Characterization Of Slaughterhouse By-Products Bottom Ash

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Abstract The objective of this study was to characterize slaughterhouse by-products bottom ash (SHASH), as well as evaluate its potential toxic effect during its use in agriculture. The investigated sample was characterized by a low volatile solids content and a high mineral content, with an evident presence of Hydroxylapatite ($Ca_5(PO_4)_3(OH)$), Whitlockite ($Ca_9(MgFe)(PO_4)_6PO_3OH$) and Quartz (SiO_2). On the other hand, metal concentrations were found not particularly elevated. Highly toxic effects on three types of plants were verified when mixing SHASH with soil at amendment rates above 2.5%.

Keywords: characterization, slaughterhouse, animal by-products, ash

1. Introduction

Animal by-products management in the European Union is conducted according to European Regulation (EC) No 1069/2009 (EU, 2009), which includes health rules regarding animal by-products and derived products not intended for human consumption and aims at protecting public health and animal health, while also protecting the environment. According to this Regulation, the correct management of processing waste provides for the collection and separation of materials into different bins depending on their risk factor. The same document includes the definition of the three categories of materials, as well as their potential disposal and/or use. As far as slaughterhouse by-products are concerned, Category 1 includes entire bodies and all body parts, including hides and skins of animals suspected of being infected by a transmissible spongiform encephalopathy (TSE) or in which the presence of a TSE has been officially confirmed, as well as mixtures of Category 1 material with either Category 2 material or Category 3 material or both. Category 2 includes manure, non-mineralised guano and digestive tract content, animal by-products containing residues of authorised substances or contaminants exceeding the permitted levels as referred to in Article 15(3) of Directive 96/23/EC, products of animal origin which have been declared unfit for human consumption due to the presence of foreign bodies in those products and mixtures of Category 2 material with Category 3 material. Finally, Category 3 includes carcasses and parts of animals slaughtered and which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons, as well as carcasses and parts originating from animals that have been slaughtered in a slaughterhouse and were considered fit for slaughter for human consumption following an ante-mortem inspection or bodies.

Management options for these types of materials, such as slaughterhouse by-products, include incineration and co-incineration, composting, rendering, anaerobic digestion and alkaline hydrolysis (Gwyther *et al*., 2011). Among these, incineration and co-incineration often are of the most widespread. Through these methods, by-products of Categories 1, 2 and 3 are thermally treated, at a temperature of 850 °C, at minimum, according to the Regulation 1774/2002 (EU, 2002). These processes result in the generation of ash materials, i.e. fly ash and bottom ash, which have been reported to represent approximately 1-5% of the initial animal remains (Gwyther *et al*., 2011). Appropriate management of ash materials has been intensively studied over the years, with some of the investigated methods focusing on their valorization. In fact, several studies conducted on ash materials of different origin (e.g. municipal solid waste, animal by-products, medical waste, etc.) report their use for the recovery of useful elements for industrial applications, e.g. as a phosphorous source (Coutand *et al*., 2008; Deydier *et al*., 2005), and for the production of construction materials as additives in cement blends or in geopolymer production (Anastasiadou *et al*., 2012; Garcia-Lodeiro *et al*., 2016; Tzanakos *et al*., 2014).

The present study focused on the characterization of bottom ash, obtained though the incineration of slaughterhouse by-products. The purpose of this procedure was to propose eventual utilization alternatives for this type of material. More specifically, the ash sample was characterized regarding its proximate and ultimate properties, as well as particle size, mineralogical composition, oxides, and metal contents. Moreover, phytotoxicity tests were conducted, in order to verify the eventual development of harmful effects on plants during the use of such a sample as a soil amendment.

2. Materials and Methods

2.1. Slaughterhouse by-products bottom ash

The slaughterhouse by-products bottom ash (SHASH) sample was obtained from the municipal slaughterhouse situated in Chania, Crete (Greece), in which mainly sheep and goats and in lesser amounts pigs and cattle are slaughtered. More specifically, SHASH originated from the incineration unit of this facility.

2.2. Characterization analyses

The SHASH sample was characterized regarding several basic characteristics. Total Solids (TS) and Volatile Solids (VS) contents were determined according to APHA (American Public Health Association) method 2540G. Elemental composition (C, H, N, S %) was determined using an EA300 Euro Vector elemental analyzer, via flash combustion at 1020 °C. The oxygen content was determined by difference, by taking into consideration the VS content of the sample. Particle size analysis was carried out by sieving an appropriate quantity of material, using sieves of pore size: 4 mm, 2 mm, 1 mm, 500 μm, 250 μm, 125 μm, 100 μm, 75 μm and 63 μm. To this regard, in order to obtain a representative sample, the material was subjected to a quartering procedure. Mineralogical analysis was performed by X-Ray Diffractometry (XRD) (Bruker), while major oxide analysis was performed by Energy Dispersive X-Ray Fluorescence (ED XRF) (Bruker) on a sample that had been ignited at 1050 ºC for Loss On Ignition (LOI) percentage determination. Total metal concentrations were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent), after conducting microwave-assisted acid digestion of the sample with HNO_3.

2.3. Phytotoxicity tests

In order to evaluate the potential toxicity of SHASH, as well as its possible suitability for soil use, phytotoxicity tests were conducted for five different amendment rates, i.e. 1, 2.5, 10, 20 and 50%. Moreover, control (0%) and reference (reference soil) tests were also conducted. The soil sample used in these assays was obtained from an agricultural area in Chania, Crete. Seeds from three plant species were used for all tests, namely the monocotyl Sorgho (*Sorghum saccharatum*), the dicotyl garden cress (*Lepidium sativum*) and the dicotyl mustard (*Sinapis alba*), resulting in three tests for each soil sample, i.e. reference sample, control sample and five amended samples.

Phytotoxicity tests were carried out by adopting the standard operational procedure of the Phytotoxkit Microbiotest (MicroBioTests Inc.), using flat and shallow transparent test plates composed of two compartments. Initially, a predetermined volume (90 mL) of soil samples was placed in the lower compartment of each plate and then, it was flattened with the aid of a flat-bottom pestle. Subsequently, the soil samples were hydrated to saturation with an appropriate amount of water, the latter corresponding to the water holding capacity of each sample. Afterwards, ten seeds of the above mentioned plants were positioned at equal distance, below the middle ridge of the test plates on filter paper, which had been placed on top of the hydrated soil samples. Finally, the plates were closed with transparent covers and vertically incubated at 25 °C for three days. Once the incubation was terminated, the plates were digitally scanned, in order to be able to determine the number of germinated seeds and the length of their roots, using the Image Tool software.

The data obtained through phytotoxicity tests were used to calculate the Germination Index (GI) (Equation 1) and the Vegetation Inhibition Factor (VIF) (Equation 2).

$$GI\ (\%) = \frac{Y}{X_0} \cdot \frac{D}{C} \cdot 100 \qquad (1)$$

$$VIF\ (\%) = \frac{A-B}{A} \cdot 100 \qquad (2)$$

where, X_0 and Y, are the average numbers of seeds germinated in the reference and test samples, respectively, C and D, are the average lengths of the roots of all seeds tested in the reference and test samples, respectively, and A and B, are the average lengths of the seeds' roots germinated in the reference and test samples, respectively.

Table 1. Physicochemical characteristics of SHASH

Solids composition [%] (w.b.)	
TS	100
VS	5.81
Elemental composition [%] (d.b.)	
C	1.92
H	0.01
N	0.34
S	< DL
O	3.55
Particle size distribution [%]	
> 4 mm	57.3
> 2 mm	18.5
> 1 mm	10.5
> 500 μm	7.1
> 250 μm	2.3
> 125 μm	1.9
> 100 μm	0.8
> 75 μm	0.8
> 63 μm	0.8

w.b.: wet basis, d.b.: dry basis, DL: Detection Limit

3. Results and Discussion

3.1. Solids, Elemental and Particle size analyses

The results obtained through solids, elemental and sieving analyses are presented in Table 1. As it can be seen from the data, SHASH was found not to contain any moisture, while its VS content was minimal. The limited organic content of SHASH was also confirmed by its low carbon content. Sieving analysis showed that most of the material (57.3%) had a particle size greater than 4 mm. This was attributed to the abundant presence of bigger bone fractions in the sample. On the other hand, a 29% of SHASH had a particle size between 1 and 4 mm, while its remaining portion (13.7%) consisted of particles smaller than 1 mm.

3.2. Mineralogical analysis

Mineralogical analysis was performed on dried (105 ºC), as well as on ignited (1050 ºC) bottom ash samples. Figure

1 depicts the X-ray diffraction patterns for these two SHASH samples.

In both patterns, three main minerals can be distinguished, namely Hydroxylapatite ($Ca_5(PO_4)_3(OH)$), Whitlockite ($Ca_9(MgFe)(PO_4)_6PO_3OH$) and Quartz (SiO_2). Hydroxylapatite is a calcium phosphate mineral and it is the main component of bones (Sobczak *et al.*, 2009). Whitlockite is another form of calcium phosphate, which can be found in the oral cavity of several vertebrates, due to its presence in their teeth (de Dios Teruel *et al.*, 2015). Finally, quartz is a silica mineral characterized by a great abundance in nature. Its presence in the investigated animal by-products bottom ash sample could be attributed to alimentation, since it is possible that soil particles may have been swallowed by the animals during grazing (Deydier *et al.*, 2015). The above mentioned mineralogical composition confirms the animal origin of the ash sample, while it also reveals its abundance in calcium and phosphorus.

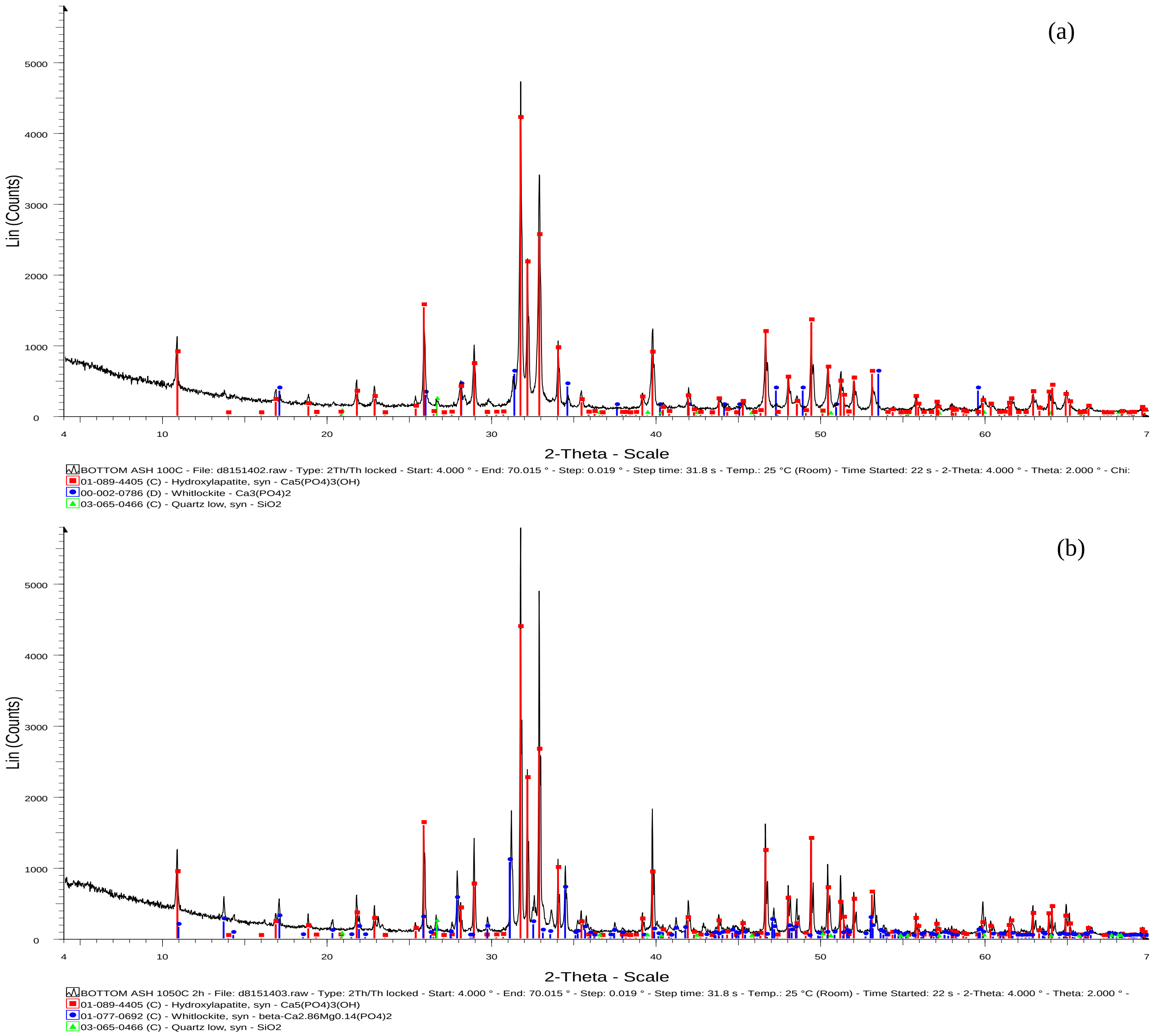

Figure 1. X-ray diffraction pattern for dried (a) and ignited (b) SHASH sample

3.3. Major oxides analysis

The contents of SHASH in its major oxides can be seen in Table 2 and were determined considering the LOI percentage (6.96%) after ignition at 1050 ºC.

CaO was found to be the most abundant of all SHASH oxide components, reaching a 63% content, with P_2O_5 following with almost 27%. K_2O and SiO_2 were found in lower contents, while Fe_2O_3, ZnO, CuO and SrO were present only in traces. These results are in agreement with similar studies regarding animal by-products bottom ash, such as the one conducted by Bahrololoom *et al.* (2009), while they differ compared with those reported for other types of bottom ash, such as medical waste ash (Anastasiadou *et al.*, 2012).

3.4. Metals analysis

The data regarding total metal concentrations of SHASH, obtained through acid digestion, are presented in Table 3.

Table 2. Major oxides of SHASH

Major oxides [%]	
CaO	63.0
P_2O_5	26.7
K_2O	1.05
SiO_2	1.04
Br	0.33
Fe_2O_3	0.24
SO_3	0.21
ZnO	0.14
Cs_2O	0.09
CuO	0.07
TeO_2	0.06
SrO	0.05
LOI	6.96

Results confirmed a high content in potassium (K) and calcium (Ca), corroborating the data obtained through the other characterization analyses. Moreover, the estimated concentrations for Cd, Cu, Hg, Ni, Pb and Zn are all in accordance with the respective limit values (i.e. 20-40, 1000-1750, 16-25, 300-400, 750-1200 and 2500-4000 mg/kg, respectively) established by the Council Directive 86/278/EEC (EU, 1986), which refers to sludge that is used in agriculture.

3.5. Phytotoxicity tests

The results obtained from phytotoxicity test can be seen in Table 4.

Germination index (GI) data revealed that in comparison with the reference soil, the soil sample used in the assays was less suitable for the development of the investigated seeds, since the GI values of the latter are lower. This numerical difference is attributed to the fact that although the same number of seeds germinated, the lengths of their roots for the control soil were lower, indicating the eventual lack of necessary nutrients and minerals. The value of the GI is used to determine the degree of phytotoxicity of a soil sample. Specifically, a sample is characterized as phytotoxic if GI > 66%. Moreover, if different concentrations of a substance that is added to the soil are investigated, the combination of the respective GI values gives a more accurate estimation regarding its phytotoxic effects. Considering these information, as well as the lower suitability of the control soil to the tested seeds, the data of Table 4 suggest that SHASH has a moderately phytotoxic effect on them. This conclusion is attributed to the fact that for each seed, GI values are found above 66% for only one of the amendment rates. Moreover, in the cases of *Lepidium sativum* and *Sinapis alba*, SHASH could also be characterized as plant stimulating at amendment rates lower than 2.5%, since the corresponding GI are higher than the respective values obtained for control assays. As far as the vegetation inhibition factor (VIF) results are concerned, these data mirror the results of the GI calculations, since vegetation inhibition appears to be more intense at higher amendment rates. More specifically, a 50% amendment rate resulted in the most elevated VIF values (> 95%) for all types of seeds, while the 20% amendment rate also exerted a relatively high negative effect on root growth, since the respective VIF values were found above 50%. A similar behavior is noticed for the 10% amendment rate, as far as *Sorghum saccharatum* and *Sinapis alba* are concerned, while on the other hand, for *Lepidium sativum*, a VIF lower than 50% is noticed, manifesting a relatively less negative effect.

Table 3. Total metals concentrations of SHASH

Metals concentrations [mg/g_{SHASH}]															
K	**Ca**	**Mg**	**Na**	**Al**	**Fe**	**Zn**	**Ba**	**Mn**	**Cu**	**Cr**	**Ni**	**Hg**	**Pb**	**As**	**Cd**
29.82	15.45	1.54	0.48	0.12	0.09	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	<DL	<DL

DL: Detection Limit

Table 4. Phytotoxicity tests results

Germination Index (GI) [%]						
	Control (0%)	**1%**	**2.5%**	**10%**	**20%**	**50%**
Sorghum saccharatum	74.9	70.2	48.0	27.5	23.6	4.3
Lepidium sativum	67.4	53.2	68.5	64.4	45.4	0.5
Sinapis alba	54.9	74.1	58.4	42.3	15.8	0.9
Vegetation Inhibition Factor (VIF) [%]						
	Control (0%)	**1%**	**2.5%**	**10%**	**20%**	**50%**
Sorghum saccharatum	25.10	29.85	52.02	72.52	76.43	95.68
Lepidium sativum	32.61	40.87	31.52	35.65	54.57	98.26
Sinapis alba	45.12	25.88	41.58	57.71	84.16	97.88

4. Conclusions

This study focused on characterizing slaughterhouse by-products bottom ash (SHASH) regarding its main properties, as well as on evaluating its potential toxicity towards three types of plants. The obtained results revealed a significant presence of Ca- and P-containing minerals and oxides in the investigated sample. These characteristics suggest that material recovery, e.g. phosphorous, as well as reuse of SHASH as an additive in construction materials (e.g. for cement blends and geopolymers manufacturing), would appear as management options that are worth investigating. On the other hand, the results of the phytotoxicity assays suggested that the use of this material as a soil amendment would not be recommended at rates above 2.5%. Nevertheless, total metal content determination indicated that such toxic effects were probably not metal-related.

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