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Industrial hemp in craft beer and biochar from waste for mercury removal

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ABSTRACT: Industrial hemp (*Cannabis sativa*) was evaluated as a partial substitute for hops (*Humulus lupulus*) in craft beer production and as a precursor for valorizing post-fermentation brewing residue. Four beer styles and four substitution levels (0, 30, 70, and 100%) were tested in a 4 × 4 factorial design with three independent brewing batches per combination. Physicochemical properties and sensory acceptability were assessed, and the selected residue was characterized for total phenolic content and converted into dried residue (T1), unmodified biochar (T2), and $FeCl_3$-modified biochar (T3) for Hg^{2+} removal. Responses to hemp substitution were style-dependent, particularly for pH and titratable acidity, while no overall substitution effect was detected for alcohol content or final density. Weissbier and Sour showed comparatively limited sensory departures from controls at 30–70% substitution. The selected residue contained 21 mg GAE g^{-1} of total phenolics. Hg^{2+} removal reached 94.0 ± 2.0% for T1, 97.0 ± 1.5% for T2, and 99.5 ± 0.3% for T3. These results support moderate hemp-flower substitution in selected beer styles and the sequential valorization of the resulting residue as an adsorbent precursor.
Keywords: *Cannabis sativa*; *Humulus lupulus*; adsorption; circular economy; phenolic compounds.

Cânhamo industrial na cerveja artesanal e biochar de resíduos para remoção de mercúrio

RESUMO: O cânhamo industrial (*Cannabis sativa*) foi avaliado como substituto parcial do lúpulo (*Humulus lupulus*) na produção de cerveja artesanal e como precursor para a valorização do resíduo cervejeiro pós-fermentação. Quatro estilos de cerveja e quatro níveis de substituição (0, 30, 70 e 100%) foram avaliados em delineamento fatorial 4 × 4, com três lotes independentes por combinação. Foram determinadas propriedades físico-químicas e a aceitabilidade sensorial, e o resíduo selecionado foi caracterizado quanto aos fenólicos totais e convertido em resíduo seco (T1), biochar não modificado (T2) e biochar modificado com $FeCl_3$ (T3) para a remoção de Hg^{2+}. As respostas à substituição dependeram do estilo, especialmente quanto ao pH e à acidez titulável, enquanto não houve efeito geral da substituição sobre o teor alcoólico nem sobre a densidade final. Weissbier e Sour apresentaram alterações sensoriais menores em comparação com os controles, na faixa de 30–70%. O resíduo selecionado apresentou 21 mg EAG g^{-1} de fenólicos totais. A remoção de Hg^{2+} atingiu 94,0 ± 2,0% para T1, 97,0 ± 1,5% para T2 e 99,5 ± 0,3% para T3. Os resultados sustentam a substituição moderada por flores de cânhamo em estilos selecionados e a valorização sequencial do resíduo gerado como precursor adsorvente.
Palavras-chave: *Cannabis sativa*; *Humulus lupulus*; adsorção; economia circular; compostos fenólicos.

1. INTRODUCTION

Craft beer has emerged as a beverage with growing acceptance in both local and international markets, thanks to its sensory diversity and the incorporation of distinctive ingredients. Within its formulation, hops (*Humulus lupulus*) perform key technological functions by providing bitterness and aroma, as well as contributing to the characteristic sensory profile of highly hopped styles (HAHN et al., 2018; RETTBERG et al., 2018). Despite the fundamental role of hops in beer production, their availability can be limited in regions where cultivation is uncommon, increasing the dependence on imports for small-scale craft breweries.

In response to these challenges, industrial hemp (*Cannabis sativa* ssp. sativa) has attracted interest as an emerging agro-industrial substitute for hops. Belonging to the same botanical family (Cannabaceae), it shares phytochemical traits that facilitate its partial integration into the brewing process, contributing terpenes and polyphenols with aromatic and functional potential (OVIDI et al., 2022; HABSCHIED et al., 2025). In reported experiences with craft beer, the partial replacement of hops with hemp flowers/inflorescences (dry-hemping) has shown sensory acceptability comparable to the control and provides volatile and phenolic compounds of interest (HABSCHIED et al., 2025). Beyond its agronomic value, industrial hemp is distinguished by the complexity of its secondary metabolites, including terpenes, flavonoids, polyphenols, and non-psychoactive cannabinoids. These compounds can influence the sensory profile and oxidative

stability of the final product and partially overlap with typical hop metabolites (HANUŠ et al., 2016; ERŽEN et al., 2021; OVIDI et al., 2022).

From an analytical perspective, phenolic compounds can be estimated using widely applied colorimetric methods such as the Folin–Ciocalteu assay (SINGLETON et al., 1999; AINSWORTH; GILLESPIE, 2007). The incorporation of alternative plant materials into brewing also generates residual biomass that may retain compounds of interest for subsequent valorization. Brewing by-products can contain recoverable phenolic fractions, while the concentration measured depends strongly on the plant matrix, processing history, solvent system, and extraction conditions (BRAVI et al., 2021; ISIDORE et al., 2021).

Beyond phytochemical recovery, lignocellulosic residues can be thermochemically converted into biochar for environmental applications. Hemp-derived biomass has been investigated as a precursor for biochar production, while modification of biochar with metal salts can alter its surface properties and sorption behavior (ANJUM et al., 2022; ŠOŠTARIĆ et al., 2024; ASSIRELLI et al., 2025). Of particular relevance to mercury remediation, Fe-modified biochars have shown enhanced Hg(II) retention in aqueous systems compared with unmodified materials (FENG et al., 2020). These approaches are consistent with circular-use strategies in which lignocellulosic agri-food residues are converted into carbonaceous materials for environmental applications (ESCUDERO-CURIEL et al., 2023; LIU et al., 2026).

In this context, the present study evaluated a sequential valorization strategy for industrial hemp (*Cannabis sativa* ssp. sativa). First, the feasibility of partially replacing hops with hemp inflorescences in four craft beer styles was assessed through physicochemical characterization and sensory analysis. The selected post-fermentation plant residue was then characterized for total phenolic content and used as a precursor for three adsorbent materials: dried non-carbonized residue, unmodified biochar, and $FeCl_3$-modified biochar. Their performance was subsequently compared for Hg^{2+} removal from aqueous solution. This approach links the use of hemp as an alternative brewing ingredient with the subsequent environmental valorization of the generated residue.

2. MATERIAL AND METHODS

The experimental methodology was structured into two sequential phases, as summarized in Figure 1. The first phase comprised the production of four craft beer styles (IPA, Weissbier, Red Ale, and Sour) with partial or total replacement of hops by dried female inflorescences of industrial hemp at substitution levels of 0, 30, 70, and 100%. The second phase focused on the environmental valorization of the post-fermentation brewing residue obtained from the selected beer treatment. This residue was recovered and conditioned for the preparation of three adsorbent materials: dried non-carbonized residue (T1), unmodified biochar produced by pyrolysis (T2), and $FeCl_3$-modified biochar (T3), which were subsequently evaluated for Hg^{2+} removal from aqueous solution.

2.1. Brewing process: replacing hops with hemp flowers

2.1.1. Raw material

Dried female inflorescences of Cherry Oregon industrial hemp were used for partial or total replacement of hops during craft beer production. Prior to use, the inflorescences were destemmed and coarsely crushed. The plant material had a moisture content below 12% and was stored in airtight amber glass containers, protected from light and under low relative humidity conditions (<60%), at room temperature (20–22 °C) for a maximum period of 30 days before experimental use.

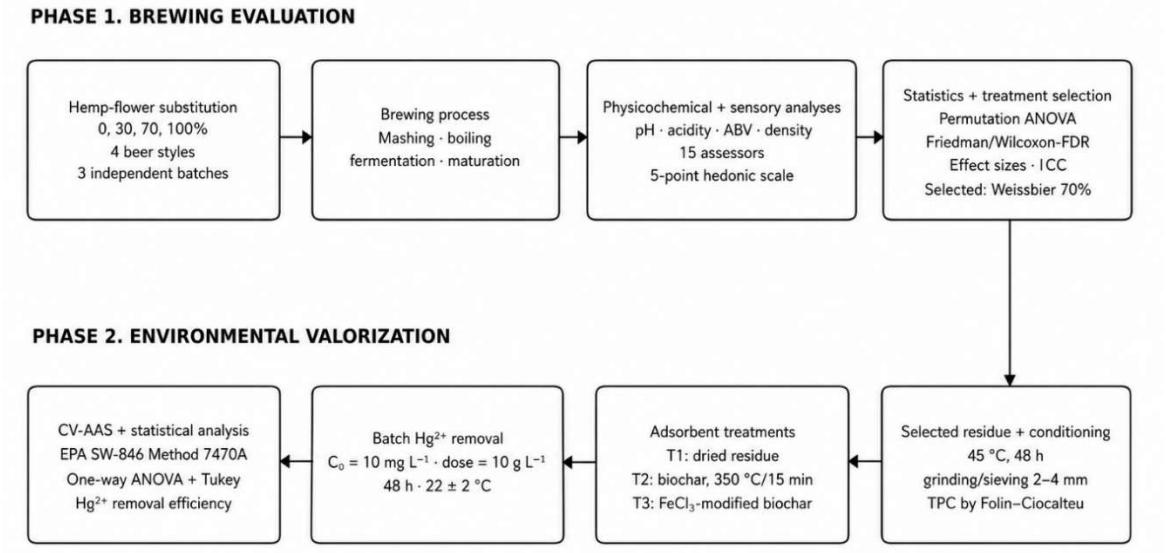

Figure 1. Experimental workflow for hop substitution with industrial hemp flower in craft beer production and subsequent environmental valorization of post-fermentation residue for Hg^{2+} removal.

Figura 1. Fluxograma experimental da substituição do lúpulo por flor de cânhamo industrial na produção de cerveja artesanal e posterior valorização ambiental do resíduo pós-fermentativo para remoção de Hg^{2+}.

2.1.2. Formulation and experimental design

Four craft beer styles were evaluated: IPA, Weissbier, Red Ale, and Sour. For each style, four levels of hop substitution with dried industrial hemp flower were established: 0% (control), 30%, 70%, and 100% (w/w). Substitution percentages were calculated relative to the total amount of hops specified in the original formulation of each beer style. The 0% treatment contained only the hops corresponding to

the original formulation, whereas hemp flower progressively replaced hops at the remaining substitution levels.

The brewing experiment followed a 4 × 4 factorial design, with beer style (four levels) and hop substitution with hemp flower (four levels) as experimental factors. Three independent brewing batches were produced for each style × substitution combination, resulting in 48 experimental units. Each batch was prepared independently from the beginning of the brewing process, including mashing, boiling, fermentation, bottling, and maturation. The independent batches, rather than repeated analytical measurements, were considered the experimental replicates for the physicochemical analyses.

2.1.3. Beer production

Each 5-L beer batch was prepared according to the formulation established for the corresponding beer style. IPA was formulated with 1.50 kg of Pale Ale malt, 0.30 kg of Munich malt, 125 g of oats, and 2.80 g of SafAle US-05 yeast; Weissbier with 0.70 kg of wheat malt, 0.70 kg of Pilsner malt, 125 g of oats, and 2.80 g of SafAle WB-06 yeast; Red Ale with 1.25 kg of Pale Ale malt, 0.125 kg of Munich malt, 0.0625 kg of Crystal malt, 0.0625 kg of Cara Ruby malt, 125 g of oats, and 2.80 g of SafAle S-04 yeast; and Sour with 0.25 kg of wheat malt, 0.25 kg of Pilsner malt, 0.75 kg of Pale Ale malt, and 1.00 g of Sour Pitch culture. Clarifier (0.50 g) was added to all batches. The formulations were adapted for the incorporation of hemp flower as an alternative brewing ingredient (HABSCHIED et al., 2025).

For IPA, Weissbier, and Red Ale, the total amount of hops and hemp flower was maintained at 17.50 g per 5-L batch. The control treatment (0%) contained 5.00 g of Nugget and 12.50 g of Willamette hops. At 30% substitution, the formulation contained 3.50 g of Nugget, 8.75 g of Willamette, and 5.25 g of hemp flower; at 70% substitution, 1.50 g of Nugget, 3.75 g of Willamette, and 12.25 g of hemp flower; and at 100% substitution, hops were completely replaced by 17.50 g of hemp flower. For Sour beer, the total amount of Cascade hops and hemp flower was maintained at 5.00 g per batch, with respective hop/hemp-flower amounts of 5.00/0, 3.50/1.50, 1.50/3.50, and 0/5.00 g at substitution levels of 0, 30, 70, and 100%.

The grains were milled and mashed at 65 °C for 60 min, followed by wort separation and boiling for 60 min. Hemp flower was added directly to the wort without prior extraction. For each formulation, the corresponding hop and/or hemp-flower amount was divided into two additions: the first was incorporated 10 min after the start of boiling, and the second 10 min before the end of the 60-min boiling period.

The clarifier was added together with the second addition. After boiling, the wort was cooled to 20 °C, transferred to sanitized fermenters, and inoculated with the fermentation culture corresponding to each beer style. Fermentation was conducted at 18–22 °C for 10–14 days, depending on the beer style. After primary fermentation, the beers were racked, bottled, and matured for 15 days before physicochemical and sensory evaluation.

2.1.4. Physicochemical analysis

Physicochemical analyses were performed on each of the three independently produced batches for each style × substitution combination using degassed beer samples. pH was measured with a calibrated pH meter, based on ASBC Beer-9. Titratable acidity was determined in 25 mL of degassed beer by titration with 0.1 N NaOH using phenolphthalein as the endpoint indicator and expressed as lactic acid, following ASBC Beer-8B. Alcohol by volume (ABV) was determined by direct aerometric measurement using a glass alcoholmeter calibrated in % v/v; samples were degassed and equilibrated to 20 °C before measurement, and the reading was recorded after hydrostatic equilibrium was reached. Final density was determined in approximately 250 mL of degassed beer using a glass hydrometer, with the reading recorded after stabilization.

2.1.5. Sensory analysis

Sensory acceptability was evaluated by 15 untrained adult assessors who were regular consumers of craft beer. All participants provided informed consent prior to participation. The same assessors evaluated all 16 beer formulations across two sensory sessions conducted on separate days, with eight samples evaluated per session. Each sample consisted of 30 mL of beer served at 20 °C. Samples were identified using randomized codes and presented in randomized order within each session. A 15-min interval was allowed between consecutive samples, and water was provided for palate cleansing. Color, odor, flavor, and turbidity were rated using a five-point hedonic scale (1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, and 5 = like very much), following the principles of hedonic testing described by Lawless; Heymann (2010).

2.1.6. Statistical analysis

Physicochemical and sensory data were analyzed separately according to their respective experimental structures. Physicochemical variables were analyzed using a two-way permutation ANOVA, with beer style (IPA, Weissbier, Red Ale, and Sour) and hemp-flower substitution level (0, 30, 70, and 100%) as fixed factors, including the style × substitution interaction. The three independently produced batches for each style × substitution combination were considered experimental replicates (n = 3). Permutation-based p-values were estimated using 9,999 permutations, and results were expressed as mean ± standard deviation (SD). To complement significance testing, standardized differences between each substitution treatment and the corresponding 0% control within each beer style were calculated using Cohen's d, based on the pooled standard deviation of the independent batches. When the pooled standard deviation was zero and the treatment and control means were identical, the effect size was assigned a value of zero; otherwise, it was not estimated.

Sensory data were analyzed as repeated observations because the same 15 assessors evaluated all 16 beer formulations. For each beer style and sensory attribute (color, odor, flavor, and turbidity), differences among the four substitution levels were evaluated using the Friedman test. When a significant overall effect was detected ($p < 0.05$), the 30, 70, and 100% substitution treatments were compared with the corresponding 0% control using the Wilcoxon signed-rank test. The resulting p-values were adjusted within each beer style and sensory attribute using the Benjamini–Hochberg false discovery rate (FDR) procedure. Paired standardized effect sizes were expressed as Cohen's dz and calculated from the mean and standard deviation of the paired treatment–control differences. Agreement in average panel ratings was assessed for each sensory attribute using a two-way random-effects, absolute-agreement, average-measures intraclass correlation coefficient [ICC(2,k)].

Statistical significance was established at $p < 0.05$. Effect sizes were interpreted separately from statistical significance, and non-significant differences were not interpreted as evidence of equivalence between treatments. All statistical analyses were performed in Python 3.13.5 (Python Software Foundation). Data handling was performed using pandas 2.2.3 and NumPy 2.3.5. Statistical tests were implemented using SciPy 1.17.0 and statsmodels 0.14.6, with Patsy 1.0.2 used for factorial model-matrix specification. Permutation-based factorial analyses and effect-size calculations were implemented using reproducible custom Python routines.

2.2. Environmental valorization of post-fermentation brewing residue for Hg^{2+} removal

2.2.1. Recovery and conditioning of post-fermentation brewing residue

Following primary fermentation and racking of the selected beer treatment, the remaining solid fraction from the three independent 5 L batches (15 L total) was recovered by decantation and filtration. Approximately 650 g of wet post-fermentation brewing residue was obtained in total and combined to form a composite sample for the environmental valorization phase. The composite material was dried in a forced-air oven at 45 °C for 48 h until a moisture content of approximately 12% was reached, yielding approximately 65 g of conditioned residue. The dried material was mechanically ground using a stainless-steel mill and sieved to obtain a uniform particle size of 2–4 mm. The conditioned residue was subsequently stored in airtight glass containers at 20–22 °C, protected from light and moisture, until its use in phytochemical characterization and preparation of the adsorbent treatments.

2.2.2. Extraction and determination of total phenolic compounds

From the approximately 65 g of dried and conditioned post-fermentation brewing residue obtained as described in Section 2.2.1, a separate 5.00 g portion was used for hydroalcoholic extraction. A 70% ethanol solution was selected as the extraction medium, consistent with hydroalcoholic extraction approaches reported for hemp matrices (BIJELIĆ et al., 2024). The sample was mixed with 50 mL of 70% ethanol (v/v), corresponding to a solid-to-liquid ratio of 1:10 (m/v), in an amber Erlenmeyer flask. The mixture was maintained under orbital shaking at 150 rpm and 20 ± 2 °C for 24 h in the dark. After extraction, the liquid phase was separated by vacuum filtration using Whatman No. 1 filter paper and stored in an amber glass container at 4 °C until analysis.

Total phenolic content (TPC) in the hydroalcoholic extract was determined using the Folin–Ciocalteu colorimetric method (AINSWORTH; GILLESPIE, 2007). A calibration curve was prepared using gallic acid standards ranging from 10 to 100 mg L^{-1}. For each determination, 200 µL of the extract was mixed with 1.0 mL of 10% Folin–Ciocalteu reagent and allowed to react for 5 min in the dark. Subsequently, 800 µL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated for 30 min at room temperature. Absorbance was measured at 765 nm using a UV–Visible spectrophotometer (UV-1800, Shimadzu, Japan). Measurements were performed in triplicate, and TPC was expressed as milligrams of gallic acid equivalents per gram of dry residue (mg GAE g^{-1}). The calibration curve showed a coefficient of determination (R^2) greater than 0.99.

2.2.3. Preparation of adsorbent treatments

Three adsorbent treatments were prepared from the dried and conditioned post-fermentation brewing residue described in Section 2.2.1 to evaluate the effect of pyrolysis and $FeCl_3$ modification on Hg^{2+} removal. The treatments were defined as follows: T1, dried post-fermentation brewing residue without carbonization; T2, unmodified biochar obtained by pyrolysis; and T3, $FeCl_3$-modified biochar obtained by chemical impregnation followed by pyrolysis.

For T2, a 5.00 g portion of the dried residue was placed in a covered crucible and subjected to pyrolysis in a muffle furnace at 350 °C for 15 min under oxygen-limited conditions. For T3, a separate 5.00 g portion of the dried residue was impregnated with 50 mL of 0.5 M $FeCl_3$ solution, corresponding to a solid-to-liquid ratio of 1:10 (m/v). The mixture was maintained under agitation at 100 rpm for 12 h at room temperature. After impregnation, the solid material was separated by filtration, washed with deionized water until neutral pH was reached, and dried at 60 °C for 12 h. The dried $FeCl_3$-treated material was subsequently pyrolyzed under the same conditions used for T2 (350 °C for 15 min under oxygen-limited conditions). After pyrolysis, T2 and T3 were allowed to cool to room temperature inside the furnace and were stored separately in airtight amber glass containers under dry and dark conditions until the Hg^{2+} removal experiments.

2.2.4. Batch Hg^{2+} removal experiments

The Hg^{2+} removal performance of the three adsorbent treatments (T1, T2, and T3) described in Section 2.2.3 was evaluated using batch adsorption experiments. An aqueous Hg^{2+} solution with an initial concentration (C_0) of 10 mg L^{-1} was prepared from mercury (II) nitrate [$Hg(NO_3)_2$] using ultrapure deionized water. For each experimental replicate, 1.00 ± 0.01 g of the corresponding adsorbent (T1, T2, or T3) was placed in an amber glass Erlenmeyer flask containing 100 mL of the Hg^{2+} solution, corresponding to an adsorbent dosage of 10 g L^{-1}. The flasks were maintained under orbital shaking at 150 rpm and 22 ± 2 °C for 48 h in the dark. Each treatment was evaluated in triplicate ($n = 3$), and a blank containing the Hg^{2+} solution without adsorbent was subjected to the same experimental conditions.

At the end of the 48-h contact period, the pH of each experimental replicate was measured using a calibrated pH meter and recorded for comparison among T1, T2, and T3. The solid and liquid phases were subsequently separated by vacuum filtration, and the filtrate was collected for determination of the residual Hg concentration (C_f). Prior to instrumental quantification, a preliminary precipitation test was performed on the filtrates obtained from T1, T2, and T3. A 2.0 mL aliquot of each filtrate was transferred to a test tube, and 6 N HCl was added dropwise until precipitation was observed. A white precipitate formed, and differences in its apparent amount were observed among treatments. Because the chemical identity of the precipitated species was not independently confirmed, this response was used only as a preliminary comparative observation and not as a selective identification or quantitative determination of Hg^{2+}. Precipitate formation was classified using a five-level ordinal scale (1–5), where 1 represented the lowest apparent precipitate formation and 5 the highest. The procedure was applied to the three experimental replicates of each treatment. Residual mercury was subsequently quantified by CV-AAS according to EPA SW-846 Method 7470A. Prior to

instrumental determination, Hg^{2+} was reduced to elemental mercury (Hg^0) using stannous chloride ($SnCl_2$), and the generated mercury vapor was quantified at 253.7 nm. The analytical method had a reported limit of detection (LOD) of 0.0002 mg L^{-1}. Hg^{2+} removal efficiency was calculated according to Eq. (1)

$$\%R_{Hg^{2+}} = \frac{C_o - C_f}{C_o} * 100 \quad (01)$$

where: C_o: represents the initial concentration of Hg^{2+} (mg L^{-1}) and C_f: represents the final concentration measured after treatment.

2.2.5. Statistical analysis

Hg^{2+} removal efficiency was calculated independently for each experimental replicate of the three adsorbent treatments (T1, T2, and T3). Results were expressed as mean ± standard deviation (SD), with three replicate adsorption assays per treatment (n = 3). Prior to inferential analysis, normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. Given the small number of replicates per treatment, the results of these assumption tests were interpreted cautiously. Differences in Hg^{2+} removal efficiency among adsorbent treatments were evaluated using one-way analysis of variance (ANOVA), with adsorbent treatment as the experimental factor. When a significant overall effect was detected ($p < 0.05$), Tukey's post hoc test was applied for pairwise comparisons among T1, T2, and T3. Statistical significance was established at $\alpha = 0.05$.

3. RESULTS

3.1. Effects of hemp on the properties of craft beer

The physicochemical response to hop substitution with hemp flower depended on beer style and the parameter evaluated (Figure 2). Values are reported as mean ± SD of three independently produced brewing batches for each style × substitution combination.

For pH (Figure 2a), the two-way permutation ANOVA showed significant effects of beer style and substitution level, together with a significant style × substitution interaction ($p < 0.001$). Sour remained relatively stable from 0 to 70% substitution (3.99 ± 0.02, 3.97 ± 0.01, and 3.97 ± 0.05, respectively) but decreased to 2.53 ± 0.01 at 100%. Weissbier decreased from 4.65 ± 0.04 in the control to 4.33 ± 0.02 at both 30 and 70% substitution and to 4.28 ± 0.01 at 100%. IPA showed comparatively small changes, whereas Red Ale fluctuated without a consistent directional trend.

Acidity also differed among beer styles and showed a significant style × substitution interaction ($p < 0.001$), whereas the overall effect of substitution level was not significant ($p > 0.05$) (Figure 2b). Sour decreased from 0.230 ± 0.026 in the control to 0.173 ± 0.000 at 30% substitution and remained at approximately 0.185–0.186 at 70 and 100%. Weissbier showed little variation across substitution levels, while IPA and Red Ale exhibited treatment-dependent fluctuations without a consistent concentration-related pattern.

ABV differed among beer styles ($p < 0.001$), but neither substitution level nor the style × substitution interaction was significant ($p > 0.05$) (Figure 2c). IPA remained at 8.00% across all substitution levels and Red Ale at 8.33%, while Weissbier ranged from 4.83 to 5.17% and Sour from 5.67 to 6.00%.

Final density differed among beer styles ($p < 0.001$), whereas no significant effect of substitution level or style × substitution interaction was detected ($p > 0.05$) (Figure 2d). Mean values ranged from 1.013 to 1.037 in IPA, 1.000 to 1.010 in Weissbier, 1.010 to 1.017 in Red Ale, and 1.040 to 1.050 in Sour.

Overall, the physicochemical response to hemp-flower substitution was parameter- and style-dependent. The clearest substitution-related responses were observed for pH and titratable acidity, whereas ABV and final density showed no overall effect of hemp-flower substitution under the conditions evaluated.

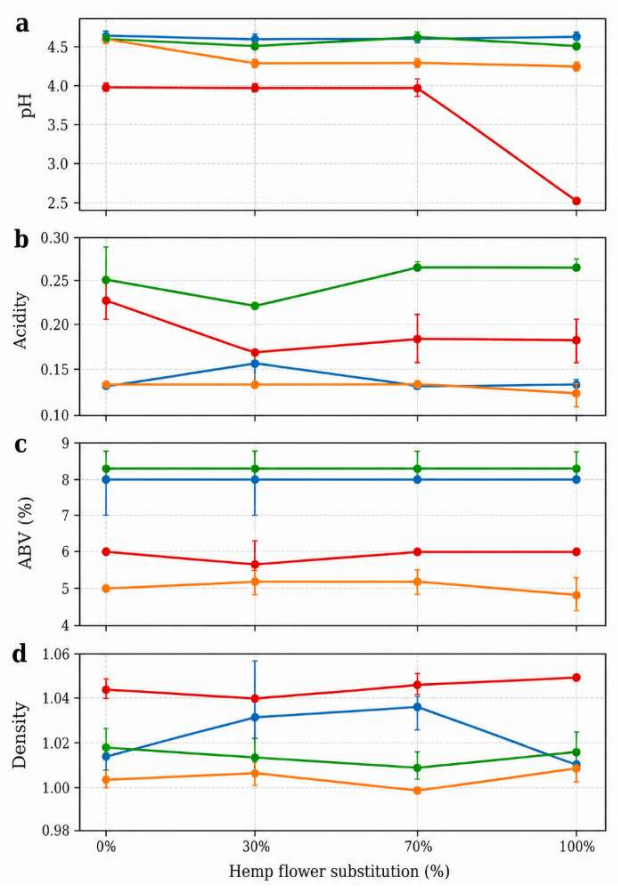

Figure 2. Trends in physicochemical parameters as a function of the level of hop substitution with hemp flower. (a) pH, (b) titratable acidity, (c) alcohol content (% v/v), and (d) final density. Values correspond to the mean ± standard deviation of three independently produced brewing batches (n = 3). The lines represent different beer styles: IPA (blue), Weissbier (orange), Red Ale (green), and Sour (red). The 0% substitution level corresponds to the control containing hops without hemp flower.

Figura 2. Tendências dos parâmetros físico-químicos em função do nível de substituição do lúpulo por flor de cânhamo. (a) pH, (b) acidez titulável, (c) teor alcoólico (% v/v) e (d) densidade final. Os valores correspondem à média ± desvio-padrão de três lotes de produção independentes (n = 3). As linhas representam diferentes estilos de cerveja: IPA (azul), Weissbier (laranja), Red Ale (verde) e Sour (vermelho). O nível de substituição de 0% corresponde ao controle que contém lúpulo, mas sem flor de cânhamo.

3.2. Physicochemical and sensory response patterns

Standardized effect-size heat maps were used to compare the magnitude and direction of physicochemical and sensory responses relative to the corresponding 0% control within each beer style (Figure 3). Cohen's d was used for

physicochemical parameters, whereas Cohen's dz was used for the paired sensory ratings.

The physicochemical effect-size patterns were consistent with the results of the permutation ANOVA. The largest departures from the control were observed for pH, particularly in Weissbier and at 100% substitution in Sour, whereas alcohol content and final density generally showed smaller and less consistent changes across substitution levels. Titratable acidity showed style-dependent responses, with larger negative effects in Sour and variable responses in IPA and Red Ale.

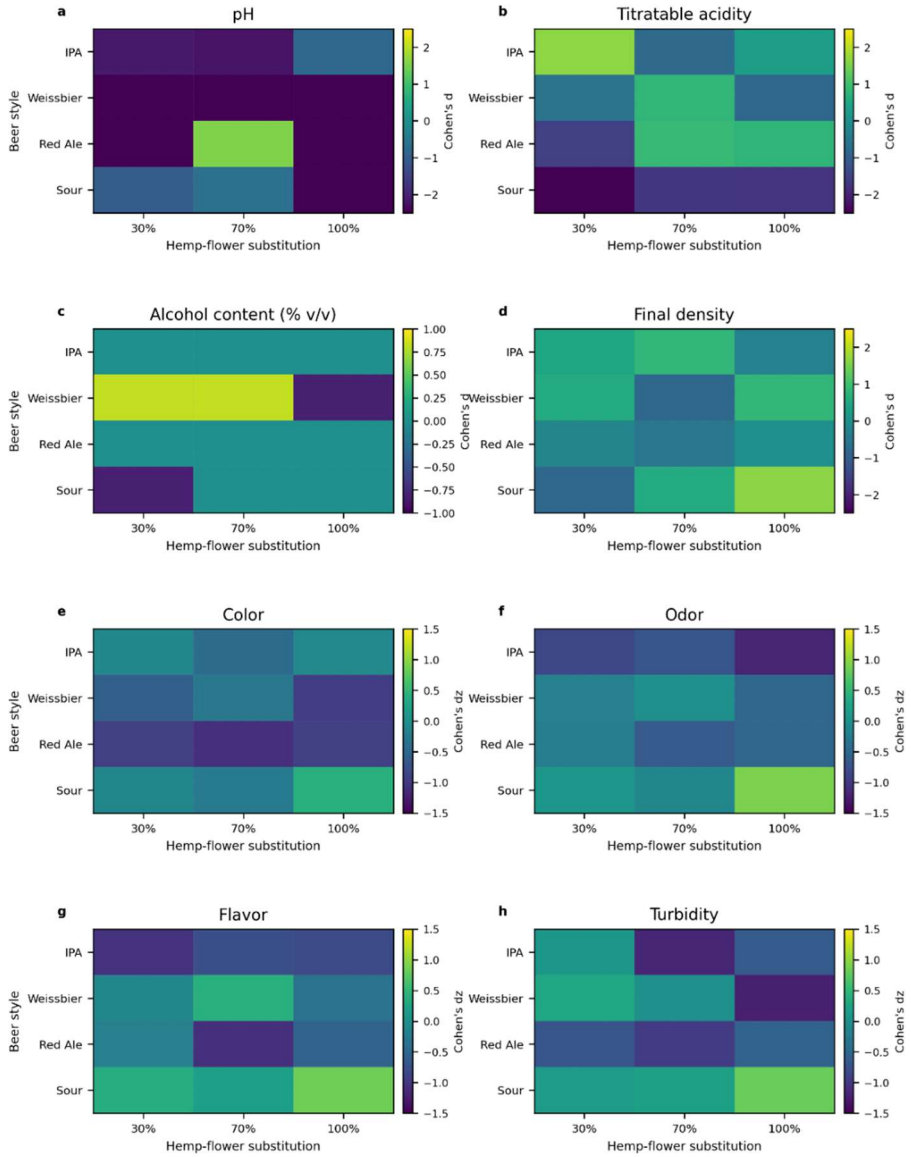

Figure 3. Heat maps of standardized effect sizes relative to the 0% control. Cohen's *d* was used for physicochemical parameters (a–d: pH, titratable acidity, alcohol content, and final density), whereas Cohen's *dz* was used for paired sensory ratings (e–h: color, odor, flavor, and turbidity). For physicochemical parameters, color scales were capped at ±2.5 where indicated for visualization purposes; values beyond these limits were mapped to the scale extremes without altering the calculated effect sizes. Positive and negative values indicate increases and decreases relative to the corresponding control, respectively.

Figura 3. Mapas de calor dos tamanhos de efeito padronizados em relação ao controle 0%. O Cohen's *d* foi utilizado para os parâmetros físico-químicos [(a–d): pH, acidez titulável, teor alcoólico e densidade final], enquanto o Cohen's *dz* foi utilizado para as avaliações sensoriais pareadas [(e–h): cor, odor, sabor e turbidez]. Para os parâmetros físico-químicos, as escalas de cor foram limitadas a ±2,5, quando indicado, apenas para fins de visualização; valores além desses limites foram representados pelos extremos da escala, sem alterar os tamanhos de efeito calculados. Valores positivos e negativos indicam, respectivamente, aumentos e reduções em relação ao controle correspondente.

For sensory acceptability, Friedman tests indicated differences among substitution levels for turbidity, odor, and flavor in IPA; for color, odor, flavor, and turbidity in Weissbier and Red Ale; and for turbidity, odor, and flavor in Sour ($p < 0.05$). No overall differences were detected for color in IPA or Sour ($p > 0.05$). After Benjamini–Hochberg FDR adjustment, no significant differences were detected between either 30 or 70% substitution and the corresponding 0% control for color, odor, flavor, or turbidity in Weissbier and Sour ($q > 0.05$). Within this substitution range, Cohen's dz values were generally small to moderate, ranging from −0.583 to 0.408 in Weissbier and from −0.270 to 0.371 in Sour. Larger departures from the corresponding controls became more evident at 100% substitution.

IPA and Red Ale showed greater sensory changes relative to their controls. In IPA, odor and flavor differed from the control at 30, 70, and 100% substitution after FDR adjustment ($q < 0.05$), while turbidity differed at 70%. In Red Ale, significant treatment–control differences were detected for color at all substitution levels, turbidity at 30 and 70%, and flavor at 70% ($q < 0.05$). ICC(2,k) values ranged from 0.835 to 0.912 across sensory attributes, indicating consistent average panel ratings.

Overall, the sensory response patterns indicated that the 30–70% substitution range produced comparatively limited departures from the corresponding controls in Weissbier and Sour, whereas IPA and Red Ale showed greater sensory sensitivity to hemp-flower substitution. Physicochemical responses remained parameter- and style-dependent, particularly for pH and titratable acidity.

Considering the combined physicochemical and sensory responses, Weissbier with 70% hop substitution was selected for the subsequent environmental valorization phase. This treatment represented a high hemp-flower incorporation level while maintaining comparatively limited sensory departures from its corresponding control. At 70% substitution, no significant differences were detected for color, odor, flavor, or turbidity after FDR adjustment, and the associated effect sizes remained within the small-to-moderate range. From a physicochemical perspective, titratable acidity showed limited variation, alcohol content and final density remained comparatively stable, and the pH observed at 70% substitution was similar to that recorded at 30%. Therefore, the 70% Weissbier formulation was selected as a suitable compromise between achieving a high level of hemp-flower substitution and maintaining the overall physicochemical and sensory characteristics of the beer.

3.3. Phytochemical characterization of the selected post-fermentation brewing residue

The gallic acid calibration curve used for the Folin–Ciocalteu assay showed high linearity ($R^2 = 0.9987$) over the concentration range evaluated. The hydroalcoholic extract obtained from the post-fermentation brewing residue showed an average absorbance of 0.235 at 765 nm, corresponding to a total phenolic content (TPC) of 21 mg GAE g^{-1} of dry residue. These results confirmed the presence of measurable phenolic compounds in the solid residue recovered after the brewing process.

3.4. Valorization of post-fermentation brewing residue

The post-fermentation brewing residue from the selected treatment was used as the precursor material for three adsorbent treatments: dried non-carbonized residue (T1), unmodified biochar (T2), and $FeCl_3$-modified biochar (T3). The following sections present the results of biochar production, the preliminary qualitative assessment of residual Hg^{2+}, pH variation after treatment, and the quantitative evaluation of Hg^{2+} removal.

3.4.1. Production of unmodified and $FeCl_3$-modified biochar

Pyrolysis of the dried post-fermentation brewing residue at 350 °C for 15 min produced the unmodified biochar (T2), characterized macroscopically by a homogeneous black appearance. Based on the dry mass of the material before and after pyrolysis, the biochar yield was approximately 42%. The $FeCl_3$-modified treatment (T3), obtained after impregnation with 0.5 M $FeCl_3$ followed by pyrolysis under the same thermal conditions, exhibited a brown-black appearance. Macroscopic differences in coloration were observed between T2 and T3. Both carbonized materials were subsequently evaluated for Hg^{2+} removal.

3.4.2. Preliminary precipitation assessment of treated filtrates

A preliminary qualitative assessment of the filtrates obtained after contact with T1, T2, and T3 showed differences in the apparent formation of white precipitate following the addition of 6 N HCl. Based on the five-level ordinal scale (1–5), T1 showed the highest mean precipitate score (4.67 ± 0.58), followed by T2 (2.67 ± 0.58), whereas T3 showed the lowest score (1.00 ± 0.00) (Table 1). Thus, the apparent formation of precipitate decreased progressively from T1 to T3. These observations were used exclusively as a preliminary semi-quantitative comparison among treatments and were subsequently complemented by quantitative determination of residual mercury using CV-AAS.

Table 1. Mean apparent precipitate score recorded after addition of 6 N HCl to filtrates from T1–T3 using a five-level ordinal scale (1–5).
Tabela 1. Pontuação média aparente de precipitação observada após a adição de HCl 6 N aos filtrados de T1–T3, utilizando uma escala ordinal de 5 níveis (1–5).

Treatment	Mean apparent precipitate score ± SD
T1	4.67 ± 0.58
T2	2.67 ± 0.58
T3	1.00 ± 0.00

3.4.3. pH variation after treatment with T1–T3

The pH values measured after 48 h of contact showed a gradual variation among the three adsorbent treatments. T1 showed a mean pH of 6.25 ± 0.05, T2 reached 6.55 ± 0.05, and T3 exhibited the highest value at 6.85 ± 0.05. A progressive increase in pH was therefore observed from the dried non-carbonized residue (T1) to the unmodified biochar (T2) and the $FeCl_3$-modified biochar (T3). Despite this variation, the solutions remained within a moderately acidic to near-neutral pH range after treatment. The distribution of the replicate measurements for each treatment is shown in Figure 4.

3.4.4. Evaluation of Hg^{2+} removal efficiency

The quantitative CV-AAS results showed a progressive increase in Hg^{2+} removal efficiency across the three adsorbent treatments (Figure 5). The dried non-carbonized residue (T1) achieved a mean removal efficiency of 94.0 ± 2.0%, corresponding to a measured mean residual Hg concentration (Cf) of 0.60 mg L^{-1}. The unmodified biochar (T2) reached 97.0 ± 1.5% removal, corresponding to a measured Cf of 0.30 mg L^{-1}, whereas the $FeCl_3$-modified biochar (T3) showed the highest removal efficiency, reaching 99.5 ± 0.3%, with a measured residual Hg concentration of 0.05 mg L^{-1}. One-way ANOVA indicated a significant overall effect of adsorbent treatment on Hg^{2+} removal efficiency ($p < 0.05$). Tukey's post hoc test showed significant pairwise differences among T1, T2, and T3, as indicated by the different letters in Figure 5.

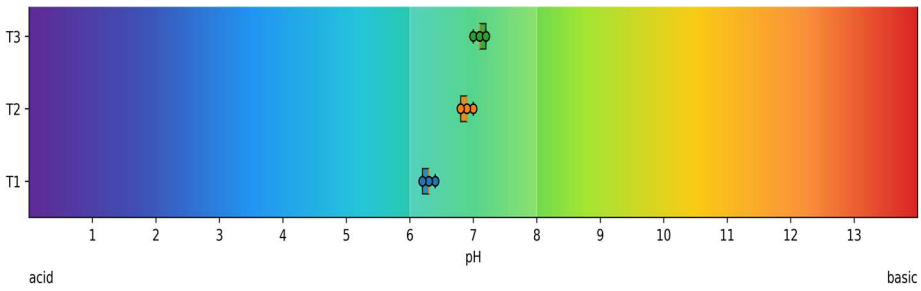

Figure 4. Distribution of pH values measured after 48 h of contact with T1, T2, and T3. Points represent replicate measurements for each adsorbent treatment (n = 3).
Figura 4. Distribuição dos valores de pH medidos após 48 h de contato com T1, T2 e T3. Os pontos representam as medições replicadas para cada tratamento adsorvente (n = 3).

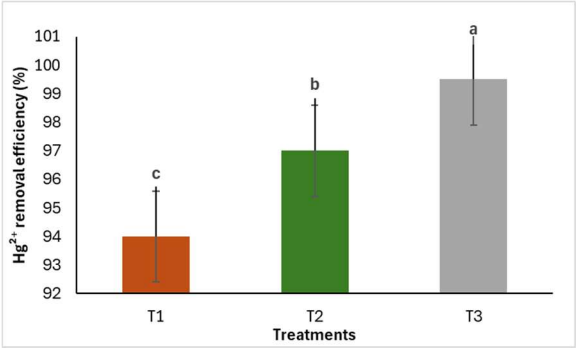

Figure 5. Mean Hg^{2+} removal efficiency for T1, T2, and T3 after 48 h of contact. Error bars represent standard deviation (n = 3). Different letters indicate significant differences among treatments according to Tukey's post hoc test ($p < 0.05$).
Figura 5. Eficiência média de remoção de Hg^{2+} para T1, T2 e T3 após 48 h de contato. As barras de erro representam o desvio padrão (n = 3). Letras diferentes indicam diferenças significativas entre os tratamentos de acordo com o teste post hoc de Tukey ($p < 0,05$).

4. DISCUSSION

The physicochemical response to hop substitution with hemp flower depended on beer style and the parameter evaluated. Significant style × substitution interactions for pH and titratable acidity indicate that hemp incorporation affected the beer matrices differently, which is consistent with differences in malt composition, fermentation microorganisms, wort characteristics, and fermentation biochemistry (PIRES; BRÁNYIK, 2015; HAHN et al., 2018). In contrast, alcohol content and final density showed no significant overall substitution effect or style × substitution interaction, suggesting a more limited influence of hemp substitution on variables associated with fermentative attenuation under the conditions evaluated.

Sensory responses were also style-dependent. In Weissbier and Sour, the 30–70% substitution range produced comparatively limited departures from the respective controls, with no significant treatment–control differences after FDR adjustment and predominantly small-to-moderate paired effect sizes. A possible explanation is the partial overlap in terpene and phenolic constituents reported for hops and hemp, which may facilitate the integration of hemp-derived aromatic and flavor notes in some beer matrices (ERŽEN et al., 2021; OVIDI et al., 2022; HABSCHIED et al., 2025). The sensory complexity of Weissbier and the acidic profile of Sour may also have influenced perceptibility, although these mechanisms were not directly evaluated because individual volatile compounds were not characterized.

IPA and Red Ale showed greater sensory departures from their controls. In IPA, odor and flavor differed at several substitution levels, consistent with the strong contribution of hops to the aromatic profile of this style (HAHN et al., 2018). Red Ale showed treatment-dependent changes in color, turbidity, and flavor. These results reinforce the influence of the beer matrix on the response to hemp incorporation and indicate that one substitution level cannot be generalized across styles. The beer results should nevertheless be interpreted as exploratory: physicochemical measurements were based on three independent batches per treatment and sensory acceptability on 15 untrained adult assessors. Thus, the absence of significant sensory differences does not demonstrate equivalence, and confirmation with additional batches and larger consumer panels is required (LAWLESS; HEYMANN, 2010).

The selected post-fermentation residue retained measurable phenolic compounds, with a total phenolic content of 21 mg GAE g^{-1} dry residue. This value is numerically within the broad range reported for hemp-derived matrices, although comparisons should be cautious because plant fraction, processing history, solvent system, extraction conditions, and analytical procedure influence the measured response (ISIDORE et al., 2021; MOTIEJAUSKAITĖ et al., 2023). Phenolic retention has also been reported in brewing by-products, supporting their consideration for subsequent valorization (BRAVI et al., 2021).

All three adsorbent treatments substantially reduced Hg^{2+} concentration under the evaluated conditions, with removal increasing from T1 (94.0 ± 2.0%) to T2 (97.0 ± 1.5%) and T3 (99.5 ± 0.3%). The same treatment order was observed in the preliminary precipitation assessment, although that test was used only as an exploratory comparison and did not replace CV-AAS. The high removal obtained with T1 shows that carbonization was not required for substantial Hg^{2+} removal. Oxygen-bearing functionalities in lignocellulosic materials can participate in metal retention through surface complexation, ion exchange, and electrostatic interactions (Qiu et al., 2022), but the specific mechanisms operating in T1 were not determined.

The additional increase after pyrolysis and $FeCl_3$ modification suggests that treatment-induced physicochemical changes favored Hg^{2+} retention. Thermal conversion can modify biochar surface chemistry and pore structure, while chemical modification can alter sorption sites

(QIU et al., 2022; ŠOŠTARIĆ et al., 2024). Enhanced Hg(II) retention has specifically been reported for Fe-modified biochars compared with unmodified materials (Feng et al., 2020), supporting the plausibility of the higher removal observed for T3. However, surface area, pore structure, functional groups, and iron speciation were not characterized, so the mechanism cannot be confirmed. Final pH increased from 6.25 ± 0.05 in T1 to 6.55 ± 0.05 in T2 and 6.85 ± 0.05 in T3. Because pH can influence surface protonation and metal–adsorbent interactions, this variation may have contributed to removal behavior, but pH was not independently manipulated, and causality cannot be established.

The environmental phase was limited to one initial Hg^{2+} concentration, one adsorbent dosage, a 48-h contact time, and three replicate assays per treatment. Consequently, the study compares T1–T3 under the tested conditions but does not establish maximum adsorption capacity, kinetics, equilibrium isotherms, or optimal operating conditions. The absence of BET, FTIR, SEM, or XRD characterization also prevents attribution of performance to specific structural or surface changes. Future work should characterize the materials, evaluate wider ranges of Hg^{2+} concentration, dosage, pH, and contact time, and assess regeneration and performance in complex aqueous matrices.

Despite these limitations, the progressive improvement from the dried residue to unmodified and $FeCl_3$-modified biochars provides preliminary evidence for sequential valorization of the brewing by-product. Converting lignocellulosic agri-food residues into carbonaceous materials for environmental applications is consistent with circular-use approaches reported in the literature (ESCUDERO-CURIEL et al., 2023; LIU et al., 2026). Hemp-derived biomass has likewise been investigated in biochar-based valorization pathways (Ottani et al., 2022; Assirelli et al., 2025), supporting the potential integration of brewing product development with subsequent environmental use of the generated residue.

5. CONCLUSIONS

Hop substitution with industrial hemp flower produced style-dependent physicochemical and sensory responses. Weissbier and Sour showed comparatively limited sensory departures from their controls within the 30–70% substitution range, whereas IPA and Red Ale were more sensitive to hemp incorporation. Responses in pH and titratable acidity were also style-dependent, while no overall substitution effect was detected for alcohol content or final density. These results support the feasibility of moderate hemp-flower substitution in selected craft beer styles under the conditions evaluated, although non-significant sensory differences should not be interpreted as equivalence.

The selected post-fermentation residue contained 21 mg GAE g^{-1} dry residue of total phenolic compounds and served a precursor for the evaluated adsorbents. Hg^{2+} removal increased from 94.0 ± 2.0% for dried non-carbonized residue (T1) to 97.0 ± 1.5% for unmodified biochar (T2) and 99.5 ± 0.3% for $FeCl_3$-modified biochar (T3), with T3 showing the highest performance under the tested conditions.

Overall, the results support a sequential valorization pathway in which industrial hemp is first used as a partial hop substitute and the resulting post-fermentation residue is subsequently evaluated for Hg^{2+} removal. The adsorption findings remain specific to the tested concentration, dosage, contact time, and replicate number. Material characterization, adsorption kinetics and equilibrium studies, broader operating conditions, regeneration tests, and validation in complex aqueous matrices are required before practical water-treatment applications can be established.

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Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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