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IN VITRO REPORTER GENE ACTIVITY OF PCDD/FS AND DL-PCBS IN WOOD ASH FROM DOMESTIC HEATING SYSTEMS (BELGIUM) USING THE H1L7.5C1 CALUX BIOASSAY

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Introduction

Wood ash can be used as a fertilizer because it contains some important nutrients (K, Mg, Ca, P) as well as certain microelements such as Fe, Mn, Cu and Zn, and this in either pure form, in a pelleted version, or in combination with organic waste to form compost. It has been demonstrated that compost amended with (wood) ash (8-16%) provides better fertilizing capacity/ies than without. As wood ash is a byproduct of the combustion of biomass, it would be profitable if these nutrient-containing ashes could return back to the ecosystem by using them as a fertilizer, and thereby making this a sustainable use of biomass[1].

There are, however, also disadvantages to the use of wood ash as fertilizer. Mainly the presence of pollutants, such as potentially toxic metals and dioxin-like compounds can be a concern. Also, the high alkalinity of these ashes, ranging in pH between 12 and 13 can be a problem since they can alter the pH of the soil and interfere with the natural biogeochemical cycle[2,3].

The chemistry of wood ash is highly dependent on a number of different factors, among them the type of wood that is being burned. For example, hardwood ash contains more macronutrients (such as K and P) than softwood ash, making it a more efficient fertilizer. There is also a difference when burning different parts of a tree, since the concentration of nutrients in the ash can differ greatly between ash generated from the bark of the tree or from the stem. Unfortunately, little is known about these differences in accumulations of POPs (such as dioxins and PCBs). Also the incineration temperature is an important variable whereby, for instance, the highest level of macronutrients in ash is retained between 500-800°C, whereas a temperature higher than 900°C can cause heavy metal volatization. There is also an important distinction between fly ash and bottom ash. Fly ash is the lightest component, which accumulates in the chimney and generally contains the highest concentration of possible harmful substances, such as heavy metals and dioxin-like compounds[4].

In this study, wood ash samples from domestic heating systems in Belgium were analysed for dioxin-like activity. Extra attention was given to samples originating from the combustion of wood pellets as these are marketed as eco-friendly, thereby driving sales and increasing their contribution to the total amount of wood used as combustion fuel. The crude wood ash samples (total extract) and the separated dioxin and PCB fractions were measured with the CALUX (Chemically Activated Luciferase gene eXpression) method. The final results were compared to legislation concerning the use of fertilizers and limit values for the use thereof to see if wood ash can be safely used for this purpose.

Materials and methods

Twenty-two different wood ash samples were collected in baked amber glass containers from domestic heating systems in Belgium. The participants were asked to fill in a questionary to gain information about the type of wood that was used, which part of the tree was burned, the dry-time of the wood, the ventilation conditions, etc. The samples were extracted with hexane/acetone (1:1) using an Accelerated Solvent Extraction (ASE) at 125°C and 1500psi over 2 extraction cycles (heat, static, purge). The whole extraction process was conducted two-fold in duplicate: one extract was analyzed in total (crude), whereas the other one was cleaned up and fractionated into a dioxin and PCB fraction.

In the case of non-crude samples, 4mL of concentrated sulphuric acid was added to remove labile components prior to preparatory column chromatography, after which an acid silica column (0.7g sodium sulphate, 1.6g silver nitrate 10% on silicagel, 3g of 33% (w/w) sulphuric acid silica gel, 0.7g sodium sulphate) followed by an activated carbon column (0.7g sodium sulphate, 0.34g X-CARB, 0.7g sodium sulphate) were used for the fractionation[5].

A third generation mouse hepatoma cell line[6] (H1L7.5c1) was used to carry out CALUX analyses, as described elsewhere[7]. Briefly, cells were maintained in alpha minimal essential medium (α-MEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and seeded in 96-well plates (200µL) at 37°C, 85% relative humidity and 5% CO2. After an incubation time of 24 hours and cells reaching a monolayer, sample extract dilutions and TCDD treatment solutions (both 1% DMSO as final concentration) were dosed in duplicate (200µL). Cells were again incubated over a 24-hour period (or 48 hours for the crude extracts) after which lysis and measurement were performed using Luciferase assay substrate and a Glomax 96-well plate reader. Data analysis was performed in Excel where statistical analysis and BEQ/EC_{50} quantification involved fitting the 4-parameter Hill equation or the Box-Cox method[8].

Results and discussion

An overview of the results of both the dioxin and PCB fractions of the wood ash samples is shown in graph 1. The PCB concentration is generally very low, and even below the LOD for 10 out of the 22 samples. This low concentration could be due to the fact that all burned wood was dried for at least two years, and no plastics were burned along side with the wood. In some cases paper was used to help light the fire, but since this paper was only present at the start of the burning process its presence had no influence on the wood ashes.

For the dioxin fractions, the sample with the highest concentration is sample 20 with a value of (10 ± 1) pg TCDD-eq./g. The fuel source in this case was wood pellets. At first sight, this high dioxin concentration related to the use of wood pellets is rather surprising, since they are marketed as an eco-friendly fuel source, because of their low NO_X and SO_X emission compared to other heating systems. Their compact form also allows easy storage and transport over long distances. A possible explanation as to why this concentration is so high is that the burning of pellets results in a very low volume of wood ashes (high efficiency incineration), resulting in a high dioxin amount over volume. Another potential explanation is that these pellets contain wood that has been treated with chemical preservatives, or that they contain other contaminants, such as glue. This high dioxin concentration could also be due to a combination of bad weather and bad burning conditions. The questionary showed that there was a lot of wind and rain during the burning process, which led to severe smoke formation. It is also stated that to end the fire, the air supply was closed off, which resulted in a shortage in oxygen supply and may have caused an incomplete combustion process.

The second highest concentration ((5.8 ± 0.7) pg TCDD-eq./g) is found in sample 5. In this case, the fuel source was wood pallets. These pallets are mainly used for transport or storage purposes. The most commonly used pallets are the europallets, which need to obey to certain guidelines, the EPAL (European PALlet Association) norms, to get the EPAL quality mark. These norms specify the assembly method, as well as the size and the weight of the pallet. It does not, however, mention anything about the types of wood that can be used to make these pallets. To be able to explain this higher dioxin concentration, more information is needed on the type of wood that was used in these pallets.

The measured dioxin activity data are similar to concentrations data found in literature. A study conducted in 2000 on the determination of the PCDD/F content in solid residue from wood combustion shows a concentration range from 0.6 to 8.6 pg TEQ/g, which is very similar to the in vitro activity range found in this study (0.2 to 10 pg TCDD-eq./g)[9]. Comparable results were found in other studies conducted on the concentration of PCDD/F in wood ash, with a dioxin concentration around 1 pg TEQ/g. In these latter studies there is also a noticeable difference between the dioxin concentration in bottom ash or fly ash, with the concentration in fly ash being around two orders of magnitude higher[10,11].

Unfortunately, no information can be found in literature on the influence that burning different tree species, different part of the trees or the burning, weather and ventilation conditions can have on the final dioxin concentrations in the wood ash.

To be able to get an idea if these wood ashes can be safely used as fertilizers, their dioxin concentrations were compared to the results of the FERTIDOX project (2011), in which a hundred different fertilizer samples, collected throughout Belgium, were analysed. The main goals of the FERTIDOX project were to analyse the dioxin content in fertilizers, and to estimate the impact that fertilization practices can have on human health.

It could be concluded from this campaign that fertilizers are of no importance in the final human exposure to dioxins, since fertilization practices generally represent less than 0.5% of the total human dioxin intake[12].Except for the high value found in sample 20, all data are in good agreement with the results found in the FERTIDIOX campaign.

No information about the PCB concentration in wood ash could be found in literature. Therefore, the concentration found in the wood ash samples was compared to the PCB content found in other fertilizers: 1.9-6.2 pg TEQ/g for sludge, 4.0 pg TEQ/g for compost and 4.8 pg TEQ/g for digestate[13,14]. The concentration range in the wood ash samples (0.23-0.79 pg TCDD-eq./g) is almost 5 times lower than the PCB concentrations found in the other fertilizers.

It can thereby be concluded that preliminary results show that these wood ashes can be safely used as fertilizers, but caution needs to be taken since there can be a lot of variation in the dioxin concentration depending on the type of wood that was burned as well as the burning conditions, and more research is needed to get an exclusive result.

All 22 samples were also analyzed as crude samples to assess activity of AhR agonists that are removed during sulfuric acid and carbon clean-up but are not metabolized after 48 hours of incubation. For these crude fractions, five samples still weren't diluted enough, even at a dilution factor of 15000. To get a quantitative determination of the concentration of these samples, the measurements need to be repeated at a higher dilution factor. An overview of the results of the crude wood ash samples is shown in graph 2.When comparing these graphs, there is a general agreement between the high BEQ of the total extracts and a high dioxin fraction (mainly samples 5,18,19,20 and 21). There is, however, a big difference between the results (factor 10 to 500). This could be explained by the presence of acid labile compounds in the total extract, such as PAHs, that are eliminated in the fractionated extracts during the clean up procedure. Due to the long incubation period of 48 hours before measuring, a part of these labile compounds are broken down, but as can be seen from the difference in results between the dioxin and total extracts, a big part of these compounds are still present and require further attention.

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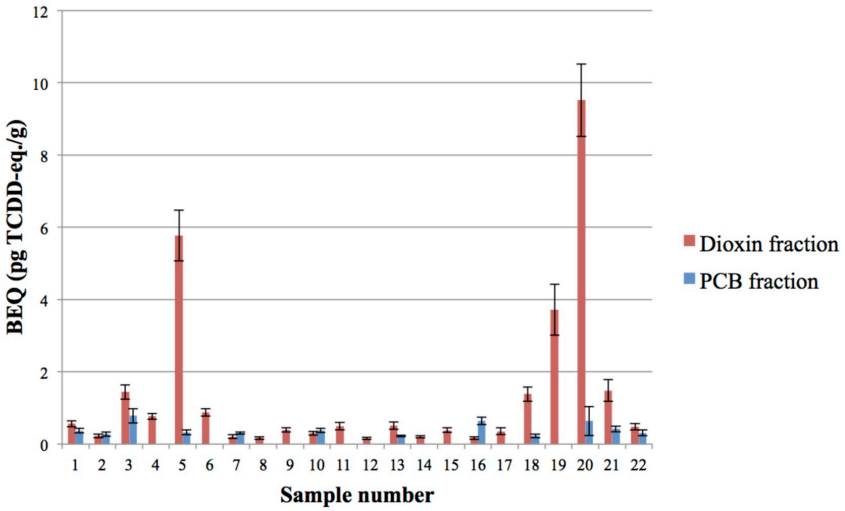
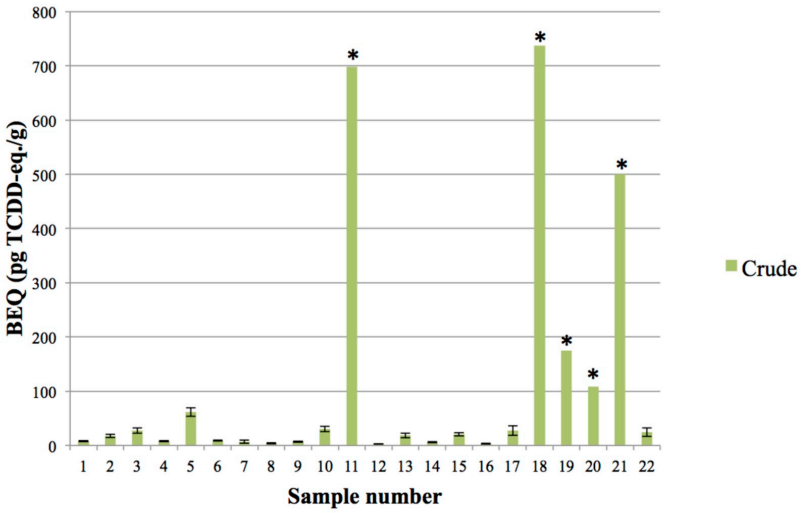


Graph 1: Bio-analytical equivalent concentration (BEQ) in pg TCDD-eq./g for PCB (blue) and dioxin (red) fractions of the wood ash samples.



Graph 2: Bio-analytical equivalent concentration (BEQ) in pg TCDD-eq./g of the crude wood ash samples. Samples with * need to be further diluted.