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RESEARCH ARTICLE

Constraints using the liquid fraction from roadside grass as a bio-based fertilizer

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Abstract

Background: Roadside grass cuttings are currently considered a waste product due to their association with road sweepings as contaminated waste, therefore, their potential as a biofertilizer is understudied.

Aim: This study aimed to determine whether grass liquid fraction (GLF) collected from a roadside verge in Maldegem, Belgium, and pressed using a screw press was suitable as a biofertilizer.

Methods: The characterization of the heavy metal content of the GLF was conducted using an ICP-OES. From May to September 2019, a pot experiment was set up using a randomized block design to compare tomato plant growth, yield, and nutrition for GLF-treated plants to two commercial fertilizers and tap water as a control.

Results: The heavy metal content of the GLF was below the maximum permissible concentrations (MPCs) for organic fertilizers as set out by the European Comission fertilizer regulation 1069/2009 and 1107/2009 (European Comission, 2019). However, despite having a fairly well-balanced nutrient content (0.1% N, 0.04% P_2O_5, and 0.2% K_2O), GLF had a negative effect on the growth, root weight, and yield of the tomato plants, killing six out of ten plants. GLF also promoted mold growth in the soil of some plants. Since the GLF was uncontaminated, heavy metal toxicity did not cause the negative effect.

Conclusions: Previous research showed that liquid fractions from some plants negatively affect the growth of others due to allelopathic chemicals; this, together with the stimulation of fungal growth, could have caused the negative effects observed. Future experiments will investigate the herbicidal property of GLF and possible treatments to potentially recover the nutrients contained within the GLF for application as a biofertilizer.

KEYWORDS
allelopathy, biofertilizer, circular economy, microbiota, tomato

1 | INTRODUCTION

Roadside grass needs to be cut at least twice per year for safety reasons, and several EU member state legislations impose a “cut-and-collect” regime, where the grass clippings have to be collected to improve the biodiversity of the roadside verges (Noordijk et al., 2009). This generates a significant amount of biomass that is currently seen as waste according to the EU Waste Framework Directive

(2008/98/EC), with only a small part being valorized into compost, a low-value product. There is, therefore, a huge potential to add a large volume of biomass to the European industry as a feedstock for higher-value products (Meyer et al., 2014), contributing to the European Commission's Bioeconomy Strategy, in which renewable biological resources are understood as essential for achieving the goals of the UN's 2030 Agenda for Sustainable Development.

One of the main value chains currently investigated for grass is the production of materials from the fiber fraction such as paper, insulation panels, biocomposites, and others. This usually entails a first fractionation step, where the solid and liquid fractions are separated (Mandl, 2010). The liquid fraction can account for up to 60% of the total fresh weight of the initial biomass (Sharma et al., 2012) and is rich in soluble nutrients, having been investigated as a growing medium for microorganisms, as a protein source, and as a substrate for biogas production (Mandl, 2010). However, such studies are still scarce and deal mostly with grasslands, with only one study reporting on the use of the grass liquid fraction (GLF) for biomethane production (Piepenschneider et al., 2016).

Since between 30 and 85% of the NPK content of grass can be found in the liquid fraction after pressing (Wachendorf et al., 2009) and, being a plant-based stream, nutrients are expected to already be in an adequate proportion for plant growth, this stream was considered suitable for application as a bio-based fertilizer. This approach is in agreement with the new Circular Economy Action Plan published in 2020 by the European Commission (EC) (2020), where it is stated that the EC wishes to ensure more sustainable application of nutrients and stimulate the markets for recovered nutrients. A fertilizer economy with its foundations in bio-based liquids would not only result in a sustainable nutrient cycle but could also reduce the carbon footprint of production when compared to inorganic mineral fertilizers (Chojnacka et al., 2020; Vaneeckhaute, Meers, Michels, Buysse, et al., 2013; Vaneeckhaute, Meers, Michels, Ghekiere, et al., 2013). However, the organic fertilizer is required to be below the European Comission fertilizer regulation 1069/2009 and 1107/2009 (European Comission, 2019) maximum permissible concentrations (MPCs) of potentially harmful metals (Cu, Cd, Cr, Pb, Ni, Zn, and Hg must be present in concentrations of less than 300, 1.5, 2, 120, 50, 800, and 1 mg kg^{-1}, respectively).

Therefore, in the present work, GLF was investigated as a bio-based plant fertilizer. Due to the significantly lower potential volumes of fertilizer production from grass if compared with the current mineral fertilizer market, the investigation was directed to indoor horticulture. An initial nutrient analysis showed that the grass liquid's concentration of NPK was similar to a tomato plant feed, which is largely cultivated in greenhouses in Belgium and the UK. The main objective of this study was, therefore, to investigate the liquid fraction of roadside grass as a liquid tomato feed, analyzing its effect on tomato plant growth, nutrient levels, and yield compared to two other liquid feeds currently in the market.

2 | MATERIAL AND METHODS

2.1 | Liquid grass production and experimental setup

The grass clippings used to obtain the liquid fraction of grass were cut and collected in June 2019 from the first meter (closest to the road) of a roadside verge on a 2.4 km street with low-to-medium traffic (Dijkstraat, Maldegem, Belgium). It was cut using a Steyr tractor with a flail mowing head (Vandaele flail head 150, head pro 680 arm, Belgium) and placed through a screw press (Rhinetech, the Netherlands) to separate the grass liquid from the fiber fraction. The liquid fraction was collected in large plastic containers, which were transferred to England within 24 h and stored at 4°C until required.

A randomized block design greenhouse study using tomatoes (Gardener's Delight, *Solanum lycopersicum*) was conducted between May and September 2019. The tomato plants were grown from seed in seed compost (Gro-sure, Westland, UK) in the greenhouse (max. temp. 40°C, min. temp. 10°C). After 3 weeks of growth, 44 of the dominant seedlings were transferred to 9-cm pots filled with compost (Levington F2 seed and modular compost, UK). When the first flowers started to appear 3 weeks later, the plants were transplanted to 30-cm pots filled with a Kettering loam soil (Norfolk Kettering Loam). This was found to be a clay loam (27% sand, 33% silt, and 40% clay, $n = 2$, Bouyoucos hydrometer method) using the soil World Reference Base standards (Food and Agriculture Organization of the United Nations, 2015). Each plant was subjected to one of four treatments: (1) water control, (2) Tomorite^{TM}, (3) Verve^{TM}, or (4) GLF. Tomorite^{TM} is an inorganic feed enriched with seaweed extract, while Verve^{TM} is an organic feed (seaweed-based) enriched with inorganic nutrients. The experiment was composed of 10 blocks of four plants, with each plant receiving one of the four treatments. The plants were trained around bamboo canes, suckers were pruned each week, and the plant height was limited to 130 cm.

2.2 | Treatments

The GLF was filtered (Whatman® Grade 1 paper filter, Merck) before use to remove the suspended solids, and its nutrient content was analyzed before the experiment began. Total N was assessed using the Kjeldahl method (Van Ranst et al., 1999) (analysis conducted by Innolab, Belgium) and total P and K were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES; analysis conducted by Innolab) following the EPA 3051A norm (United States Environmental Protection Agency, 1998). To match the N concentration of the GLF with the N concentration of the inorganic feed (Tomorite), which provides 0.4 g of *N* per feed as per the instructions on the bottle, 310 mL of GLF were added to each tomato plant per feed. The organic feed (Verve) provided 0.3 g of N per plant per feed, as per the instructions on the bottle. The GLF provided less P and K (0.126 g P and 0.7 g K) than the inorganic feed (0.3 g P and 0.8 g K) but more than the organic feed (0.075 g P and 0.5 g K) per feed. All feeds were diluted to 2.25 L with tap water and 2.25 L of water was given to the plants in the water control treatment per feed. The plants were fed on a rotating 2-week cycle: Monday and Friday in week 1 and Wednesday in week 2, giving an average of 0.6 g of N per week for the GLF and inorganic feed treatment and 0.45 g of N per week for organic feed as per the instructions on the bottle. During the hottest weeks, the plants were also watered between these feeds. During the course of the experiment, it was observed that the GLF was not taken up by the tomato plants, pooling instead in the pot saucer, in contrast with the other fertilizers, which were completely taken up by the plants within 24 h of feeding. The saucers were drained of the GLF 24 h after each feed to prevent waterlogging of the soil. The pH (pH meter, Orion Star A211, Thermo Scientific, USA) of the GLF was 4.5 while the commercial feeds had a pH of around 7, but this was not deemed to be detrimental, as low pH nutrient solutions have been used in other tomato nutrient research (Heeb et al., 2005, 2006).

2.3 | GLF macronutrients, aluminum, and heavy metal analysis

The macronutrients other than NPK (Ca, Mg, Na, S), aluminum, and heavy metal concentrations (Cr, Cu, Cd, Fe, Pb, Ni, Mn, Co, Zn) of the GLF were measured using ICP-OES (Premier Analytical Services, UK) and Hg was measured using a direct mercury analyzer (Milestone DMA80, Italy; analysis conducted at Premier Analytical Services). BCR®-certified reference material was used to ensure measurements on the ICP-OES were accurate (Merck, Germany). Total organic carbon was measured with a TOC device that used catalytic oxidation combustion to convert organic carbon into measurable CO_2 (Shimadzu, Japan).

2.4 | Tomato plant growth and tomato harvest

The height (up to 130 cm) and the number of flowers per tomato plant were recorded once a week for the first month of the experiment. The leaf color grade was also noted over the course of the whole experiment as a measure of plant health, where 5 = dark green, 4 = green, 3 = light green, 2 = greeny-yellow, 1 = yellow, 0 = browny-yellow, adapted from leaf color charts by Varinderpal-Singh et al. (2010). Tomatoes were harvested for 5 weeks starting from day 96. Only ripe tomatoes at maturity stage 6, which corresponds to 90% of the tomato being red, were picked (Gierson & Kader, 1986). The number and diameter of the fruits were recorded, and the dry weight of the tomatoes was calculated by oven-drying at 60°C for 48 h. The final harvest of all the green tomatoes was completed on day 131, and their number and dry weight were recorded. At the end of the experiment, the roots were cut from the tomato plant and cleaned of soil by gentle washing. The dry weight of the roots was recorded after oven drying at 60°C for 48 h.

2.5 | Tomato and leaf analyses

The nutrient and heavy metal concentrations of the ripe tomatoes were analyzed. Duma N was measured by an Elementar Rapid Max N Exceed DUMAS system (analysis conducted at Premier Analytical Services) and the total macronutrient and heavy metal content were analyzed using ICP-OES (Perkin Elmer Optima 8000, USA) following microwave digestion (Berghof, Speedwave, Germany) of 500 mg of oven-dried tomato in 6 mL HNO_3 (65%), following the manufacturer's recommendation.

Leaf nutrient concentration was also assessed; Duma N was measured using the above method and total P and K were analyzed using ICP-OES (Perkin Elmer Optima 8000, Germany) following microwave digestion (Berghof Speedwave) of 400 mg of leaf sample in 5 mL HNO_3 (65%) and 3 mL H_2O_2 (35%), following the manufacturer's recommendation.

2.6 | Soil analysis

At the end of the experiment, a pooled sample of soil taken from the top, middle, and bottom of each pot was analyzed for total N, P, K, heavy metals, and KCl-extracted nitrate and ammonium. The pH, electrical conductivity (EC), and organic matter content were also measured. Total N was determined using the Kjeldahl procedure (DET, Kent, UK). Phosphorous, K, and the heavy metals were analyzed using ICP-OES (Perkin Elmer Optima 8000, USA) after oven drying the soil (105°C for 24 h) and following hot plate digestion of 1 g of soil in aqua regia solution (2.5 mL of HNO_3 [65%] and 7.5 mL of HCl [37%]). A total of 150 mL of 1 M KCl was added to 30 g of soil, shaken for 1 h (180 rpm, New Brunswick Scientific, UK), filtered, and analyzed for the nitrate-N and ammonium-N concentration by discrete analysis (Gallery discrete analyzer, Thermo Scientific, UK). For pH and EC analysis, 40 mL of distilled water was added to 10 g of soil and shaken for 2 h (180 rpm, New Brunswick Scientific), then allowed to settle for 10 minutes. The pH was measured using a pH probe (HI9126, Hanna Instruments, UK) and EC with a conductivity probe (Orion, Thermo Scientific, USA). The soil organic matter content was measured by the loss on ignition method; 2 g of oven-dried soil was added to crucibles and placed into a muffle furnace (Carbolite, UK) at 500°C for 5 h (De Leenheer et al., 1957).

2.7 | Statistical analysis

A one-way ANOVA followed by a post-hoc Tukey test was used to identify significant differences between the means of the four different treatments. A Kruskal–Wallis test was carried out on the leaf color grade from different treatments, and a Chi-squared test was used to understand statistical differences between the total number of tomatoes produced.

TABLE 1 Macronutrient and heavy metal content of the grass liquid fraction ($n = 3$).

	GLF (mg kg^{-1} FM)	GLF (mg kg^{-1} DM)	MPC (mg kg^{-1} DM)
N	1280 ± 100	38788 ± 3030	
P_2O_5	406 ± 5	12303 ± 152	
K_2O	2260 ± 126	68485 ± 3818	
Ca	1160 ± 52	35151 ± 1575	
Mg	376 ± 15	11393 ± 454	
S	258 ± 13	7828 ± 393	
Na	219 ± 6	6636 ± 182	
Al	5.54 ± 0.99	167 ± 30	–
Fe	6.98 ± 0.73	211 ± 22	–
Co	0.20 ± 0.01	6.1 ± 0.4	–
Mn	6.17 ± 0.15	187 ± 4	–
Cu	0.59 ± 0.34	18 ± 10	300
Cd	0.07 ± 0.02	2.2 ± 0.7	1.5
Cr	0.08 ± 0.03	2.4 ± 0.9	2
Pb	0.50 ± 0.25	15 ± 8	120
Ni	0.23 ± 0.01	7.0 ± 0.2	50
Zn	4.93 ± 0.15	149 ± 4	800
Hg	0.00 ± 0.00	0.03 ± 0.00	1

Heavy metals are compared to maximum permissible concentration (MPC) of fertilizers according to the new European Comission fertilizer regulation 1069/2009 and 1107/2009 (European Comission, 2019).

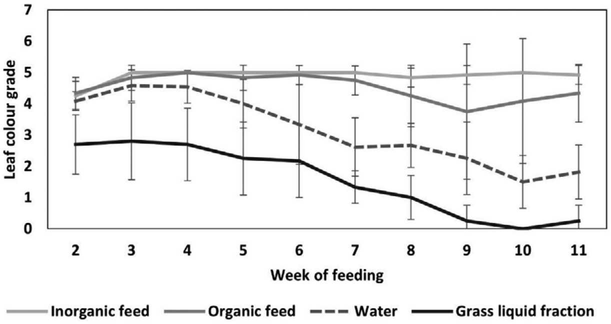

FIGURE 1 Leaf color grade from the four different treatments over the course of the experiment. 5 = dark green, 4 = green, 3 = light green, 2 = greeny-yellow, 1 = yellow, 0 = browny-yellow.

3 | RESULTS

3.1 | Suitability of the GLF as a liquid organic fertilizer

The new European Comission fertilizer regulation 1069/2009 and 1107/2009 dictate the minimum amount of nutrients that a product must have to be recognized as a liquid organic fertilizer (European Commission, 2019). According to this legislation, when a liquid organic fertilizer has more than one primary nutrient, which is the case of GLF, (1) it must contain at least 1% of N, P_2O_5, or K_2O, (2) the sum of those nutrient contents has to be at least 3%, and (3) its organic carbon content has to be at least 5%. GLF has only an N content of 0.1%, a P_2O_5 content of 0.04%, a K_2O content of 0.2% (Table 1), a sum of NPK of 0.34%, and an organic carbon content of 3%, which means that this stream would need to be concentrated 5× (based on K_2O content) to be considered a marketable liquid organic fertilizer. Nevertheless, its NPK ratio of 3:1:6 is close to the proportion found in commercial tomato feeds, 3:1.5:5 for an organic feed, and 2:1.5:4 for an inorganic feed, indicating that the GLF might be a suitable tomato feed.

One of the reasons roadside grass is considered a waste while clippings issued from natural grasslands are considered a feedstock is a fear that grass grown on roadside verges will be contaminated by aluminum and heavy metals emitted by vehicles, which will then be transferred to products derived from this material. Therefore, a characterization of the aluminum and heavy metal content of the GLF was conducted and compared against current legislation for organic fertilizers. Table 1 shows the aluminum and heavy metal concentrations of GLF compared to the MPCs for organic fertilizers as set out by the new European Comission (2019).

The concentrations found in GLF were much lower than the MPC for most of the tested metals, except Cd and Cr, which were still within the maximum amount when the standard deviation was considered. To the best of our knowledge, there is no previous research on the aluminum and heavy metal content of a liquid fraction made from roadside vegetation, and further investigation is needed to understand the impact of the location of the roadside verge on the aluminum and heavy metal content of GLF. The proximity to farmland could also affect the heavy metal and metalloid content of GLF, since synthetic fertilizers, biosolids and manures, herbicides, pesticides, and wastewater applied to arable land has been associated with elevated concentrations of Co, Cu, Cd, Cr, Pb, Ni, and Zn, amongst others (Wuana & Okieman, 2011). In this experiment, part of the road verge was next to arable and pastoral land. Although the elevation of the verge means that run-off is unlikely, accidental contamination (i.e., during spraying) is possible. Nevertheless, our results indicate that this stream can be used as a fertilizer after a concentration step, following the new European Comission fertilizer regulation 1069/2009 and 1107/2009.

3.2 | Plant health assessment and tomato production

In the early stages of the experiment, the leaves of the tomato plants fed with GLF showed signs of yellowing (grades 2–3) whereas the plants growing in the other treatments had green or dark green leaves (grades 4–5) (Figure 1). The plants of the water control showed signs of mild chlorosis (light green, grade 3) after 4 weeks and moderate chlorosis (grade 2) by the end of the experiment (Figure 1). The tomato plants of the inorganic feed treatment, however, remained healthy with dark green leaves throughout the whole experiment (grade 5) and those given the organic feed had dark green leaves during the experiment but green leaves at the end. This contrasts with the plants given the GLF, all of which had brownish-yellow leaves by the end of the experiment.

TABLE 2 Average height and number of flowers per plant at week 3 ± standard deviation ($n = 10$).

	Water	Inorganic feed	Organic feed	Grass liquid fraction
Height (cm) after 3 weeks	123.5 ± 10.1A	126.3 ± 10.5A	127.4 ± 8.6A	111.4 ± 12.9B
Number of flowers after 3 weeks	22.6 ± 4.5AB	24.3 ± 3.2A	22.9 ± 7.1AB	17.2 ± 5.5B
Total number of ripe tomatoes per treatment	179	189	178	75*
Total number of ripe and unripe tomatoes	240	285	274	95*
Average number of ripe tomatoes per plant	17.9 ± 3.45AB	18.9 ± 4.03A	17.8 ± 4.34AB	12.5 ± 5.40B
Average number of ripe and unripe per plant	24.0 ± 3.46A	28.5 ± 6.41A	27.4 ± 4.27A	15.8 ± 8.13B
Average dry weight (g) of ripe tomatoes per plant	40.9 ± 8.80A	38.0 ± 8.62A	39.1 ± 8.62A	24.31 ± 10.10B
Average diameter (cm) of ripe tomatoes per plant	35.2 ± 1.26A	35.1 ± 1.43A	34.2 ± 2.12A	30.41 ± 1.61B
Average dry weight (g) of roots per plant	37.0 ± 7.28A	43.8 ± 19.6A	49.5 ± 16.9A	4.4 ± 1.88B

Tomato harvest ($n = 10$ for all treatments apart from GLF, where $n = 6$), dry weight of ripe tomatoes per plant, and average diameter of ripe tomatoes per plant. Dry weight of roots ± standard deviation ($n = 10$). Different letters show significant differences between treatments according to a one way ANOVA followed by a post hoc Tukey test. * shows a significant difference according to a Chi-squared test.

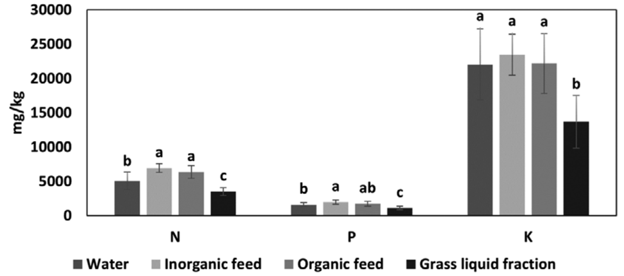

FIGURE 2 Leaf nutrient content from the four different treatments at week 3 of feeding. Error bars show standard deviation, $n = 4$. Different letters on "B" show significant differences between treatments according to a one-way ANOVA followed by a post-hoc Tukey test.

On day 60, after 3 weeks of feeding, when differences in the health of the plants became apparent, leaf samples were taken from the lower part of the plants for nutrient analysis, and the results can be seen in Figure 2. Both commercial fertilizers significantly ($p < 0.05$) increased the leaf N compared to the control, and the inorganic feed also significantly ($p < 0.05$) increased the leaf P compared to the control, concurring with the dark green leaves found in these treatments. Leaves harvested from tomato plants from the GLF treatment contained significantly ($p < 0.05$) less N, P, and K than all the other treatments including the water control (Figure 2). This can explain their yellowish color, as N deficiency results in less production of chlorophyll and yellowing of leaves (Rezende Fontes & de Araujo, 2006).

Table 2 compares the plants' height and the number of flowers after 3 weeks of feeding and the results of the tomato harvest and root dry weight obtained at the end of the experiment. Interestingly, there were no significant differences for any of the measured parameters between the inorganic feed, organic feed, and water treatments. The plants fed with GLF were significantly shorter than all the other treatments ($p < 0.05$). They had a similar number of flowers when compared to the control treatment and the plants treated with the organic feed but significantly fewer when compared to the inorganic feed ($p < 0.05$).

Within 2 weeks of feeding the plants, mold started to grow on the soil of seven out of 10 GLF plants, and, after 3 weeks, the mold was approximately 1 inch deep on the soil of four of the GLF pots (Supporting Information Figure S1). By the fourth week of feeding, two GLF plants had died (all the leaves were brown and shriveled and the branches were yellow) and were removed from the greenhouse, and further GLF plants died on the fifth, sixth, seventh, and ninth week of feeding. At the end of the experiment, six out of 10 tomato plants fed with GLF had died and the four left were in very bad health, but this was not directly correlated with the presence of mold, as some plants where mold was not observed also died. Even though the GLF caused plant death, all of the surviving plants fed with GLF produced tomatoes (Table 2).

Four out of 10 GLF tomato plants died before producing any tomatoes, leaving six tomato-producing plants in total, two of which died after producing just seven tomatoes each. Over the course of the experiment, the number of ripe GLF tomatoes peaked earlier than the other treatments, at seven weeks after feeding (31 tomatoes produced by five tomato plants), after which the number of tomatoes produced reduced considerably (Supporting Information Figure S2). The total number of tomatoes produced by the GLF plants was significantly lower than the number of fruits obtained in all other treatments ($p < 0.05$); however, when considering the lower number of GLF plants that survived and produced tomatoes, the tomato production per plant was similar for the GLF, the water, and the plant-based fertilizer treatments (Table 2). However, the dry weight and diameter of the GLF tomatoes per plant were significantly lower than the other treatments ($p < 0.01$, $p < 0.001$, respectively).

Finally, the roots of the GLF plants also weighed significantly less ($p < 0.05$) than the other treatments (Table 3). The size difference was particularly noted when removing the dead tomato plants from the greenhouse; the roots had not grown since the plant was transplanted from the 9-to-30-cm pot just before feeding was started (Supporting Information Figure S3).

TABLE 3 Total nutrient and heavy metal concentration ± standard deviation of tomatoes taken from the four different treatments ($n = 6$).

(mg kg^{-1})	Water	Inorganic feed	Organic feed	Grass liquid fraction
N	15271.1 ± 1098.1**B**	24514.5 ± 1406.4**A**	22442.9 ± 1798.9**A**	16257.1 ± 3146.9**B**
P	1400.4 ± 278.4**B**	1983.2 ± 335.4**A**	1791.6 ± 148.0A**B**	1607.3 ± 245.4A**B**
K	24806.9 ± 2571	24542.6 ± 2234	26804.5 ± 2312	28450.4 ± 3382
Ca	1284.3 ± 202.6	1185.2 ± 168.9	1362.3 ± 106.8	1188.9 ± 214.0
Mg	794.7 ± 49.3	627.4 ± 62.4	698.2 ± 90.3	712.0 ± 122.7
S	1358.4 ± 102.1**B**	1504.4 ± 165.9**A**	1520.9 ± 115.6**A**	1238.1 ± 143.3**B**
Na	153.3 ± 19.8**B**	237.8 ± 87.6A**B**	193.8 ± 63.8**B**	318.3 ± 88.2**A**
Co	13.7 ± 2.82	14.0 ± 1.84	12.2 ± 1.35	12.2 ± 1.51
Cu	15.2 ± 2.4	14.0 ± 0.80	18.8 ± 2.0	13.9 ± 2.63
Cd	1.56 ± 0.24	0.80 ± 0.24	1.38 ± 0.54	1.00 ± 0.31
Cr	42.3 ± 2.27	45.8 ± 1.60	43.6 ± 1.88	41.2 ± 2.52
Pb	25.6 ± 3.85	25.6 ± 4.21	23.7 ± 4.38	24.9 ± 4.43
Ni	30.3 ± 1.72	30.5 ± 1.5	30.2 ± 2.11	28.6 ± 1.97
Zn	76.8 ± 5.67	80.5 ± 3.46	83.1 ± 10.1	77.8 ± 4.61

Different letters show statistical ($p < 0.05$) differences between the treatments for each nutrient and heavy metal according to a Tukey post-hoc test after a one-way ANOVA. Rows without letters are not significantly different.

3.3 | Tomato nutrient and heavy metal analysis

The tomato nutrient and heavy metal results are summarized in Table 3. The NPK content of the tomatoes produced by plants from the GLF treatment was not significantly different from the water control. However, when compared with the commercial feeds, the GLF treatment resulted in tomatoes with significantly ($p < 0.05$) lower N content. Micronutrient concentrations were not especially affected by the GLF treatment in comparison to the control and both commercial feeds tested. Finally, the heavy metal concentrations in the tomatoes grown in different treatments were not significantly different from each other, concurring with the low heavy metal contents found in the GLF (Table 1).

3.4 | Understanding the effect of GLF on plant health

The use of GLF as a tomato feed caused slow growth of the tomato plants, severe chlorosis of the leaves, the death of six out of 10 tomato plants, and severely poor plant health in the other four. It decreased the tomato yield per plant and the average size of the ripe tomatoes harvested compared to all the other treatments including the water control. To better understand these, a full characterization of the soil at the end of the experiment was conducted.

The pH of GLF was much lower (4.5) when compared to the commercial fertilizers used (around 7). Nevertheless, the final pH of the soil was similar in the different treatments (6.9—7.1) (Table 4). Regarding EC, the GLF treatment resulted in a significantly higher final value when compared to the other treatments ($p < 0.05$), even though all values were in the same order of magnitude. Tomato plants fed with GLF were suffering from N deficiency, which was confirmed by the analysis of the leaves after 3 weeks of feeding (Figure 2). The results showed that GLF plants had even lower N levels in their leaves than plants grown solely on water. However, soil analysis indicated that the N content for the soil at the end of the four treatments was statistically similar ($p > 0.05$) and the GLF treatment even had higher ammonium concentrations than the other treatments (Table 4). Finally, the fungus growing in the GLF soil was sampled, observed under a microscope, and found to be a common phytopathogenic fungus belonging to the genus *Aspergillus*.

4 | DISCUSSION

4.1 | Causes of the negative effects of GLF on plant health

Possible causes for these negative effects on tomato plant growth, yield, and nutrition could be attributed to (1) pH, EC, aluminum and heavy metal toxicity; (2) N bioavailability; (3) stimulation of growth of pathogens or unbalance in the soil microbiota; and/or (4) presence of allelopathic chemicals.

4.2 | pH, EC, aluminum, and heavy metal toxicity

Since the final pH of the soil was similar in the different treatments, the soil buffer system was able to overcome the low pH and maintain an adequate value for plant growth. Similarly, most studies

TABLE 4 Properties, nutrient concentration, and heavy metal concentration of soil from the four different treatments ± standard deviation, ($n = 10$ for all treatments apart from the grass liquid fraction, in which $n = 6$).

	Water	Inorganic feed	Organic feed	Grass liquid fraction
pH	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.2
EC (µS cm^{-1})	259 ± 83C	538 ± 158B	476 ± 50B	846 ± 245A
Organic matter content (%)	4.74 ± 0.31B	5.02 ± 0.43AB	5.45 ± 0.43A	5.4 ± 0.30A
N (g kg^{-1})	1.83 ± 0.41	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Ammonium N (mg kg^{-1})	8.27 ± 1.38B	9.90 ± 3.05B	9.52 ± 0.96B	61.6 ± 13.2A
Nitrate N (mg kg^{-1})	0.32 ± 0.36B	47.1 ± 21.9A	16.5 ± 13.1B	4.51 ± 5.07B
P (g kg^{-1})	0.72 ± 0.04C	0.97 ± 0.06A	0.79 ± 0.05BC	0.81 ± 0.09B
K (g kg^{-1})	3.49 ± 0.26C	4.37 ± 0.22B	3.84 ± 0.23C	5.08 ± 0.50A
Ca (g kg^{-1})	11.46 ± 0.968	11.83 ± 0.335	11.28 ± 0.689	12.10 ± 1.02
Mg (g kg^{-1})	2.764 ± 0.167	2.901 ± 0.134	2.774 ± 0.152	2.848 ± 0.215
S (g kg^{-1})	0.35 ± 0.03B	0.34 ± 0.02B	0.37 ± 0.06B	0.48 ± 0.04A
Na (mg kg^{-1})	46.7 ± 16.9C	140.3 ± 38.8A	96.3 ± 19.1B	109.9 ± 36.8AB
Co (mg kg^{-1})	13.8 ± 2.83	14.0 ± 1.83	12.2 ± 1.35	12.2 ± 1.51
Cu (mg kg^{-1})	15.2 ± 2.41	14.0 ± 0.80	18.6 ± 2.00	13.9 ± 2.63
Cd (mg kg^{-1})	1.56 ± 0.24	0.80 ± 0.24	1.38 ± 0.54	1.00 ± 0.31
Cr (mg kg^{-1})	42.3 ± 2.26	45.8 ± 1.60	43.6 ± 1.88	41.2 ± 2.52
Pb (mg kg^{-1})	25.6 ± 3.85	25.6 ± 4.21	23.7 ± 4.38	24.9 ± 4.43
Ni (mg kg^{-1})	30.4 ± 1.72	30.5 ± 1.50	30.2 ± 2.11	28.6 ± 1.97
Zn (mg kg^{-1})	76.8 ± 5.67	80.5 ± 3.46	83.1 ± 10.0	77.7 ± 4.61

Different letters show significant differences ($p < 0.05$) between treatments for each nutrient and heavy metal across the rows according to a one-way ANOVA followed by a post-hoc Tukey test. Rows without letters are not significantly different.

examine the effect of different ECs on the nutrient solution for tomatoes grown hydroponically. In this configuration, EC values between 2000 and 5000 µS cm^{-1} are recommended; therefore, the slight increase in soil EC caused by the GLF treatment was probably not responsible for the negative results obtained (Dorai et al., 2001).

Much research has been conducted on the heavy metal contamination of roadside soil (Bäckström et al., 2004; Pagotto et al., 2001; Wang & Zhang, 2018; Wawer et al., 2015; Werkenthin et al., 2014) and a limited number of papers have also evaluated the heavy metal content of the roadside vegetation itself (Garcia & Millán, 1998; Kalavrouziotis et al., 2007; Meyer et al., 2014; Mosweu & Letshwenyo, 2013; Piepenschneider et al., 2015; Shephard et al., 2022), concluding that the contamination of the roadside vegetation was low and not exceeding legislative levels for agricultural fertilizer use and as animal feed. The present paper found that concentrations of heavy metals were below the European Comission fertilizer regulation 1069/2009 and 1107/2009 (European Comission, 2019) maximum permissible limits in fertilizer (Table 1). The concentrations of heavy metals were also not elevated in the tomatoes from plants fed with GLF (Table 3) or in the soil to which the GLF was added (Table 4). Therefore, heavy metal toxicity was also excluded as the factor responsible for the detrimental results found with GLF.

4.3 | N bioavailability

Most of GLF nitrogen content is not present in the form of ammonium and nitrate, but rather as proteins (total N: 1280 mg L^{-1}; N-NH_4: 95 mg L^{-1}; N-NO_3: 160 mg L^{-1}). Therefore, the high ammonium concentration found in the GLF soil could be resulting from amino acid degradation (Jones & Kielland, 2012) and it is an indication that protein conversion was taking place in the soil. Tomato plants can take up both ammonium acids and ammonium, and even though nitrate seems to be the preferred form (Ge et al., 2009), it does not seem likely that a lack of bioavailable N was responsible for the negative results obtained. Root damage was probably the reason why nutrients were not being properly taken up by the GLF plants, as the roots from the GLF treatment were not able to grow beyond their initial size (Table 2).

4.4 | Stimulation of the growth of pathogens or unbalance in the soil microbiota

Previous studies have shown that *Aspergillus* can reduce root growth and can also release cell-wall degrading enzymes (Bansal et al., 2012; Huang et al., 2019). This suggests that the fungi could be implicated in the negative effect of the GLF on the tomato plants.

A possible explanation for the stimulated fungal growth in the GLF-fed pots is the high sugar concentrations found in the liquid fraction (80 g L^{-1}). Other work on GLFs has also noted a high sugar content (McGrath, 1991; Slewinski, 2012; Xiu et al., 2017). In fact, both the GLF and the organic feed increased the organic matter of the soil significantly ($p < 0.05$) compared to the control, as can be expected from organic fertilizers. The lack of fungal growth in the organic treatment might be a consequence of the form of carbon present—the organic feed was made with seaweed extract, and seaweeds only have complex carbohydrates in their composition that need to undergo a hydrolysis step to release free sugars (Jang et al., 2012). On the other hand, it has long been known that grass juice is relatively rich in soluble carbohydrates (Wilson & Webb, 1937).

It has been suggested that sugar concentrations over a certain threshold mainly stimulate fungal growth in soil (Reischke et al., 2014) but it is also probable that bacterial growth was stimulated in the GLF pots. In fact, bacterial biofilms were observed in the saucers where GLF was pooled. Nevertheless, it has been shown that an increase in sugar concentration in the soil can end up causing the destruction of native bacteria (Wu et al., 1993), which could in turn cause an imbalance in the soil microbiota and in the transformation of soil nutrients into bioavailable forms.

Additional experiments should be performed with a processed GLF, where the high sugar concentration would be reduced before its application as a fertilizer, to better assess the hypothesis of microbial growth and destruction as a reason for the negative results found in the present study.

4.5 | Presence of allelopathic chemicals

When adding liquid fractions made from plants or weeds to a variety of plants, several researchers have observed an inhibitory and phytotoxic effect similar to that found in this study (Arowosegbe et al., 2012; Kadioglu et al., 2005; H. H. Li et al., 1993; Z. H. Li et al., 2010; Ye et al., 2006). With regards to those studying tomato plants, Mersie and Singh (1988) found that extracts of Ragweed parthenium (*Partenium hysterophorus*) reduced the root and shoot dry weight of the plants and reduced leaf nitrogen and phosphorus. Arowosegbe et al. (2012) found that the aqueous root extract of *Aloe Ferox* inhibited the germination of tomato seeds, reduced the root and shoot elongation of tomato seedlings, and decreased the plant's leaf nutrients.

The inhibitory effect of the plant extracts in these articles was attributed to the presence of allelopathic phytochemicals (Arowosegbe et al., 2012; H. H. Li et al., 1993; Z. H. Li et al., 2010) and this was shown clearly by Mersie and Singh (1988), who compared the effect of the Ragweed extract to the effect of the constituent phenolic compounds such as vanillic, *p*-courmaric, chlorogenic, and ferulic acid on tomato plants. All the phenolic acids tested reduced root and shoot dry weight and leaf nitrogen and phosphorus in the same way as the extract.

This concurs with the negative impact of the GLF on the tomato plants, suggesting that the GLF could contain allelopathic compounds that resulted in the reduction of growth, the chlorosis, and toxicity in the plants (brown marks found up the stems), the reduction in root growth, the decrease in the nutrient content of the leaves, and the inhibition of nutrient and water uptake, finally leading to the reduced yield and the death of the tomato plants.

If indeed, allelopathic compounds are responsible for the results found when feeding GLF to tomato plants rather than the stimulation of phytopathogenic fungi growth, this opens up the possibility of using the GLF as a herbicide rather than as a fertilizer after a treatment to reduce its soluble sugar content. The efficacy of plant extracts in plant disease management is now well documented (Gurjar et al., 2012) and the use of bio-based herbicides is a growing field (Amini et al., 2014, 2016; Appiah et al., 2015; Fujii et al., 2003).

5 | CONCLUSIONS

The GLF obtained from pressed roadside grasses showed a balanced NPK ratio for feeding tomato plants with low heavy metal contents. Nevertheless, its NPK content was too low to be considered a marketable organic liquid fertilizer in light of the new European Comission fertilizer regulation 1069/2009 and 1107/2009, requiring a concentration step before commercialization. Even though it had a balanced nutrient content, results showed that GLF could not be used as a tomato plant feed in its unprocessed form. Its application killed six out of 10 tomato plants and reduced leaf nutrient concentration, tomato yield, and size. This negative effect could have resulted from the pathogenic fungal growth stimulated by the high sugar content of GLF and/or the presence of allelopathic chemicals. These two hypotheses need further investigation; nevertheless, the removal and reduction of the GLF sugar content is needed to turn this stream into a commercial product for plant application, either as an organic fertilizer or a natural herbicide.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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