Cation Exchange Capacity of biochar: an urgent method modification

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Abstract

Biochar, produced through pyrolysis of organic matter, is negatively charged, thus contributing to electrostatic adsorption of cations. However, due to its porous structure and contents of alkaline ashes, the determination of the cation exchange capacity (CEC) is challenging. Literature values for the CEC of biochar are surprisingly variable and are often poorly reproducible, suggesting methodological problems. Here, we modify and critically assess different steps in the existing ammonium acetate (NH4OAc) method (pH 7), where ammonium (NH4+) is displaced by potassium chloride (KCl), following removal of excess NH4OAc with isopropanol, in batch mode. We used pigeon pea biochar to develop the method and conducted a test on three additional biochars with different acid neutralizing capacity. A pretreatment step of biochar was introduced, using diluted hydrochloric acid, to decrease biochar pH to near neutral, so that 1 M NH4OAc effectively buffers the biochar suspension pH at 7. This allows the CEC of all biochars to be determined at pH 7, which is crucial for biochar comparison. The dissolution of ashes may cause relatively large weight losses (e.g. for cacao shell biochar), which need to be accounted for when computing the CEC of raw biochar. The sum of NH4OAC-extractable base cations provided a smaller and better estimate of the CEC than KCl-extractable NH4+. We hypothesize that the overestimation of the CEC based on KCl-extractable NH4+ is due to the ineffectiveness of the relatively large isopropanol molecules to remove excess NH4OAc in biochars rich in micro-pores, due to size exclusion. The amount of base cations removed in the pretreatment was about three (rice husk biochar) to ten times (pigeon pea biochar) greater than the amount of exchangeable cations. The CEC values of biochar increased from 10.8 cmol/Kg carbon to 119.6 cmol/Kg carbon. These values are smaller than reported CEC values of soil organic carbon.
1. Introduction

Biochar is a carbon-rich product made by pyrolysis of organic waste, which may be used as a soil enhancer. Particularly, in tropical soils biochar has been shown to have a positive impact on soil fertility, including increased potassium (K\(^+\)) content, pH, water retention capacity, and cation exchange capacity (Jeffery et al., 2011; Liang et al., 2006; Martinsen et al., 2015), which all contribute to increased crop yield (Jeffery et al., 2017). Moreover, biochar is emerging as an alternative for heavy metal remediation in soil and water (Ahmad et al., 2014). For the assessment of the effect of biochar on soil properties and its potential as remediation agent, it is important to have reliable and accurate determination of its contribution to the soil’s cation exchange capacity (CEC). However, in the literature CEC estimates for biochar are highly variable, commonly ranging from 5 to 50 cmol(+) Kg\(^{-1}\) (Agegnehu et al., 2016; Berek and Hue, 2016; Budai et al., 2014; Gamage et al., 2016; Nelissen et al., 2015; Singh et al., 2010; Song and Guo, 2012) and even reaching values as high as 69 to 204 cmol(+) Kg\(^{-1}\) (Lou et al., 2016; Mukherjee et al., 2011; Pandit et al., 2018; Yuan et al., 2011). The large variability in CEC may be due to the number of factors affecting the surface properties of biochar, such as charring temperature and feedstock (Budai et al., 2014; Suliman et al., 2016). However, it also may be attributed to errors in the analytical method, which have not yet been properly addressed. Previous research (Graber et al., 2017) summarized the potential sources of error in CEC determination of biochar, which may relate to the presence of ashes, porosity and the intrinsic hydrophobicity of biochar:

1. Incomplete saturation of exchange sites, due to hydrophobicity of biochar, which may cause poor wetting of the sample and thus underestimation of the CEC.

2. Slow diffusion of replacing cations, due to the micro-porous structure of biochar, which can prolong the equilibration time, potentially causing underestimation of the CEC.
3. Floating and non-settling particles can increase substantially the mass losses during the decantation process may cause underestimation of the CEC.

4. Release of base cations coming from the dissolution of carbonates and silicates present in ashes can interfere with the sum of exchangeable base cations (overestimation of CEC).

5. Soluble carbonates in biochar can change the pH of the replacing solution, with increasing pH causing an increase in CEC.

Methodologies, where displacement after washing is applied are commonly used for CEC analysis in soil (Bache, 1976; Rhoades, 1982) and more recently in biochar (Grabber et al., 2017). Often, base cations extracted by NH$_4$OAc at pH 7 (Chapman, 1965; Hendershot et al., 2008; Schollenberger, 1945) are used to estimate sum of exchangeable base cations, which, in near-neutral soils, often is assumed to be equal to CEC. Alternatively, the CEC is based on displaced NH$_4^+$ (CEC-NH$_4^+$) by KCl in a subsequent step, after washing with an organic solvent, such as isopropanol or ethanol. Advantages of NH$_4$OAc include the avoidance of hydrolysis problems of non-neutral salts, the high wetting and penetration capacity (e.g. compared with barium chloride), and the inexpensive reagents (Schollenberger, 1945). Moreover, NH$_4$OAc (pH 7) allows comparison of different biochars at neutral pH, which is important, due to the pH dependency of CEC and the high variability in surface properties among different biochars (Mukherjee et al., 2011). However, biochar’s CEC may differ considerably between its natural pH and pH 7. In addition, the alkalinity of biochar is highly variable and for some chars it may be needed to adjust pH with acid before using NH$_4$OAc (pH 7). This may cause problems such as removal of some cations, electrostatically bound to weak acid functional groups at the biochar surface, due to protonation of these groups in response to acid addition, and thus, affecting CEC based on sum of base cations (CEC-BC).
Graber et al. (2017) modified a two-step procedure for determination of CEC of biochar using 1M NH₄OAc (pH 7), isopropanol and 2M KCl. In brief, biochar was shaken with 1 M NH₄OAc at pH 7 in batch mode and then transferred into a mechanical extractor, where more NH₄OAc was added, before washing with alcohol and subsequently with KCl. According to these authors the method has a number of aspects which need further development, including: 1) The biochar: solution (B:S) ratio required for the saturation with NH₄⁺ and displacement with K⁺. 2) The equilibration time of the saturation of exchange sites with NH₄⁺ and its subsequent displacement with K⁺. 3) Amount of extraction steps required to get full saturation and subsequent removal of NH₄⁺. In the present study we explore most of these aspects and suggest ways forward. The batch approach ensures contact between the biochar and the solution, which is important when assessing the reaction time with NH₄OAc, KCl and isopropanol and it avoids problems such as preferential flow in the column.

In our study we will critically assess (A) the B:S ratio required for the saturation with NH₄⁺ and displacement with K⁺, the equilibrium time with 1 M NH₄OAc and 2M KCl, the volume of isopropanol needed to wash the excess NH₄⁺, the optimal volume of 1M NH₄OAc to sufficiently displace cations on biochar surface and the volume of KCl to replace NH₄⁺ from the exchange sites, (B) to assess the relative contribution of readily soluble salts vs. exchangeable cations and (C) to compare CEC estimates based on displacement of NH₄⁺ by K⁺ and CEC based on sum of base cations.

2. Materials and Methods

2.1 Biochar samples
Four types of biochar were used in this study: pigeon pea biochar (made at 600 °C in an earth-mound kiln at Mkushi, Zambia), cacao shell biochar (made in a locally constructed kiln at Bogor, Indonesia at 350 °C (Hale et al., 2013)), corncob biochar (made in a traditional earth-mound kiln in Zambia at 400 °C during 7 days (Cornelissen et al., 2013)) and rice husk biochar (made at 400-500 °C in a drum retort kiln at Chisamba, Zambia at 350 °C (Obia et al., 2016)). The biochar production methods are discussed in the references. In addition, two certified soils materials (NCS DC85101a and NCSDC85113 (NCS, 2017)) were included in the batch experiment. Biochar samples of about 5 L were taken to the Norwegian University of Life Sciences (NMBU), where they were crushed and sieved to 0.5 mm to 2mm.

2.2 Biochar pretreatment

For some of the biochars, the buffered NH₄OAc (pH 7) solution may not adjust the pH to 7 (this is especially important for biochars with high alkalinity). Therefore, CEC estimates with and without pretreatment may diverge, since CEC is pH-dependent. To circumvent this problem, the biochar samples were washed with deionized water and the pH values adjusted to pH 7, using 0.05 M hydrochloric acid (HCl), thus removing readily soluble cations associated with salts, ashes and weak acid functional groups. First, one gram of biochar (1.0 ±0.005) and 20 ml of deionized water were added to 50 ml polypropylene tubes. The tubes were shaken at 200 rpm in a horizontal shaker overnight to ensure a proper wetting of the sample. Next, 0.05 M HCl was gradually added to the tubes until reaching pH 7.0 ± 0.3. Subsequently, the tubes were shaken during 24 additional hours. The amount of acid added was recorded and the amount of H⁺ to reach pH 7 was calculated to determine the acid neutralizing capacity (ANCₚH7). Next, the tubes were centrifuged at 1700g during 15 minutes, the supernatants were removed using a 10 ml pipette and the biochar slurry in the tubes was washed twice more with 20 ml deionized water and shaken for 24 hours each time.
The supernatant in the third washing should have EC values $< 0.2 \text{ mS cm}^{-1}$ to avoid significant amounts of base cations in the slurry prior to NH$_4$OAc addition, which may cause overestimation of exchangeable base cations in the NH$_4$OAc extracts. The total amount of water and dilute HCl used for each biochar were recorded and the supernatants were stored for analysis of base cations by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3 Release of exchangeable cations in NH$_4$OAc (pH 7)

To know the adequate amount of NH$_4$OAc needed to extract all exchangeable cations, the four types of biochar and two certified soil materials (1 g dry weight of each) were extracted four times consecutively with 20 ml 1M NH$_4$OAc each time. In the first extraction, the tubes were shaken horizontally during 24 hours at 200prm, then centrifuged at 1700 g during 15 minutes and the supernatants collected and stored until analysis. In the three subsequent extractions, the supernatants were collected after 2 hours shaking. The extracts were analyzed for Ca$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, Fe, Al and Mn by ICP-OES and the CEC calculated based on sum of exchangeable base cations.

2.4 Washing of excess NH$_4$OAc with isopropanol and subsequent NH$_4^+$ extraction with KCl

The adequate amount of isopropanol needed to remove excess NH$_4^+$ after shaking with NH$_4$OAc, was determined, using pigeon pea biochar. The test involved four washings steps with 20 ml 99% isopropanol in triplicate. The biochar was pre-treated as described above; subsequently, 20 ml of 1M NH$_4$OAc was added and the tubes were shaken at 200 rpm during 24 hours. After extraction with NH$_4$OAc, the biochar slurry was washed with 20 ml 99% isopropanol, once, twice, three and four times, respectively. In each case, 20 ml of 2 M KCl were added subsequently and the tubes were shaken during 24 hours at 200 rpm. The tubes were centrifuged at 1700 g, and the
supernatants were analyzed for NH$_4^+$ (for details see next paragraph). In addition, we determined
NH$_4^+$ in isopropanol extracts after three and four washings in the other biochar types and certified
soils.

2.4.1 Biochar to solution ratios and equilibration time for KCl extraction

A factorial 3x3x2 experiment was set up to assess the importance of diffusion of replacing cations
(NH$_4^+$ and K$^+$) in micro-pores over prolonged time by equilibrating pigeon pea biochar with 1M
NH$_4$OAc (pH7) for 1 and 7 days, respectively, and for 1, 3 and 7 days with 2M KCl. In addition,
the effect of three different biochar to solution (B:S) ratios (1:15, 1:30 and 1:45) on the final CEC
estimates, based on displaced NH$_4^+$ in the KCl extract was assessed. One gram of biochar and
15ml of NH$_4$OAc and KCl were used for the treatment 1:15, while 0.5 g and 15ml and 22.5ml
were used in the 1:30 and 1:45 treatments, respectively. Only one loading with 1M NH$_4$OAc and
one with 2M KCl was done. The pH adjustment and the washing with water was done in the same
way as described above. Every treatment was assessed in triplicates. All the CEC estimates were
based on displaced NH$_4^+$ by K$^+$.

2.4.2 Extractions of NH$_4^+$ by K$^+$

The amount of 2M KCl needed to displace NH$_4^+$ by K$^+$ was determined through three subsequent
extractions with 20 ml 2M KCl each time. One gram of the four types of biochar and the two
certified soils was weighed in 50 ml plastic tubes. For biochars, the pH and EC adjustment was
done as described above. The certified soils were not pretreated. Subsequently, the samples were
saturated with NH$_4$OAc, washed four times with 20ml of 99% isopropanol and then extracted three
consecutive times with 2M KCl in triplicates. The CEC was estimated based on displaced NH$_4^+$ in
the KCl extracts.
2.5 Chemical analysis

The NH₄OAc extracts were analyzed for base cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) by ICP-OES. Displaced NH₄⁺ was analyzed in 2M KCl extracts spectrophotometrically (see SM section). All results were corrected for dry matter content of biochar and background concentration in the sequential extractions. Moreover, the biochars were analyzed for total amount of Ca, Mg, K, Na and after decomposition with nitric acid (HNO₃) and hydrofluoric acid (HF).

2.6 Reproducibility

We evaluated the reproducibility of the CEC-NH₄⁺ estimates by using coefficients of variation (CV), i.e. the relative standard deviation, of those experiments in which we carried out 3 and 4 washings with isopropanol. For CEC-BC we used CV of experiment 2 (Table 1), in which four consecutive extractions with 1M NH₄OAc (pH 7) were done.

2.7 Mass loss

After the extraction with 2M KCl, the biochar slurries were washed 3 times with 35ml deionized water to remove the excess of salt, until EC ≈ 0.4 mS cm⁻¹ and dried during a week at 70 °C. The mass loss during all the procedure was determined and the CEC values referred to the initial raw biochar, corrected for dry matter content.

2.8 Statistical analysis

Statistical analysis of the experiment described in section 2.6 was done using the R software (R-Core-Team, 2017). The CEC estimates of the samples shaken for 1 and 7 days with NH₄OAc, 1, 3 and 7 days with 2M KCl and the three different B:S were subjected to one-way ANOVA.
Table 1. Overview of the consecutive experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Type of biochar</th>
<th>Objective</th>
<th>Description/Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>Biochar pretreatment</strong></td>
<td>Pigeon pea, rice husk, cacao shell and corncob</td>
<td>To Remove acid-soluble salts and adjust pH</td>
<td>pH adjustment to 7 and washing of samples until EC&lt; 0.2 mS.cm⁻¹</td>
</tr>
<tr>
<td>2. <strong>Release of exchangeable cations in NH₄OAc (pH7)</strong></td>
<td>Pigeon pea, rice husk, cacao shell and corncob</td>
<td>To know the amount of NH₄OAc needed to exchange the base cations</td>
<td>Four consecutive extractions with 20ml NH₄OAc in each extraction.</td>
</tr>
<tr>
<td>3. <strong>Washing of excess NH₄OAc with isopropanol and subsequent NH₄⁺ extraction with KCl</strong></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; part: Pigeon pea</td>
<td>To know the right amount of isopropanol needed to remove the excess NH₄⁺</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; part: 1, 2 and 3 washings with 20ml isopropanol each time. Analysis of NH₄⁺ in KCl extracts of pigeon pea biochar.</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; part: Pigeon pea, rice husk, cacao shell and corncob</td>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; part: analysis of NH₄⁺ in isopropanol extracts in the 3&lt;sup&gt;rd&lt;/sup&gt; and 4&lt;sup&gt;th&lt;/sup&gt; washing for all the biochars.</td>
</tr>
<tr>
<td>3.1 <strong>Biochar to solution ratios and equilibration time for KCl extraction</strong></td>
<td>Pigeon pea</td>
<td>To assess the diffusion of NH₄⁺ and K⁺ in micro-pores and the effect of three different B:S on the CEC estimates, based on NH₄⁺ in KCl extract</td>
<td>B:S : 1:15/1:30/1:45 NH₄OAc: 1 and 2 days KCl: 1, 3 and 7 days</td>
</tr>
<tr>
<td>3.2 <strong>Extractions of NH₄⁺ by K⁺</strong></td>
<td>Pigeon pea, rice husk, cacao shell and corncob</td>
<td>To know the amount of KCl needed to fully displace NH₄⁺</td>
<td>Three consecutive extractions with 20ml KCl in each extraction</td>
</tr>
</tbody>
</table>
3. Results and discussion

3.1 Pretreatment

The ANC_{pH7} was largest in cacao shell biochar followed by that of pigeon pea biochar despite the higher pH of the latter (Table 2). Rice husk and corncob biochar had a relatively small ANC_{pH7}, although also these biochars had pH > 8.5. In accordance with this, the cumulative amount of base cations (expressed in cmol(+/)+/Kg) removed in three consecutive washings and the initial EC decreased in the same order with cacao shell biochar > pigeon pea biochar > corncob biochar > rice husk biochar (Table 2). In the third washing of the pretreatment the removal of base cations declined to only 7.5 cmol(+/)+/Kg\(^{-1}\) (cacao shell biochar), 2 cmol(+/)+/Kg\(^{-1}\) (pigeon pea biochar), 1.3 cmol(+/)+/Kg\(^{-1}\) (corncob biochar), 1 cmol(+/)+/Kg\(^{-1}\) (rice husk biochar), thus contributing little to ANC_{pH7}. The cumulative amount of removed cations in the three washings exceeded ANC_{pH7} (Table 2) indicating that a considerable proportion of the extracted cations are due to dissolution of salts (47 to 71%, except pigeon pea biochar for which this was 14%).
Table 2. pH, electrical conductivity (EC), acid neutralizing capacity (ANC_{pH7}) and the accumulated sum of base cations (Ca, Mg, K, Na) removed during the three washings of the pretreatment of biochar with water and acid. The first washing was done with water and acid followed by two consecutive washings with 20ml deionized water each time. The values are referred to raw biochar.

<table>
<thead>
<tr>
<th></th>
<th>1pH</th>
<th>s.d</th>
<th>EC</th>
<th>s.d</th>
<th>ANC_{pH7}</th>
<th>1st cmol(+) Kg^{-1}</th>
<th>2nd cmol(+) Kg^{-1}</th>
<th>3rd cmol(+) Kg^{-1}</th>
<th>3Δ cmol(+) Kg^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon Pea</td>
<td>10.4</td>
<td>0.03</td>
<td>1.4</td>
<td>0.02</td>
<td>49</td>
<td>47.2 ± 0.3</td>
<td>7.4 ± 0.2</td>
<td>2.0 ± 0.07</td>
<td>7.6</td>
</tr>
<tr>
<td>Cacao Shell</td>
<td>9.6</td>
<td>0.03</td>
<td>8.9</td>
<td>0.18</td>
<td>134</td>
<td>219 ± 5.0</td>
<td>24.5 ± 0.4</td>
<td>7.5 ± 0.4</td>
<td>117</td>
</tr>
<tr>
<td>Corncob</td>
<td>8.5</td>
<td>0.04</td>
<td>0.8</td>
<td>0.01</td>
<td>7</td>
<td>15.7 ± 0.4</td>
<td>3.4 ± 0.2</td>
<td>1.3 ± 0.09</td>
<td>13.4</td>
</tr>
<tr>
<td>Rice husk</td>
<td>8.7</td>
<td>0.05</td>
<td>0.4</td>
<td>0.01</td>
<td>4</td>
<td>10.1 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>1.0 ± 0.05</td>
<td>9.8</td>
</tr>
</tbody>
</table>

1 Measured in 1:5 deionized water and raw biochar.
2 Measured in 1:20 deionized water using raw biochar.
3 Difference between accumulated sum of cations removed in the pretreatment and ANC_{pH7}.

3.2.1 Release of exchangeable cations in the NH_4OAc (pH 7) extract

On average, 83% of all exchangeable base cations of the biochar samples were extracted in the first extraction with 20 ml NH_4OAc (Table 3). Both certified soils had very similar patterns as pigeon pea and rice husk BC, with 88% of the base cations removed in the first extraction. After four extractions, the sum of exchangeable base cations decreased in the order cacao shell biochar > pigeon pea biochar > rice husk biochar > corncob biochar (Table 3). The average sum of exchangeable base cations of the certified soils was 20.7 and 26.9 cmol(+) Kg^{-1} for DC85101a and...
DC85113, respectively. The certified values of sum of exchangeable cations for these soils are
23.4±2.4 and 29±1.4 cmol(+)/Kg, respectively (NCS, 2017).

Table 3. Percentage of exchangeable base cations extracted in four consecutive extractions with
20ml 1M NH₄OAc (pH 7) for four types of biochars and two certified soils. CEC values are
referred to raw biochar.

<table>
<thead>
<tr>
<th>Biochar</th>
<th>CEC-BC cmol(+) Kg⁻¹</th>
<th>First s.d (%)</th>
<th>Second s.d (%)</th>
<th>Third s.d (%)</th>
<th>Fourth s.d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon Pea</td>
<td>6.6</td>
<td>1.6</td>
<td>77.5</td>
<td>0.3</td>
<td>13.6</td>
</tr>
<tr>
<td>Cacao Shell</td>
<td>59.1</td>
<td>4.2</td>
<td>75.6</td>
<td>2.5</td>
<td>12.2</td>
</tr>
<tr>
<td>Corncob</td>
<td>6.0</td>
<td>1.8</td>
<td>90.1</td>
<td>1.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Rice husk</td>
<td>6.2</td>
<td>0.03</td>
<td>87.7</td>
<td>1.4</td>
<td>7.8</td>
</tr>
<tr>
<td>DC85101a</td>
<td>20.7</td>
<td>0.5</td>
<td>88.5</td>
<td>0.4</td>
<td>7.6</td>
</tr>
<tr>
<td>DC85113</td>
<td>26.9</td>
<td>0.5</td>
<td>87.6</td>
<td>0.7</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Among the exchangeable base cations, Ca²⁺ is the most abundant in cacao shell and pigeon pea
biochars (63 and 53 % of the total respectively), followed by Mg²⁺ (20.8 and 30%) K⁺ (15.5 and
16.6%) and Na⁺ (0.15 and 0.14%) (Figure 1 b). In corncob and rice husk biochars, K⁺ was the most
abundant (48 and 63% respectively), followed by Ca²⁺ (41 and 27%) Mg²⁺ (10 and 9.6 %) and Na⁺
(0.2 and 0.9%). This difference in Ca²⁺ and K⁺ saturation is related to the type of feedstock, with
grass-type feedstocks being richer in K⁺ than the other biochars used in this study. In both soil
samples Ca²⁺ represented about 70% of the total exchangeable cations, whereas this was about
24%, 3% and 1% for Mg²⁺, K⁺, and Na⁺, respectively. As expected at high pH, exchangeable Fe,
Al and Mn in the biochars were small (Table S1).

3.2.2 Cations removed in the pretreatment vs exchangeable cations
The amount of cations removed during the pretreatment was higher than the amount of exchangeable cations for all the biochars (Table S2). When combining the exchangeable cations and the cations removed in the pretreatment, the exchangeable fraction accounts for less than 31% (Figure 1c.) with the highest values observed for rice husk biochar (31%), followed by corncob (22.7%), cacao shell (19%) and pigeon pea (10.4%) biochar. The proportion of individual base cations relative to total exchangeable cations differed from that observed in the pretreatment (Figure 1 a. and b.). In general, K⁺ is the most abundant base cation removed in the pretreatment (> 68%) for all the biochars (Figure 1a), while Ca²⁺ was more important in the exchangeable fraction, particularly for pigeon pea and cacao shell biochars as indicated above (Figure 1b). The proportion of Mg²⁺ to the total readily soluble cations was 3.9% for corncob, 7.9% for rice husk, 13.3% for cacao shell and 18.2% for pigeon pea biochar. In general, Mg²⁺ is more abundant in the exchangeable fraction of cations than in the readily soluble ones. The contribution of Na⁺ to the exchangeable fractions and the readily soluble cations was minor, being the highest for rice husk biochar with 1.6% of the exchangeable cations and 1.4% of the cations removed in the pretreatment.

By pretreating biochar, we washed away the readily soluble cations, associated with ashes and salts, which otherwise would have dissolved in NH₄OAc (pH 7). The amount of base cations removed in the third washing of the pretreatment (Table 2) was considerably lower than CEC-BC (Table 3). This was the reason for setting 0.2 mS cm⁻¹ as EC threshold in the pretreatment. Previous research (Martinsen et al., 2015) using a sub sample of the same cacao shell biochar showed that CEC-BC without any pretreatment was 197 cmol(+)Kg⁻¹, while in our experiment this was 59.1 cmol(+) Kg⁻¹ with pretreatment. Furthermore, Graber et al. (2017) found significant differences between CEC-BC and CEC- NH₄⁺ for some of the biochars they analyzed. Without pretreatment,
they found that for wheat straw biochar made at 550 °C and at 700 °C, the sum of base cations in NH$_4$OAc was 4 and 5 times greater than CEC-NH$_4^+$. Therefore, when the sum of exchangeable cations is used to estimate CEC, a pretreatment removing readily soluble salts is compulsory, to prevent the contribution of soluble components of ashes, especially for biochars with high EC.
Figure 1. a) Contribution of individual cations to the total amount readily soluble cations removed in the pretreatment, b) Contribution of individual cations to the total amount of exchangeable cations. c) Relative contribution of cations removed during the pretreatment and the exchangeable cations.

3.3 Washing of excess NH₄OAc with isopropanol and subsequent NH₄⁺ extraction with KCl

The 2M KCl extractable NH₄⁺ significantly decreased upon washing with isopropanol with a reduction of 66% from the first to the third washing (Figure S 1). The analysis of NH₄⁺ in isopropanol extracts after washing three and four times revealed small amounts of excess NH₄⁺ for all biochars and soils (Table S3).

3.3.1 Biochar to solution ratios and equilibration time for KCl extraction

There were no significant ($p = 0.64$, Table S4) differences in the CEC estimates of pigeon pea biochar based on KCl extractable NH₄⁺, if the biochar had been shaken with NH₄OAc for 1 or 7 days (27.3 (±2.3) cmol(+) Kg⁻¹ and 27.0 (±2.4) cmol(+) Kg⁻¹, respectively). This indicates that in there was no diffusion limitation to saturate biochar with NH₄⁺ despite the presence of micro-pores that could restrict the entrance of NH₄⁺ ions. In addition, there was no significant ($p = 0.88$, Table S4) difference in CEC estimates of pigeon pea biochar if shaken with 2M KCl, for 1, 3 or 7 days. This indicates that within one day there is full displacement of NH₄⁺. Moreover, there were no significant differences between the three B:S ratios evaluated ($p = 0.54$, Table S4). The average CEC values were 26.6 (±2.2) cmol(+) Kg⁻¹, 27.4 (±1.6) cmol(+) Kg⁻¹ and 27.4 (±3.0) cmol(+) Kg⁻¹ for 1:15, 1:30 and 1:45 treatments, respectively. In all cases, the B:S ratio was enough to fully saturate the exchange sites. To have ample solution volume for chemical analysis of the extracts, we used 20 ml of 1M NH₄OAc and 2M KCl.
3.3.2 Extractions of $NH_4^+$ by $K^+$

CEC-$NH_4^+$ decreased in the order cacao shell biochar > rice husk biochar > pigeon pea biochar > corncob biochar (Table 4). The first extraction with 20 ml 2M KCl following isopropanol washing removed 93% of the adsorbed $NH_4^+$ for all the biochars (Table 4) and 98.7% for the certified soils. The certified CEC values for soils are 21.6±1.6 and 31±1 cmol(+) Kg$^{-1}$; (NCS, 2017); note that the certified CEC values are different than sum of exchangeable base cations and our results summing the three consecutive extractions were 23.8 and 34 cmol(+) Kg$^{-1}$ for DC85101a and DC85113. However if we only consider the value from the first extraction with 2M KCl, the CEC values fit in the certified intervals. This indicates that at least one extraction with 20ml 2M KCl is enough to have a reasonable estimate of CEC-$NH_4^+$ values of biochar and soil samples.

Table 4. CEC based on displaced $NH_4^+$ (CEC-$NH_4^+$) and percentage of $NH_4^+$ extracted in 3 consecutive extractions with 2M KCl. CEC values are referred to raw biochar.
3.4 Mass loss

On average, the mass loss of the raw biochars during the batch procedure was 26%, 1.7%, 1.6% and 10% of the raw biochar for cacao shell biochar, corncob biochar, rice husk biochar and pigeon pea biochar, respectively. The pretreatment data indicate that a significant amount of Ca, Mg and K was lost as salts and also through dissolution of carbonates and silicates (included in $\text{ANC}_\text{pH7}$). Based on the removal of Ca, Mg, K and Na in the pretreatment we estimated a weight loss of 8.6%, 0.7%, 0.5 % and 1.8% for cacao shell, corncob, rice husk and pigeon pea biochars, respectively. Presumably the loss is at least twice as much, because the dissolution of cations is coupled with the dissolution of anions like chlorides, sulfates, carbonates and silicates. Based on this it is likely that nearly all mass loss is due to the removal of salts, carbonates and silicates in the pretreatment, whereas the loss of pure biochar was limited.

During the pretreatment, the supernatant of corncob biochar was the only with dark color (Figure S2), however, the mass loss of this biochar was very low. Probably the loss of pure biochar was small, as the extracts were carefully pipetted instead of decanted and because the ionic strength of the extracting solutions caused flocculation and settling of the brown colored colloids of the biochar suspensions. In addition, three additional washings with water were done after the extraction with 2M KCl in order to remove the excess of salt and to estimate the total mass loss. Thus, the total mass loss during the batch procedure is an overestimation.

3.5 Comparison of CEC-BC and CEC-NH$_4^+$ for biochar

For cacao shell biochar and the two certified soils, CEC-NH$_4^+$ to CEC-BC ratios were 1.0, 1.1 and 1.2 respectively, indicating reasonable correspondence between both methods (Figure 2). However, for the other biochars, CEC-NH$_4^+$ was 3.9, 4.1 and 3.2 times greater than CEC-BC
A likely explanation is that these biochars are rich in micro-pores (not analyzed) and isopropanol does not effectively remove excess NH$_4^+$ trapped in the micro-pores, presumably because of size exclusion of isopropanol molecules. Exclusion of organic molecules from small pores due to steric effects was reported earlier for wood charcoals (Zhu et al., 2005). Previous research (Pignatello et al., 2006) described a molecular sieving effect in which large natural organic compounds were more restricted to the external sites of environmental black carbon and thus, blocking the entrance of N$_2$ to the interior of the narrowest pores when analyzing surface properties. Moreover, previous research indicated that biomass-based activated carbon was dominated by pores in the size range of 3.5 – 15 Å (Amstaetter et al., 2012). Also torrefied maize and cotton stalks made at 290°C had a significant amount of pores between 4 and 5 Å (Chen et al., 2014). Considering that the molecular diameter of isopropanol is 5.2 Å (Van der Bruggen et al., 1999), it is likely that it cannot penetrate the smallest pores, thus reducing its effectiveness to remove excess NH$_4$OAc. Therefore, we recommend to wash excess NH$_4$OAc in biochar with solvents having smaller molecular size than isopropanol, e.g. ethanol or even water. To our knowledge there is no study of the effectiveness of different solvents to remove excess index cations in CEC methodologies for biochar. Due to the limitations of using isopropanol as a washing agent, we believe that CEC-BC after pretreatment of biochar provides a better estimate of its CEC than CEC-NH$_4^+$. 

The CEC-BC was 119.6 cmol(+) Kg C$^{-1}$, 11.6 cmol(+) Kg C$^{-1}$, 10.8 cmol(+) Kg C$^{-1}$, and 14.1 cmol(+) Kg C$^{-1}$ for cacao shell biochar, pigeon pea biochar, corncob biochar and rice husk biochar, respectively. These values are smaller compared to those reported for soil organic C, (221-330 cmol(+) Kg C$^{-1}$ (Parfitt et al., 2008); 370-500 cmol(+) Kg C$^{-1}$ (Gruba and Mulder, 2015);540-810 cmol(+) Kg C$^{-1}$ (Martinsen et al., 2017)) and closer to what other studies have published for biochar.
(75 cmol(+) Kg C\(^{-1}\), (Silber et al., 2010)). Total organic carbon of the biochars are given in Table S5.

Figure 2. CEC-NH\(_4^+\) (cmol(+) Kg\(^{-1}\)) as function of CEC-BC (cmol(+) Kg\(^{-1}\)). The line is the 1:1 line.

3.6 Reproducibility

The reproducibility of CEC-BC was good for the biochars of rice husk and cacao shell, and rice husk biochar, but less so for corncob and pigeon pea biochars (Table 5). Probably, the CV values for CEC-BC were relatively high, because they are a summation of four cations, each adding to the uncertainty of the sum. Although, representing an overestimation, due to ineffective washing of excess NH\(_4\)OAc, the reproducibility of CEC-NH\(_4^+\) was superior and if a more appropriate solvent is found it is to be preferred over CEC-BC. Even if CEC-NH\(_4^+\) estimates with 3 and 4 washings with isopropanol were bulked (Table 5), CV was lower than 10% for cacao shell, pigeon pea biochar and rice husk biochar, while this was slightly higher for corncob biochar (14.5%, Table
5). CV values of CEC-NH$_4^+$ may be decreased further for all the biochars if only the values from the experiment with four isopropanol washings are included (Table 4). The experiments with 3 and 4 washings with isopropanol were combined because there were only three replicates using 4 washings with isopropanol.

Table 5. CV of CEC-NH$_4^+$ combining all the experiments in which 3 and 4 washing with isopropanol were done and CV of CEC-BC of the experiment with four consecutive extractions with 1M NH$_4$OAc.

![Table 5](image)

4. Conclusions

The NH$_4$OAc (pH 7) method for CEC determination based on displacement of NH$_4^+$ by KCl was tested for biochar in batch mode and compared with CEC values obtained from the sum of extractable cations in NH$_4$OAc.

A compulsory pretreatment was shown to be necessary to remove readily soluble cations from salts, carbonates and silicates and adjust biochar pH to near neutral. The amount of readily soluble cations removed in the pretreatment was several times greater than the exchangeable fraction for all biochars. Skipping the pretreatment causes a major overestimation of the CEC of biochar.

Pretreatment of biochar also causes major mass losses, due to the removal of salts, carbonates and silicates. These mass losses have to be quantified in order to be able to compute the CEC of the initial raw biochar. The most abundant cation in the readily soluble fraction of the pretreatment of...
all biochars was K+. The exchangeable cations (CEC-BC) were dominated by K+ for rice husk and corncob biochars, whereas this was Ca\textsuperscript{2+} for pigeon pea and cacao shell biochars.

This study shows that shaking 1 g of biochar, following pretreatment, with 1M NH\textsubscript{4}OAc during 24 hours was enough to saturate the exchange sites with NH\textsubscript{4}\textsuperscript{+}. Additional 1M NH\textsubscript{4}OAc was needed to fully extract the exchangeable cations. There was no additional NH\textsubscript{4}OAc removed after four consecutive washings with 20ml isopropanol. However our results suggest that isopropanol may not penetrate the smallest pores of some biochars and we conclude that other liquid compounds are required to fully remove excess NH\textsubscript{4}OAc. Shaking with 2M KCl during 24 hours fully displaced NH\textsubscript{4}\textsuperscript{+} from the exchange sites. The biochar to solution ratios did not affect the CEC estimates.

CEC-NH\textsubscript{4}\textsuperscript{+}, although representing an overestimation due to the inefficient removal of excess NH\textsubscript{4}OAC by isopropanol, was more reproducible than CEC-BC.

A detailed description of the protocol for the modified method in batch is presented in the SM.

Acknowledgements

The study was funded by NMBU PhD internal financing to the first author and by the Faculty of Environmental Sciences and Nature Resource Management at NMBU as part of the stipend to Vegard Martinsen granted for young talented scientists. We are thankful to Magdalena Rygalska, Irene E. Dahl and Oddny Gimmingsrud for technical assistance. The authors thank the three anonymous reviewers who provided valuable comments and suggestions to improve the quality of this paper.

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