality and $PM_{2.5}$ (Laden et al., 2000; Ostro et al., 2006) as well as the relative effects of $PM_{2.5}$ chemical constituents (Ostro et al., 2007). Epidemiologic analyses of specific constituents of $PM_{2.5}$ have indicated that elemental and organic carbon (EC and OC) and several transition metals are associated with mortality (Burnett et al., 2000; Mar et al., 2000). One major source of these constituents is wood combustion in domestic residences (Boman et al., 2003; Heringa et al., 2011). The different toxic potentials of constituents as well as the mixture of compositions of $PM_{2.5}$ can have important implications for both the establishment of ambient air quality standards and for more targeted PM control strategies. In particular, by focusing regulations on the most toxic of the $PM_{2.5}$ constituents and their sources it could be possible to protect public health more efficiently than by adhering the current PM mass based standards (Mauderly et al., 2010). This is of special interest since there has been significant expansion in domestic use of wood combustion in U.S. and European Union (EU) countries (Alliance for green heat, 2011; European Bioenergy Outlook, 2013).

Zn is a ubiquitous transition metal often associated with industrial emissions (smelters, steel mills and waste incinerators) (Lina and Wendt, 1993; Voutsa and Samara, 2002; Amodio et al., 2013) and traffic (Thorpe and Harrison, 2008). In fact, Zn is one of the most abundant transition metals present in wood fuel, with concentrations ranging from 1 to 300 mg/kg (Sippula et al., 2007; Chandrasekaran et al., 2012; Jones et al., 2014; Phyllis2, 2014). During wood combustion, a large percentage of the Zn is released into the emission gas as elemental Zn-vapor which subsequently becomes oxidized to form ZnO nanoparticles (NPs) (Sippula et al., 2009; Torvela et al., 2014a). Consequently, Zn typically appears in the form of ZnO in wood combustion emissions. ZnO is recognized as an occupational hazard (Madl and Pinkerton, 2009; Kendall and Holgate, 2012). Moreover, in epidemiological studies the Zn content of urban air PM has been associated with a cardiopulmonary mortality and increased health care admissions (Tsai et al., 2000; Hirshon et al., 2008; Chen and Lippmann, 2009). Epidemiological findings are supported by both *in vivo* and *in vitro* experiments where exposure to ZnO NPs and Zn ions (Zn^{2+}) has claimed to exert many harmful effects, *e.g.* inflammation, cytotoxicity, and oxidative stress (Xia, et al., 2008; Wang et al., 2008; Raemy et al., 2012; Adamcakova-Dodd et al., 2014). There is a very limited amount of information available on the involvement of Zn in the toxicity of wood combustion emissions. Recently we have published *in vivo* and *in vitro* data pointing to a putative toxic role of Zn in emission PM_1 (particles <1 μm in aerodynamic diameter, PM_1) from residential wood combustion (Uski et al., 2012, 2014; Happo et al., 2013; Torvela et al., 2014b). This study now demonstrates that Zn is the factor in emission PM_1 from pellet combustion responsible for much of the toxicity detected in *in vitro* and *in vivo* tests.

In order to study toxic role of Zn in pellet combustion emissions we investigated four types of the wood pellet fuels: native pellets (Zn concentration being 11 mg Zn/kg) and pellets enriched with Zn, resulting in total concentrations of 170, 480 and 2300 mg Zn/kg of fuel. The combustion appliance was a modern pellet boiler that has been described previously by Lamberg et al. (2013). The effects of the PM_1 were investigated in a RAW 264.7 macrophage cell line using four dose levels 15, 50,150 and 300 μg ml^{-1}. Several endpoints were monitored including cytotoxicity, cell cycle analysis and the formation of reactive-oxygen and -nitrogen species (ROS and RNS) as assessed by a flow cytometer. Genotoxicity was studied using the comet assay. The release of proinflammatory mediators tumor necrosis factor-alpha (TNF-α) and macrophage inflammatory protein 2 (MIP-2) was measured with an enzyme-linked immunosorbent assay (ELISA).

2. Materials and methods

2.1. Fuel preparation, wood combustion appliance and particle sampling

Pellet raw material (shavings and sawdust from pine stem wood) was first ground with a mill and passed through a 3 mm sieve. Thereafter elemental Zn powder (Sigma Aldrich Corp.) was added, which resulted in 170, 480 and 2300 mg Zn/kg concentrations of Zn in the fuel material. When the fuel was left unaltered, Zn concentration was 11 mg Zn/kg. Finally, the mixtures were pelletized (Hylicpress M 60, Konepaja M. Pappinen Ltd.) for usage as a fuel. The fuel Zn content was determined using ICP-OES spectrometry (Perkin Elmer Optima 2000 DV). The effective heating value of the native pellet was 18.95 MJ/kg, ash content (at 550 °C) 0.35% and fuel moisture content 7.0%.

The 170 mg Zn/kg content corresponded to a realistic Zn concentration of biomass fuel (Sippula et al., 2007; Chandrasekaran et al., 2012; Jones et al., 2014; Phyllis2, 2014), whereas the two higher Zn concentrations would be more commonly encountered in waste incineration (Krook et al., 2006; Jones et al., 2014). The combustion experiments were performed in a laboratory environment using a pellet boiler (Biotech GmbH, model PZ-RL 25) with 25 kW nominal output. The gaseous emissions (40 relevant calibrated compounds, *e.g.*, CO, NO_x, and CO_2) were measured continuously directly from the stack during PM sampling by a Fourier Transform Infrared analyzer (FTIR, Gasmet) as described in more detail in Leskinen et al. (2014).

PM samples for toxicological and chemical analysis were collected from diluted flue gas with a dilution ratio of 9.3–11.8 on polytetrafluoroethylene (PTFE) filters (Millipore Corp., Billerica, MA, USA) with Dekati® Gravimetric Impactor (DGI, Dekati Ltd., Tampere, Finland) by using previously validated methods (Ruusunen et al., 2011). Blank filter control substrates were treated similarly as the actual sample substrates.

2.2. PM sample preparation

The emission particles from four combustion experiments – native, Zn-low, Zn-medium and Zn-high – were prepared for the toxicological analyses as follows. Before particle sampling, the sampling substrates were washed twice with HPLC grade methanol (99.9%, Mallinckrodt Baker B.V. Inc., Phillipsburg, NJ, USA) and dried at +50 °C. Prior to the weighing before and after sampling the PTFE filters substrates were