Essential oil and linalool contents in basil (*Ocimum basilicum*) irrigated with reclaimed water

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**ABSTRACT**

Essential oils (EO) are volatile compounds with complex chemical compositions that are derived from the secondary metabolism of aromatic herbs. There are several applications of EO in the industrial and medical sectors. Basil (*Ocimum* sp.) is one of the most important EO-producing aromatic herbs. In this study, EO content (EOC) and linalool content (LC) in basil irrigated with reclaimed water were investigated. Plant development parameters, nutrient absorption and crude protein (CP) content were also evaluated. The experiment was conducted in a greenhouse, with five different treatments and five repetitions each. Compared with treatments only using water, the results of those with reclaimed water irrigation showed higher nutrient absorption, CP contents (in their foliar tissue), length of plant branches, foliar biomass weights, and EOC and LC. EOC reached up to 0.58% of the dry biomass and LC was up to 5.84% of EO mass. In practice, it is estimated that around 5.8 kg of EO and 0.34 kg of linalool can be obtained from one ton of dry basil biomass.

**Key words:** aromatic herbs, crude protein, diluted wastewater, foliar biomass, nutrients, reuse

**HIGHLIGHTS**

- Higher essential oil and linalool contents were obtained in plants irrigated with higher percentage of reclaimed water used.
- Reclaimed water use resulted in the highest length of branches and biomass production of dry leaves, up to 30 cm and 28 g per plant, respectively.
- Crude protein content in leaves increased proportionally to the percentage of reclaimed water used.
- Approximately 5.8 kg of EO and 0.34 kg of linalool can be obtained per ton of basil dry mass.
INTRODUCTION

Water is a scarce resource of vital importance in arid and semi-arid regions, and climate changes have brought additional stress on water sources. Therefore, water recycling becomes a key component of water supply in those regions, besides being of great social value and contributing to water safety. In this context, treated wastewater has been considered suitable for use mainly for non-potable purposes, such as fertigation of crops (Gil-Meseguer et al. 2019).

The Brazilian northeastern region covers about 18% of country territory with an area of more than 1.5 million km², of which 62% (969,589.4 km²) is inserted in the semi-arid zone. The Brazilian semi-arid zone presents long periods of scarce rains, the rivers are non-perennial, and the soil is relatively nutrient poor for agriculture crops (Araújo 2011). The region is also known to have the lowest indexes of income, health and food. The use of treated wastewater in agriculture has been recommended to improve the hydric demands of that arid zone. Beside the content of components of agronomical importance, such as the dissolved nutrients in the treated wastewater solution, the use can provide the increase of the productivity of diverse cultures, as well as to benefit the regional agricultural economy, especially for the small and medium farmers.

Aromatic herbs produce essential oils (EO) that are the odoriferous, volatile, complex compounds responsible for their characteristic aromas. EO are related to the secondary metabolism of plants, and they can be obtained from leaves, flowers, roots, wood barks and seeds (Couto et al. 2019). EO interact with other organisms and serve various ecological functions, such as self-defense against predators and pests, attraction of pollinators, protection against water stress and temperature raising.

The main constituents of EO are terpenes, which contain oxygen in the form of hydroxyl, ether, aldehyde, ketone or carboxyl. Additionally, 80% of them are represented by the monoterpenes, which have widely been used as flavorings and fragrances in the food and cosmetic industries. Linalool is a monoterpeno found in the EO of several aromatic herbs and it is known to have relevant bioactive properties, such as antibacterial activity (Pereira et al. 2018).

Basil (Ocimum sp.) is considered a popular culinary herb, widely consumed across the world, which belongs to the Lamiaceae family. Around 150 species of basil are known and cultivated in different countries. Due to the high economic value and richness in phenolic compounds, it has been used worldwide in the pharmaceutical, food and cosmetic industries.

The increasing demand for natural products like basil increases the need for higher quality and productivity. Since basil is a species with substantial nutritional needs, an adequate fertilizer selection is recommended (Burducea et al. 2018). An option can be the use of treated wastewaters for irrigation of agricultural crops since satisfactory productivity has been achieved with many cultures. In the case of EO, increased production from aromatic herbs irrigated with pond effluent was justified by its richness in nutrient and trace elements that were taken up by the plants (Bensahab et al. 2015).

Nutrients like N, P and K play an important role in EO biosynthesis. They are components of proteins and photosynthetic structures, acting in the metabolic and regulatory processes of plants and activating enzymes involved in EO synthesis (Zhong et al. 2017). N can increase EO content (EOC) in aromatic plants by increasing biomass content per unit area, leaf area development and photosynthetic rate. P is considered a key factor that controls crop growth and is capable of changing EO yield and composition. K is essential to the enzymatic activation of volatile oil synthesis. Furthermore, a rise in the K supply rate contributes to improve the EO production in aromatic plants and to increase the percentage of linalool in the EO (Khan et al. 2017).

Higher availability of macronutrients enables increased EO concentrations in basil plants, accordingly with the percentage of wastewater used (Villanova et al. 2018). These authors reported that the supply of major N concentrations to Ocimum gratissimum L. improved the growth and EO production by the plants. The use of wastewater, beside preventing environmental pollution, promotes sustainable agriculture. In previous studies, it has been reported that reclaimed water had a beneficial effect on plants and could increase EO production in herbs because of the richness of the nutrients that can be harnessed, leading to a rise in crop productivity (Bensahab et al. 2015).

Therefore, the aim of the present study was to investigate the effects of irrigation with reclaimed water on the development of basil, focusing on EO and linalool production, plant growth parameters, nutrient absorption and crude protein (CP) content in leaf tissue. Basil (Ocimum basilicum) was chosen as the test plant because it is an aromatic herb that is widespread and cultivated around the world, mainly in temperate semi-arid regions (Akbari et al. 2018).

METHODS

Experimental design and monitoring

The experiment was carried out in a greenhouse (CAA-UFPE campus, Caruaru, Brazil) covered with canvas (200 μm thick) and a 70% shading screen. Basil plants were cultivated in drilled, polypropylene 3-L pots (working volume) with a 2-cm basal
gravel layer, to provide good drainage conditions, and a 5-cm top free space, in order to benefit the irrigation and seedlings phase. All the pots were randomly placed in the greenhouse, following a completely randomized statistical design, with five treatments (T) and five repetitions for each treatment.

The plants were cultivated in a soil typically classified as Planossolo. Since Pereira & Moreira (2011) reported that basil growing is preferentially organic, the experiment was designed according to the treatments (T1–T5) in Table 1. The irrigation was carried out with tap water (W) in the control run (T1) and reclaimed water (RW); RW was diluted with tap water in different volumetric proportions (T2–T5). There was no addition of inorganic fertilizers in any of the treatments. The dilution of RW provided the assessment of the effects of different nutrient contents on the plants. The water was stemmed from the local municipal water supply system and collected in a tap of the university campus (CAA-UFPE), and the RW was collected in the effluent of the polishing ponds of the Rendeiras Wastewater Treatment Plant (WWTP) in Caruaru, Brazil.

The Rendeiras WWTP is one of the facilities in the city of Caruaru, Pernambuco state. The plant’s capacity was 450 L·s⁻¹ and the sewerage covered 14 districts, which corresponded to 40% of the urban area of Caruaru. The treatment system was composed of a UASB reactor type and three ponds in series (aerated, facultative and maturation). The treated effluent has been used to irrigate urban gardens and in the own WWTP for cleaning purposes and to irrigate the internal grasses and gardens.

Plant irrigation was performed manually, and the demand was estimated by crop evapotranspiration. The frequency was defined by a tension-controlling device installed in the soil at 20 cm depth (Proplus model, Blumat Digital, USA). A total watering plate of 347 mm was applied during the experimental period of 56 days. To avoid any negative effects on the plants due to naturally excessive temperature increases during the day, irrigation was conducted at sunset.

The basil seedlings were previously selected from adult plants and inserted into pots with tap water, to stimulate the growth of white roots during a 15-day period. Thereafter, the basil seedlings with better appearance were selected and, for each experimental pot, three of them were transferred, totalling 75 plants in this experiment. The start of the experimental period (lasting 56 days) was based on the first day after transplantation (DAT). Pruning was carried out at the beginning of the first inflorescence to preserve the maximum possible EOC, since it decreases during the bloom. The plant heights and horizontal lengths were measured daily with a graduated ruler.

At the 56th DAT, the plant leaves were removed from the branches and weighed to obtain the fresh leaves’ biomass. Subsequently, they were transferred into paper bags, dried in an aerated oven at 40 °C and then weighed to determine the dry-leaves biomass. A fraction of the dry, ground biomass was reserved for EO extraction and another was used to determine the primary macronutrients (N, P and K) and CP contents in the leaf tissue. The crop development parameters (height of plants, horizontal length, and fresh and dry biomass weights), nutrient content and CP content were evaluated to verify the deviations among the treatments by analysis of variance (ANOVA), F and Tukey’s tests (with ρ < 0.05 and ρ < 0.01). All gravimetric measurements were conducted in an analytical balance (model M214A, Bel, Brazil).

**Chemical and physical characteristics of the water and reclaimed wastewater and soil**

The laboratory analyses of W and RW used for irrigation were performed in the LEA and LSA laboratories of UFPE, following the procedures set out in Standard Methods (APHA 2012). The determined parameters were pH, temperature (T), electrical conductivity (EC), salinity, turbidity, chemical oxygen demand (COD), nitrogen (N), phosphorous (P), sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺) and thermotolerant coliforms. The average values from eight samples are shown in Table 2. The soil’s physical and chemical characteristics are presented in Tables 3 and 4, respectively. Using the watering plate of 347 mm, it was calculated by the nutrient inputs to the plants based on the macronutrient concentrations contained in the wastewater.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Irrigation</th>
<th>Ratio W:RW*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Irrigation with tap water only</td>
<td>1:0</td>
</tr>
<tr>
<td>T2</td>
<td>Irrigation with tap water and reclaimed wastewater</td>
<td>3:1</td>
</tr>
<tr>
<td>T3</td>
<td>Irrigation with tap water and reclaimed wastewater</td>
<td>1:1</td>
</tr>
<tr>
<td>T4</td>
<td>Irrigation with tap water and reclaimed wastewater</td>
<td>1:3</td>
</tr>
<tr>
<td>T5</td>
<td>Irrigation with reclaimed wastewater only</td>
<td>0:1</td>
</tr>
</tbody>
</table>

*Ratio tap water: reclaimed water (volume/volume).
in the W and RW (diluted and non-diluted) (Table 5). The results of chemical analyses showed that there were no nutrients in the tap water; therefore, in the case of treatment T1, the plants took in nutrients only from the soil, in which the contents were: 72.4 kg NH₄⁺-ha⁻¹, 98.2 kg NO₃⁻-ha⁻¹, 1,698.8 kg PO₄³⁻-ha⁻¹ and 0.6 kg K⁺-ha⁻¹. Plants irrigated with RW (diluted or non-diluted) took in nutrients from both, RW and soil.

### Chemical analyses of the plants

Chemical analyses for the determination of primary macronutrients (N, P and K) and CP contents were conducted according to Bezerra Neto & Barreto (2011). EO was extracted by hydro-distillation in a Clevenger-type extractor using dry leaves. The dried leaves were ground and poured into a 2-L flask, containing 1 L of distilled water. After coupling the flask to the extractor and heating it for 90 min at 98.5 ± 0.14 °C, the EO was obtained separately from the hydrolat.
The EO was transferred to amber vials and weighed; EOC was determined based on the relation between the mass of EO and that of the dry biomass (Akbari et al. 2019). Analysis of EO was carried out in a gas chromatograph (GC) (model 7890A, Agilent, USA) coupled to a mass spectrometer (model 7975C, Agilent, USA). An analytical column HP-5 of 30 mm × 0.25 μm (J&W Scientific, USA) was used in the GC. As analytical linalool, a standard with 97% purity was used (Sigma Aldrich, Merck, Germany). Linalool was the only measured EO because it is the major monoterpene alcohol found in several plant species, especially in the Lamiaceae plant family, as basil (Prakash et al. 2019). Linalool content (LC) was calculated by the ratio between the mass of linalool and that of EO.

RESULTS AND DISCUSSION

Plant growth parameters

The average values of plant heights and horizontal lengths at the end of the experiment (DAT 56) are shown in Table 5. The plant growth in terms of height and length during the experiment is shown in Supplementary Figure S1 (see Online Resource).

Concerning the plant height development, the values varied between 46.0 and 50.6 cm, but no significant difference was observed among the five treatments (Figure 1(a)). Therefore, irrigation with RW at any dilution ratio (T2–T5) did not influence the plant height, compared with that of T1 (water only). At the 12th and 40th DAT, pruning was carried out to slow down the growth.

Table 5 | Inputs of nutrients for plants due to the water (T1) or reclaimed water (T2 to T5) (in kg·ha⁻¹)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1* W:RW = 1:0</th>
<th>T2 W:RW = 3:1</th>
<th>T3 W:RW = 1:1</th>
<th>T4 W:RW = 1:3</th>
<th>T5 W:RW = 0:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-NH₄⁺</td>
<td>BDLb</td>
<td>41.64</td>
<td>76.42</td>
<td>67.11</td>
<td>69.12</td>
</tr>
<tr>
<td>N-NO₃⁻</td>
<td>BDLb</td>
<td>3.16</td>
<td>5.16</td>
<td>5.83</td>
<td>14.89</td>
</tr>
<tr>
<td>P-PO₄³⁻</td>
<td>BDLb</td>
<td>51.39</td>
<td>52.38</td>
<td>74.73</td>
<td>78.96</td>
</tr>
<tr>
<td>K⁺</td>
<td>BDLb</td>
<td>0.03</td>
<td>0.35</td>
<td>0.45</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Inputs of nutrients to the plants were only by the soil (72.40); N-NH₄⁺ (72.40); N-NO₃⁻ (98.20); P-PO₄³⁻ (1698.8); K⁺ (0.60).

bBDL: values below the detection limit (10.0 mg N-NH₄⁺ ·L⁻¹; 0.5 mg N-NO₃⁻ ·L⁻¹; 1.0 mg P-PO₄³⁻ ·L⁻¹; 2.0 mg K⁺ ·L⁻¹).

Figure 1 | Height (a) and length (b) of plants for the five treatments at the end of the experimental period (results without letter or with the same letter mean no significant differences among the treatments for the same parameter; capital letters mean significance at 1%, p < 0.01).
flowering of the plants; soon after pruning, a linear increase in the plant height was observed for all the treatments (Supplementary Figure S1 in the Online Resource). Plants had their inflorescence structures removed earlier, since the leaves were collected prior to the flowering stage, to benefit compounds, such as linalool, that are usually biosynthesized in the early stages of a basil plant. However, in the case of the branches, they developed more in the plants irrigated with RW, and consequently, a greater horizontal length was obtained in the treatments T2–T5 (Figure 1(b)), the highest being in T5 (38 cm), where only RW was used.

Higher horizontal length means higher leaf biomass area, which can be justified by the higher input of nutrients; they are related to plant biomass production and protein synthesis. Increased sprouts, roots and EO yield were obtained in basil cultivated with tannery sludge due to the addition of organic carbon and major essential nutrients (N, P and K) in the used soil (Chand et al. 2015). The absence of water stress and available N and K were the main factors attributed to the increased biometric characteristics of basil irrigated with vinasse (Palaretti et al. 2015).

Concerning the fresh and dry biomass of the leaves, as expected, the highest values were obtained in the pots irrigated only with RW (T5), whereas the lowest values were found in those irrigated only with W (T1). Intermediate treatments (T2, T3 and T4) showed no, or at least less significant, differences among them (Figure 2). Fertigation with reclaimed water has positively influenced the foliar yield favoring the commercial interest of basil cultivation because of the extraction of EO, which is also aimed at pharmaceutical and cosmetic industries.

The growth and development of agricultural plants is potentially promoted by nutrient supply. For instance, N is involved in widening new cells and tissue production, and it is responsible for improving the plant growing characteristics. An increment in basil development was observed when using NPK-rich fertilization and, consequently, an increased area and mass of leaves (Yokoda et al. 2015).

**Nutrients and crude protein content in the tissue of leaves**

A supply of nutrients is needed in agriculture and each one of them plays an important role in a plant’s development. Nitrogen is important for the synthesis of proteins, and it is related to the number of emerging leaves and the rate of leaf expansion. Moreover, most of the N content in the leaves is present in chlorophyll and in proteins. Thus, it has a positive impact on the photosynthesis, resulting in intense green leaves that are resistant to plague attack (Koch et al. 2020). Phosphorus is one the most important limiting macronutrients for agricultural production; it plays an important role in cellular metabolism, respiration, photosynthesis and

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**Figure 2** | Average weight of fresh (a) and dry (b) leaf biomass per pot at the end of the experiment (results with the same letter mean no significant differences among the treatments for the same parameter; capital letters mean significance at 1%, $p < 0.01$; lowercase letters mean significance at 5%, $p < 0.05$).
growth from seed until grain formation and maturity (Malhotra et al. 2018). Potassium is crucial for crop development; it controls the cell osmoregulation, and it is a key element for photosynthesis and the distribution of photosynthates by the phloem. K deficiencies can lead to an accumulation of sucrose in leaves that can stimulate the occurrence of pests (Koch et al. 2020).

The contents of macronutrients (N, P, K) and CP in the tissue of leaves are shown in Figure 3. The highest N content of 4.34 g·kg⁻¹ was found in T5 (RW) (Figure 3(a)), and the corresponding CP content was 2.71% of the N content (Figure 3(b)). These values were expected, and they were significantly different from those in the treatments T1 and T2. CP contents observed in T3 (1.75%), T4 (2.19%) and T5 (2.71%) were higher than the contents of CP expected for 100 g of eatable part (2 g·100⁻¹·g⁻¹) of basil (TBCA 2020). Furthermore, higher N inputs lead to an increasing number of branches and leaves. The values obtained for N and CP are also in accordance with the productivity parameters related to the higher biomass of leaves obtained in T3, T4 and T5 (Figure 3).

The highest P content was found in the pots irrigated with RW (T2–T5), but only T5 showed statistical differences compared with T1 (Figure 3(c)). The great majority of the P naturally present in the soil is in an insoluble form; therefore, only a small amount becomes available to plants. In this experiment, the results showed that the dissolved P present in RW facilitated its uptake by plants. In relation to K content, there was no significant difference among the treatments (Figure 3(d)). This indicates that its presence in the soil solution was enough for the plants’ requirements and that RW did not significantly influence K uptake by the plants.

The studies on using diluted RW have been reported in terms of the effects of nutrients, salinity and metals on soils and plants. Ahmad et al. (2013) investigated the impact of irrigation with freshwater (control) and wastewater (diluted with fresh water at ratios varying from 10 to 100%) on the growth and productivity parameters of canola (Brassica napus L.). They concluded that irrigation with wastewater diluted at a 50% ratio resulted in a considerable absorption of nutrients with an increased growth and yield of the plants.

EOC and LC

The results of EO and LC are shown in Figure 4. Higher yields of both were obtained with the treatments T4 and T5 that received higher amounts of RW. Concerning EO, only the results obtained by plants submitted to these two treatments were statistically different from those of the other treatments (Figure 4(a)). In the case of LC extracted from plants, almost all the treatment results were statistically different from each other, suggesting that the irrigation with RW positively influenced the linalool synthesis in basil plants (Figure 4(b)). These results can certainly be attributed to the macronutrients supplied to the plants, which occurred in treatment T5 with the highest RW (see Table 5).

![Figure 3](http://iwaponline.com/aqua/article-pdf/70/5/773/913943/jws0700773.pdf)

Figure 3 | Contents of macronutrients (a, c, and d) and CP (b) in the tissue of leaves for the five treatments at the end of the experiment (values of N, P and K are in g·kg⁻¹ of dry leaves; results without letter or with the same letter mean no significant differences among the treatments for the same parameter; capital letters mean significance at 1%, p < 0.01).
EO production depends on the plant physiology, particularly concerning the tissue synthesis and metabolic processes. Several factors may affect EO production and its chemical composition; some of them are the method and material used for the extraction (dry or fresh leaves, roots and flowering tops), the age of the plants, nutrient availability, type of fertilization and seasonality (Bistgani et al. 2018; Couto et al. 2019). In the present study, EOCs of the four treatments irrigated with RW were 0.36–0.58% (Figure 4(a)). By using chemical fertilizers in different cultivar groups of basil plants, the extraction potential of EO ranged from 0.60 to 1.00% (Rahimi et al. 2019). Bistgani et al. (2018), by fertilizing thyme (Thymus daenensis Celak), observed that EO yield increased 52% in relation to plants cropped without fertilization. In the present study, the results of the treatments T4 (0.53%) and T5 (0.58%) are close to those in the range reported by Rahimi et al. (2019); and those EOCs in T4 and T5 increased significantly when compared with T1 (0.28%). Therefore, converting wastewater into irrigation water can represent an alternative to closing the loop for a circular economy.

Considering the contents obtained in T5, approximately 5.8 kg of EO and 0.34 kg of linalool can be obtained from one ton of dry foliar biomass. For pharmaceutical and cosmetic uses, the high yields found in this work may represent an interesting economical alternative for farmers, since 1 kg of EO and natural linalool oxides from basil may reach prices of US$ 110 and US$ 750, respectively, based on the international market (Bormann et al. 2012). Additionally, Do et al. (2015) reported that the cumulative sales for EO industries have been within the range of several billion dollars since 2008.

The fertigation with reclaimed water significantly improved the production of the studied parameters in this experiment under the environmental conditions of a semi-arid region like that of the Brazilian Northeast; there and in many parts of the world, most of the crops are carried out in rainfed systems, sometimes without any guarantee of a good harvest. Therefore, the use of treated domestic wastewater for the aim of irrigation can be a sustainable alternative for basil cultivation in regions with water scarcity. Cultivation of aromatic herbs is recommended by a family farming system since it can also improve the farmers’ income.

CONCLUSIONS

The continuous supply of nutrients in reclaimed water stimulated EO production and increased LC in basil plants. When submitted to irrigation treatments with 75 or 100% RW, EOCs were 0.53 and 0.58% of the dry biomass, respectively; and LCs were 5.26 and 5.84% of EO mass, respectively. These results can be attributed to the high nutrient content, which positively influenced the biochemical processes for the synthesis of volatile compounds in the basil plants. Furthermore, the EOC
LCs observed (even in the treatments with 25 and 50% of RW) were close to or higher than some values reported in the literature and, therefore, they become attractive options to be applied to basil cultivation.

The irrigation with the highest RW concentrations (T3, T4 and T5) resulted in a high expansion of branches (up to 30 cm) and good production of fresh (up to 70 g) and dry (up to 28 g) biomass of leaves per plant. Nutrient contents found in the tissue of the leaves in T5 (4.34 g N·kg⁻¹; 2.30 g P·kg⁻¹; 6.10 g K·kg⁻¹), T4 (5.50 g N·kg⁻¹; 2.12 g P·kg⁻¹; 5.69 g K·kg⁻¹) and T3 (2.80 g N·kg⁻¹; 1.42 g P·kg⁻¹; 5.53 g K·kg⁻¹) were also the highest among the studied treatments. CP contents also increased proportionally to the increase in reclaimed water used: 1.75% in T3, 2.19% in T4 and 2.71% in T5.

Therefore, irrigation with reclaimed water positively influenced the metabolism and biochemical processes of basil, resulting in higher values of nutrients (N, P and K), CP, EOC and LC in the tissue of leaves. In practice, it is estimated that around 5.8 kg of EO and 0.34 kg of linalool can be obtained from one ton of dry basil biomass.

The use of treated wastewater in the irrigation of basil, under the environmental conditions of a semi-arid region, can significantly improve the basil’s productivity parameters, especially EO yield. Consequently, the fertigation of basil with reclaimed water can be a sustainable alternative for regions with water scarcity and to increase the income of farmers in the family farming system of these regions.

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AUTHORS’ CONTRIBUTIONS

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COMPETING INTEREST
The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

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