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Black carbon assessment using benzene polycarboxylic acids: Limitations for organic-rich matrices

Arne Kappenberg *, Melanie Bläsing, Eva Lehndorff, Wulf Amelung

Institute of Crop Science and Resource Conservation – Soil Science and Soil Ecology, University of Bonn, Nussallee 13, 53115 Bonn, Germany

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ABSTRACT

For the assessment of black carbon (BC), its oxidation to benzene polycarboxylic acids (BPCAs) is an established method. However, doubts about biological precursors remain and not all published data were obtained at low carbon concentration. We hypothesised that a considerable proportion of BC may be produced during sample treatment in the presence of a high amount of organic carbon (OC). We therefore tested whether and to which degree (i) BC-free material from stems of *Zea mays* L. (maize straw) and leaves of *Capsicum annuum* L. (bell pepper), as well as (ii) cyclic and non-cyclic carbon forms (chlorophyllin, ellagic acid and β-carotene) afford BPCAs when method protocols are overloaded with a sample above the recommended amount of 5 mg OC. The results showed that small amounts (< 2 g/kg OC) of BPCAs with three and four carboxyl groups may be formed even at low sample weight (< 5 mg OC), thereby falsely representing biological BC production. When this threshold was exceeded, all BPCA forms were detected. The artificial BPCA production yield in g OC increased with increasing amount of OC ($R^2 \geqslant 0.81$), adding up to 8.7 g/kg OC (19.7 g BC/kg OC) artificial production. We therefore strongly recommend that a threshold of 5 mg OC sample concentration be maintained in future studies and that future BC assessments be restricted to BPCAs with five and six carboxyl groups. This constrains the application of the BPCA method for organic rich samples and for samples expected to contain a relatively low amount of BC.

1. Introduction

Black carbon (BC) is formed by incomplete combustion of biomass and fossil fuel and can be found ubiquitously in soils, sediments and water, and as an aerosol in the atmosphere (e.g. Goldberg, 1985; Crutzen and Andreae, 1990; Jaffé et al., 2013). It is persistent in the environment (Goldberg, 1985; Glaser et al., 2000; Brodowski et al., 2007) and may thus contribute to a global carbon sink (Czimczik and Masiello, 2007; Rodionov et al., 2010; Santín et al., 2015). Different methods have been suggested for its detection in soil, all having limitations (Hammes et al., 2007; Roth et al., 2012). Among them, converting BC to benzene polycarboxylic acids (BPCAs) as a proxy for pyrogenic organic carbon (OC) input and as specific markers for combustion processes has gained increasing attraction because it is the only routine method that provides information on BC composition, by indicating the degree of aromatic condensation (Glaser et al., 1998; Brodowski et al., 2005) and hence the temperature of BC formation in the environment (Schneider et al., 2010, 2013; Wolf et al., 2013). Total BPCA content has been suggested as a proxy for BC content, employing a minimum conversion factor of 2.27 for the loss of C during oxidation (Glaser et al., 1998). This factor assumes that all BPCAs detected stem from BC.

Intriguingly, BPCA yield correlated repeatedly and linearly with total organic matter (OM) content of soils and sediments (e.g. Gustafsson and Gschwend, 1998; Glaser and Amelung, 2003; Cornelissen et al., 2005). One explanation for co-occurrence of BC and soil OM (SOM) is that the production of BC is highest at sites with a high amount of biomass production (Glaser and Amelung, 2003; Czimczik and Masiello, 2007; Lehndorff et al., 2015). Another explanation could be either a method artefact (minimised from alteration of the method by Brodowski et al., 2005) or production of BPCAs from biological substances. The latter theory was substantiated by Glaser and Knorr (2008), who found that up to 25% of the isolated BC fraction from soils contained a ^{13}C label added as non-pyrogenic C during lab and field studies. There are, however, no studies that could validate a biological BC-forming mechanism.

In theory, *Aspergillus niger*, for instance, is able to produce mellitic acid (B6CA) biologically (Brodowski et al., 2005). Some of the B6CA could occur in a free form. Such a free form exists in soil

* Corresponding author. Tel.: +49 228 732194; fax: +49 228 732782.
E-mail address: arne.kappenberg@uni-bonn.de (A. Kappenberg).

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(Möller et al., 2000; Haumaier, 2010), but should be removed during the sample preparation steps in the routine methodology (Brodowski et al., 2005). Besides, the total quantity of *A. niger* is likely not sufficient to explain the occurrence of B6CA (Brodowski et al., 2005). Moreover, we are not aware of any study that found other microbial strains able to condense OM to BC-like macromolecules. We therefore felt that neither the reason for some close correlations between BC and OC, nor for the high ^{13}C labelling extent in BPCAs in the work of Glaser and Knorr (2008) was resolved satisfactorily. We concluded that potential biological precursors and the original method should be revisited a third time to better elucidate the risk of non-pyrogenic BPCA formation.

Biological substances close to aromatic carbon structures are cyclic carbon forms, e.g. natural pigments like chlorophyll and carotene. Chlorophylls occur in plants, algae and cyanobacteria and may therefore contribute significantly to organic-rich soils and sediments. Large amounts of chlorophyll have been detected particularly in lake sediments (Möller and Scharf, 1986), paralleling elevated content of BC (Lehndorff et al., 2015). Nevertheless, Ziolkowski et al. (2011) found that aromatic carbon forms with fewer than three benzene rings did not afford BPCAs, making it unlikely that cyclic carbon forms produced in nature (e.g. carotene, chlorophyll derivatives) were a source for the production of BPCAs. Also, Brodowski et al. (2005) tested different plants and biological sources, such as the pigment aspergillin, for production of BPCAs. They found only a marginal amount of BPCAs (< 1% OC), but recommended that samples expected to contain > 200 g BC/kg OC and/or 100 g/kg OC, should be oxidised using a maximum of only 5 mg OC. This limit could be easily exceeded when samples contain an inherently high amount of OC, e.g. organic-rich lake sediments or peat and vegetation. Besides, it remained uncertain how degradation products of, e.g. carotene- and chlorophyll-related structures would behave under conditions of the exceeded concentration limit.

The present study aimed at (i) identifying whether and which BPCAs could be produced artificially from non-pyrogenic OM from samples that exceeded the 5 mg OC boundary for the BPCA method, as suggested by Brodowski et al. (2005) and (ii) re-elucidating BPCA formation from potential biological precursors under the high temperature and pressure conditions of the BPCA method. In addition, we aimed at (iii) evaluating the influence of such a high OC load on using the distribution of BPCAs as a proxy for BC combustion temperature.

2. Materials and methods

2.1. Sample description

First, to test possible BPCA production from biological material we used BC-free material from stems of *Zea mays* L. and from leaves of *Capsicum annuum* L. The *Z. mays* L. stems were peeled and the leaves from *C. annuum* L. were grown in a high purity greenhouse in order to exclude the risk of contamination with atmospheric BC. The fresh material was dried at 40 °C and milled. Second, we re-tested production of BPCAs from potential biogenic precursor material using the cyclic carbon form, ellagic acid (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione), the synthetic, cyclic compound chlorophyllin and the unsaturated long chain hydrocarbon β-carotene. We chose these substances due to their structure and their abundance in nature, i.e. chlorophyllin, a tetrapyrrole, was tested due to the high abundance of chlorophylls in organic rich sediments (e.g. lake sediments). Chlorophyllin is a synthetic product but it is structurally comparable to natural chlorophyll forms. β-Carotene, a plant pigment, is a tetraterpene with two ionone rings connected by a chain of nine double bonded carbons. Ellagic acid, a polyphenol with two aromatic rings, occurs in plants. All these compounds have structures, which are not obvious direct precursors for BPCA formation, unlike high molecular weight polycyclic aromatic carbon compounds, for instance (Glaser et al., 1998). However, all these compounds might well be degraded in HNO_3 to potential precursor materials for aromatic re-condensation (e.g. Smith and Baran, 2015), which could then be the source of BPCAs.

2.2. BPCA method

All samples were processed as outlined by Glaser et al. (1998) and Brodowski et al. (2005), at an OC weight < 5 mg, as recommended by Brodowski et al. (2005), as well as at elevated amount. Briefly, to remove polyvalent cations, samples were treated with 10 ml 4 M CF_3CO_2H. The residue was collected on glass fibre filters (GF 6, Hahnemühle FineArt GmbH, Dassel, Germany), and oxidised to BPCAs with hot HNO_3 (8 h, 170 °C) within a high pressure digestion apparatus. After cleanup via a cation exchange column (Dowex 50WX8, 200–400 mesh, Fluka, Steinheim, Germany), the BPCAs were silylated and separated and quantified using gas chromatography (GC; Hewlett Packard 6890; Hewlett Packard GmbH, Waldbronn, Germany) equipped with a HP-5 column (30 m × 0.32 mm i.d. 0.25 μm film thickness; Macherey-Nagel, Düren, Germany) and flame ionisation detection (FID). Injection was with a split ratio of 1:50 and 1:10 respectively when OC < 31 mg and < 5 mg OC were oxidised (Table 1 and Fig. 1). The detection limit for BPCAs was 7 ng injection amount (Brodowski et al., 2005). Precision from duplicate analysis was < 5% and recovery of the internal standard (citric acid) was always > 75%.

3. Results and discussion

To identify whether and which BPCAs could be produced artificially from non-pyrogenic OM from samples that exceeded the 5 mg OC boundary for the BPCA method of Brodowski et al. (2005), we used plant material as a precursor, subjected the BC-free stems of *Z. mays* L. and leaves of *C. annuum* L. to OC and BPCA analysis. OC concentration was 430 g/kg in *Z. mays* L. and 385 g/kg in *C. annuum* L., respectively (dry wt). To explore potential condensation reactions we extended the range of weight to 0.6–215 mg OC for the stems of *Z. mays* L. and to 0.4–192 mg OC for the leaves of *C. annuum* L. (Fig. 1). For the *C. annuum* L. leaves, BPCA detection started at sample weight exceeding 5 mg OC. For the stems of *Z. mays* L., however, we detected B3CA and B4CA even at sample weight < 5 mg OC. We therefore concluded that, at excess OC amount in the reaction tubes, B3CA and B4CA may be artificially formed, i.e. they should not be considered in BC evaluation.

We did not detected B5CA and B6CA at a sample weight < 5 mg OC. As long as the use of BPCA analysis as a proxy for combustion temperature relies solely on these BPCAs (ratio B5CA to B6CA; Wolf et al., 2013), the reconstruction of burning conditions from the BPCA pattern should not be affected.

The total yield of BPCAs relative to OC increased with sample weight and hence with OC weight (R^2 0.81, $p < 0.001$ for *Z. mays* L. and R^2 0.91, $p < 0.001$ for *C. annuum* L., respectively). The amount of BPCAs increased systematically when the concentration of OC exceeded 10 mg OC in 2 ml HNO_3. The maximum amount detected was ca. 8.7 mg BPCAs/g OC (Fig. 1). Using a conversion factor of 2.27, this accounted for up to 19.7 mg BC/g OC, i.e. a BC contribution of 2% to OC. Soil usually contains 10–30% aromatic carbon in the SOM (Kögel-Knabner and Amelung, 2014), i.e. in most cases artificial BPCA formation would remain insignificant even if the recommended threshold value for BC oxidation of 5 mg OC (Brodowski et al., 2005) were exceeded. Yet, in some fire-protected