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Research article

# Can microalgae grown in wastewater reduce the use of inorganic fertilizers?

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A B S T R A C T

Alternatives to conventional inorganic fertilizers are needed to cope with the growing global population and contamination due to the production and use of those inorganic compounds. The recovery of nutrients from wastewater and organic wastes is a promising option to provide fertilization in a circular economy approach. In this context, microalgae-based systems are an alternative to conventional wastewater treatment systems, reducing the treatment costs and improving the sustainability of the process, while producing nutrient-rich microalgal biomass. The aim of the present study is to evaluate the use of microalgal biomass produced during domestic wastewater treatment in high rate algal ponds as a biofertilizer in basil crops (*Ocimum basilicum* L.). Wastewater was successfully treated, with removal efficiencies in the secondary treatment of 69, 91 and 81% in terms of chemical oxygen demand (COD), total inorganic nitrogen (TIN) and phosphates ($PO_4^{3-}$–P), respectively. The microalgal biomass, composed mainly by *Scenedesmus*, presented the following composition: 12% of dry weight and nutrients concentration of 7.6% nitrogen (N), 1.6% phosphorus (P) and 0.9% potassium (K). The study compared the performance of 3 different fertilizers: 1) microalgae fertilizer (MF), 2) inorganic fertilizer (IF) as positive control and 3) the combination of both microalgae and inorganic fertilizer (MF + IF). Comparable plant growth (i.e., number of leaves, shoot fresh and dry weight and leaf fresh weight) was observed among treatments, except for leaf dry weight, which was significantly higher in the IF + MF and MF treatments (28 and 27%, respectively) in comparison with the control. However, the microalgae treatment provided the lowest chlorophyll, N and K leaf content. In conclusion, this study suggests that combining microalgae grown in wastewater with an inorganic fertilizer is a promising nutrients source for basil crops, enhancing the circular bioeconomy.

## 1. Introduction

The enhancement of a sustainable agriculture requires alternatives to inorganic fertilizers, which are currently being extensively used. In 2019, 62.7 kg of nitrogen (N) per hectare of cropland were globally applied as chemical fertilizer, which is 3.5 times higher than the N provided in 1961 (FAO, 2021). Indeed, 80% of the global N produced goes to inorganic fertilizers (Liu et al., 2010), which are obtained with high energy-consuming processes. Moreover, it is estimated that 40% of the N provided as inorganic fertilizer is lost (Liu et al., 2010). And high nutrients concentrations in water bodies have important ecological consequences on aquatic ecosystem functions, processes and structures (Mohsin et al., 2021). Phosphorus (P) is also an important nutrient for plant's growth (Solovchenko et al., 2016). Globally, 21 kg of P ($P_2O_5$) per hectare of cropland were applied as chemical fertilizer in 2019, which is 2 times higher than the P provided in 1961 (FAO, 2021). As a non-renewable element, it seems essential to look for solutions to recover P. In this context, growing microalgae in wastewater, which presents high concentrations of both N and P, is a sustainable technology to recover nutrients by assimilation in the biomass (Günther et al.,

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2018).

Wastewater treatment systems based on the combination of microalgae and bacteria have gained interest in recent years, among other reasons due to the potential production of value-added products from the biomass (Young et al., 2017). In high rate algal ponds (HRAPs), pollutants are removed from wastewater by an algae-bacteria symbiosis in which heterotrophic bacteria oxidize organic contaminants using the oxygen released by microalgae that absorb nutrients (mainly N and P) and capture $CO_2$ from bacterial respiration (Muñoz and Guieysse, 2006). In such systems, the biomass harvested can be used to produce different bioproducts such as pigments, bioplastics and biofertilizers (Arias et al., 2017; Arashiro et al., 2022), recovering nutrients from wastewater while reducing the cost and carbon footprint of conventional microalgae cultivation (Li et al., 2021).

Biofertilizers are products containing living or dormant microorganisms (bacteria, fungi and algae) alone or in combination (Dineshkumar et al., 2018), which represent an alternative to chemical fertilizers. Photosynthetic microorganisms present different advantages as they increase soil fertility by fixing atmospheric N (heterocystous cyanobacteria) and release nutrients into the soil (non-heterocystous cyanobacteria and green algae) (Dineshkumar et al., 2018; Mahapatra et al., 2018). Microalgae biomass is considered as an organic slow release fertilizer, preventing nutrient losses from soil through a gradual release of macro- and micronutrients. In this sense, Mulbry et al. (2005) found that, after microalgal biomass fertilization, the N available for plants increased from 1-4%–41% after 63 days. It is therefore expected that N from microalgae will not exceed the crop nutrient demand. This is an advantage in comparation to other ways of recovering nutrients from organic wastes, such as the application of manure in agriculture, which can cause N contamination in surface and ground water (Carpenter et al., 1998).

Thus, wastewater grown microalgae seem to be a promising alternative to conventional inorganic fertilizers, enhancing the circular economy. For instance, Sharma et al. (2021) found that *Chlorella* biomass grown in wastewater treatment improved the spinach and baby corn yield when applied as biofertilizer. Previous studies have addressed the effect of microalgae fertilization on plant growth and yield (Ronga et al., 2019; Kang et al., 2021). However, the use of microalgae biomass grown in wastewater for crop production is still quite scarce (Kang et al., 2021). From an economic point of view, attention should be paid to the fact that this biofertilizer comes from a residue generated in a microalgae-based wastewater treatment process, and typically wastewater treatment plants have to pay for the disposal of these wastes, so it would not represent any cost for the farmer. Moreover, it is worth highlighting that this wastewater treatment technology has similar or lower costs than conventional wastewater treatments (Morais et al., 2021).

Basil (*Ocimum basilicum* L.) is a plant with high economic interest, used for different scopes. First, for culinary purposes: to flavour foods using the fresh herb or even the flowers which are edible (Makri and Kintzios, 2008). Second, basil extracts were found to have antioxidant properties due to the presence of phenolic compounds, which may have health benefits (Hossain et al., 2010). Third, it is a source of essential oils and aromas, which may be used in cosmetics or pharmaceutical industry (Makri and Kintzios, 2008). Lee and Scagel (2009) showed the presence of chicorid acid in basil plants, which has commercial interest mainly in US as a dietary component. Basil has been successfully grown using different types of fertilizers, including inorganic, organic or even biological fertilizers such as plant growth promoting rhizobacteria (PGPR) (Ordookhani et al., 2011).

This study evaluated, for the first time, the effect of wastewater grown microalgae and their combination with an inorganic fertilizer on basil crop. The aim of the study is to assess the potential use of microalgae biomass as a biofertilizer. The effect of this organic fertilizer was compared with an inorganic fertilizer in terms of fresh and dry shoot weight, fresh and dry leaves weight, number of leaves, and leaves nutrients content. The novelty of this study lays in the concept of circular economy here proposed. Indeed, a residue from a wastewater treatment technology (microalgal biomass) is valorized as biofertilizer to partially replace inorganic fertilizers for basil production.

## 2. Material and methods

### 2.1. Microalgae-based wastewater treatment system

Real domestic wastewater was treated in a pilot plant located outdoors at the Universitat Politècnica de Catalunya campus (Barcelona, Spain, 41°23′18″N 2°06′38″E). A schematic diagram of the pilot plant is included as supplementary material (Figure S3). The system consisted of two HRAPs, which were operated for several months since February 2021. For the purpose of this research, the performance of the HRAPs in terms of wastewater treatment and biomass production was monitored during 90 days (from May to July 2021). Domestic wastewater was daily pumped from a municipal sewer into a homogenization tank with a volume of 1.2 $m^3$, which was constantly stirred. Then, pretreated wastewater was pumped into a primary settling tank, which consisted of a PVC cylinder with an effective volume of 3 L and a hydraulic retention time (HRT) of 41 min. Afterwards, the primary effluent from the settler was pumped into two HRAPs by means of two peristaltic pumps (Damova, MP-3). Each HRAP had a volume of 470 L, a surface area of 1.54 $m^2$ and a depth of 0.3 m. The mixed liquor was constantly stirred with a paddle wheel with an average velocity of 10 cm/s. Both HRAPs were operated with the same HRT, which was changed depending on the weather conditions as suggested by Passos et al. (2017) The HRT was therefore set to 6 days during May–June and 4 days during June–July. Subsequently, microalgal biomass was harvested in two secondary settling tanks, which consisted of two cylindrical polypropylene tanks with a volume of 200 L. This biomass was further thickened in Imhoff cones at 4 °C and centrifuged (4200 rpm, 7 min, UniCen21, OrtoAlesa, Spain). Finally, the microalgae biofertilizer obtained was stored at 4 °C until it was used for the agronomic assays.

### 2.2. Wastewater and biomass characterization

Grab samples of the primary effluent and mixed liquor from the HRAPs were weekly collected and immediately analysed in order to monitor the performance of the pilot plant. pH (Crison 506, Spain), turbidity (Hanna HI 93703, USA), volatile suspended solids (VSS), total and soluble chemical oxygen demand (tCOD and sCOD) were analysed according to Standard Methods (APHA-AWWS-WEF, 2012), and ammonium-N ($NH_4^+$-N) according to the Solórzano method (Solórzano, 1969). Nitrite ($NO_2^-$-N), nitrate ($NO_3^-$-N) and phosphate ($PO_4^{3-}$-P) were measured through isocratic mode with carbonate-based eluents at a temperature of 30 °C and a flow of 1 mL/min (ICS-1000, Dionex Corporation, USA); the limits of detection (LOD) were: 0.9 mg/L of $NO_2^-$-N, 1.12 of $NO_3^-$-N, and 0.8 mg/L of $PO_4^{3-}$-P).

$NH_4^+$-N, $PO_4^{3}$–P and COD were the parameters taken into account to assess the nutrient and organic matter removal in the HRAPs. Total inorganic N (TIN) was calculated as the sum of $NO_2^-$-N, $NO_3^-$-N and $NH_4^+$-N. To determine the COD removal, primary effluent samples were measured without filtration (tCOD) while mixed liquor samples were measured after filtration (sCOD) through glass fiber filters (47 mm and average pore size 1 μm) so as to prevent microalgae contribution (Gutiérrez et al., 2016). $NH_4^+$-N and $PO_4^{3}$–P were also measured in samples after filtration.

Grab samples of the mixed liquor were weekly collected and analysed with an optic microscope (BA310, Motic, China) in order to observe the dominant species in the culture. Biomass productivity (g VSS/$m^2$d) was calculated from the weekly measure of the VSS concentration in the mixed liquor of both HRAPs (equations 1-4 in supplementary material).

The biofertilizer obtained from the harvested microalgal biomass was characterized by measuring dry weight and organic matter (OM) (AOAC, 2005). The N content was measured as total Kjeldahl N (AOAC, 2005). The K content was measured with a flame spectrophotometer (Corning 410C, Halstead, Essex, England), according to AOAC (2005). P was measured with a spectrophotometer (Agilent Cary 60, Mulgrave, Victoria, Australia), according to Bray and Kurtz (1945).

### 2.3. Agronomic assay

The fertilization assay was conducted with Basil (*Ocimum basilicum* L.) in a greenhouse located in Castelldefels (Barcelona, Spain, 41°16′32″N 1°59′08″E) during June and July 2021, under natural sunlight with a light:dark cycle of 15:9 (corresponding to the natural photoperiod during this time of the year). The greenhouse was equipped with an automated ventilation. Air temperature and relative humidity were recorded over the experimental period (Supplementary Material Fig. S2).

In this experiment, three different fertilizers were tested: 1) microalgae fertilizer (MF), 2) inorganic fertilizer (IF) as positive control and 3) the combination of microalgae and inorganic fertilizer (MF + IF). The experiment was set out in a completely randomized block design with three replicates and 10 pots per replicate. A mixture of peat and perlite (1:1; v/v), amended with 3 kg/m$^3$ of calcium carbonate in order to reach an optimal pH, was used as a substrate. Each pot had a volume of 1 L and 0.9 L of this substrate.

For both microalgae fertilizer treatments, microalgae biomass was added to the substrate at the beginning of the experiment, at a depth of 30 mm, and covered with the remaining substrate. In each pot, 14 and 28 g of fresh centrifuged microalgae biomass were applied in IF + MF treatment and MF treatment, respectively. This biomass could potentially provide a total N of 9.6 and 19.2 g N/m$^2$ in the IF + MF and MF treatments, respectively (Table 1). It was assumed that approximately 60% of algal N would be released into the soil, according to Rupawalla et al. (2021).Thereafter, basil seedlings were transplanted to individual pots. For the IF treatment, a modified Hoagland's solution (Hoagland and Arnon, 1950) was applied once a week at a dose of 100 mL/pot. In addition, 100 mL of the diluted Hoagland solution (50%) were weekly applied in the IF + MF treatment, whereas 100 mL of tap water were weekly applied to the MF treatment. Irrigation was applied daily on each pot using 2 L/h drippers. The amount of water was the same for all the treatments. Irrigation was supplied daily and the amount of irrigation water was adjusted weekly to prevent water deficits while achieving a minimum drain in order to prevent nutrient loss through leaching.

At the end of the experiment, the Chlorophyll Content Index (CCI, relative units) was measured using a Chlorophyll Content Meter (CCM-200plus, Opti-Sciences, USA). Measurements were taken on the uppermost expanded mature leaf.

After 5 weeks of experiment, aboveground biomass was harvested and the leaf number, and leaf and shoot fresh weight per plant were determined. Subsequently, plants were oven-dried at 80 °C for 48 h and then weighed to determine shoot and leaf dry weight per plant. The leaf nutrient concentration was also analysed: N, P and K were measured as described in section 2.2; calcium (Ca), magnesium (Mg) and iron (Fe) were measured with atomic absorption (Varian SpectrAA-110, Mulgrave, Victoria, Australia), according to AOAC (2005). Sodium (Na) was measured with a flame spectrophotometer (Corning 410C, Halstead, Essex, England), according to AOAC (2005). Sulphur (S) was measured with a spectrophotometer at 420 nmmL (Agilent Cary 60, Mulgrave, Victoria, Australia), according to AOAC (2005).

Prior to these measurements, dried leaves were pooled for each replicate and finely ground to powder. Analyses were carried out in 3 replicates.

**Table 1**
Nitrogen content provided per treatment: IF (inorganic fertilizer), IF + MF (inorganic fertilizer and microalgae fertilizer), MF (microalgae fertilizer).

| | IF (g N/m$^2$) | IF + MF (g N/m$^2$) | MF (g N/m$^2$) |
|---|---|---|---|
| Inorganic source | 9.5 | 4.75 | – |
| Microalgae source | – | 9.6 | 19.2 |

### 2.4. Statistical analysis

Data from biometrical parameters measures and leaf chlorophyll content were tested with the one-way analysis of variance (ANOVA) test processed by R software, version 4.1.0. Tukey's post hoc test was used to analyse differences among treatments ($\alpha = 0.05$). The mean value of the different parameters is represented in the graphs and bars indicate the Standard Error of the Mean (SEM).

## 3. Results and discussion

### 3.1. Wastewater treatment efficiency and biomass productivity

The main water quality parameters of the primary effluent and the HRAP mixed liquor are summarized in Table 2, along with the average removal efficiencies (%) of organic matter (COD) and nutrients. Overall, the organic matter and nutrients removal efficiencies in the secondary treatment were rather high, with average values of 69% for COD, 95% for $NH_4^+$-N, 83% for TIN and 81% for $PO_4^{3-}$-P. Gutiérrez et al. (2016) and Arashiro et al. (2019) obtained similar nutrient removal efficiencies using the same HRAPs during the warm season.

During the experiment, *Scenedesmus* sp. (Fig. 1A) was the most abundant species in both HRAPs. Ciliate and flagellate protozoans were also observed (Fig. 1B), in bgreement with previous studies carried out in the same HRAPs (Arashiro et al., 2019). The average VSS in the mixed liquor was 231 mg/L, and the average biomass productivity 13.5 g VSS/m$^2$d, in line with those obtained by Arashiro et al. (2019) in the same pilot plant during the warm season.

### 3.2. Biofertilizer characterization

The composition of the biofertilizer obtained from the microalgal biomass produced in the wastewater treatment system is shown in the supplementary material (Table S1). Thus, the N:P ratio was 5:1. Park et al. (2011) reported N:P ratios in microalgal biomass from wastewater treatment systems from 4:1 to 40:1, depending on the species and nutrient availability. The nutrients ratios of other studies using microalgae grown in wastewater and other organic wastes, such as manure or compost, as biofertilizers are summarized in Table 3. As can be seen, other researchers reported NPK ratios similar to that obtained with *Scenedesmus* biomass in this study (1:0.21:0.12). On the other hand, microalgae biomass contains a higher proportion of N in relation to P and K when compared to manure or compost.

**Table 2**
Influent wastewater (primary effluent) and high rate algal ponds (HRAP) mixed liquor characterization over a period of 90 days (n = 16). Values are calculated as the average and standard deviation. Acronyms: VSS (volatile suspended solids), COD (chemical oxygen demand), TIN (total inorganic nitrogen). Average of the removal efficiencies of nutrients and COD. *COD in the mixed liquor was measured as soluble COD.

| | Influent | HRAP | Removal efficiency (%) |
|---|---|---|---|
| pH | 7.9 ± 0.2 | 9.4 ± 0.6 | |
| VSS (mg/L) | 198 ± 91 | 231 ± 103 | |
| Turbidity (NTU) | 127 ± 55 | 129 ± 66 | |
| COD (mg/L) | 353 ± 182 | 105 ± 38* | 69 ± 13 |
| $NH_4^+$-N (mg/L) | 23.8 ± 10.5 | 1.4 ± 1.4 | 95 ± 4 |
| TIN (mg/L) | 27.3 ± 14.6 | 2.7 ± 2.5 | 83 ± 11 |
| $PO_4^{3-}$-P (mg/L) | 4.4 ± 1.1 | 0.8 ± 0.7 | 81 ± 18 |

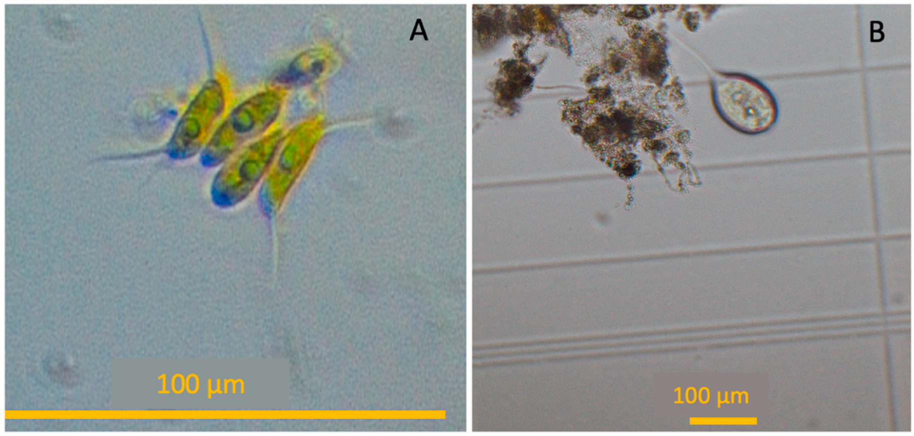


**Fig. 1.** Images of the high rate algal ponds mixed liquor observed in bright light microscopy. A) Scenedesmus sp., B) mixed liquor with flagellate protozoans.

**Table 3**
Nitrogen, phosphorus and potassium (NPK) ratio in different biofertilizers.

| Biofertilizer | Growth medium | NPK ratio | Reference |
|---|---|---|---|
| Microalgae | Municipal wastewater | 1:0.21:0.12 | This study |
| Microalgae | Municipal wastewater | 1:0.20:0.47<br>1:0.12:0.36 | Renuka et al. (2016) |
| Microalgae | Municipal wastewater | 1:0.17:0.08 | Sharma et al. (2021) |
| Microalgae | Meat processing industry wastewater | 1:0.21:0.03 | Castro et al. (2020) |
| Compost from a mixture of grasses, stubbles and plant leaves<br>Farm manure (cattle) | | 1:0.23:0.89<br>1:0.46:0.38 | Celik et al. (2004) |
| Manure (pure pig manure) | | 1:0.64:0.62 | Cai et al. (2019) |

### 3.3. Effect of the microalgae biofertilizer on basil plants growth

#### 3.3.1. Plant biometrical parameters

The maximum shoot fresh weight was observed in the IF + MF treatment (13 g) while the minimum was obtained in IF treatment (12 g). Likewise, the maximum shoot dry weight was measured in the IF + MF treatment (2 g) while the IF treatment presented the minimum (2 g). However, the statistical analysis revealed that there were no significant differences among treatments regarding the shoot fresh and dry weight (Fig. 2A, B).

Regarding the leaves, the maximum fresh weight was produced in the MF treatment (9 g) while the minimum was obtained in IF treatment (8 g). IF + MF and MF treatments presented a similar dry weight (1 g and 1 g, respectively) (Fig. 2C). Once again, the IF treatment showed the minimum dry weight (1 g). No significant differences were observed among treatments in relation to the leaves fresh weight. However, the leaves dry weight was statistically higher ($p < 0.05$) in the IF + MF (with an increase of 28%) and MF (with an increase of 27%) treatments, in comparison with the IF treatment (Fig. 2D). The number of leaves was similar in the three treatments: IF + MF (52), MF (48) and IF (47). No significant differences were found among them (Fig. 2E).

The IF treatment presented the minimum mean in all plant biometrical parameters, although only the dry leaves weight was significantly lower ($\alpha < 0.05$), suggesting that microalgae biomass grown in municipal wastewater has a positive effect on basil growth and can be used as biofertilizer. Previous studies have investigated the use of wastewater grown microalgae as slow release biofertilizer. For instance, Renuka et al. (2017) found that the plant growth parameters, yield and nutritional status of wheat crop were enhanced by adding sewage grown microalgae as biofertilizer. Indeed, the grain yield was 48% and 37% higher in microalgae treatments than in the control with a full dose of inorganic fertilizer. González et al. (2020) showed that the germination index in barley seeds was improved by rising the concentration of wastewater grown microalgae supplied as biofertilizer. They also reported an increase in dry weight (120% in ryegrass and 143% in barley) when applying wastewater grown microalgae as biofertilizer in comparison with an ammonium sulphate treatment. Recently, Sharma et al. (2021) compared the efficiency of wastewater grown microalgae biofertilizer with an equivalent dose of a NPK inorganic fertilizer in spinach. The growth parameters measured in spinach revealed that leaf fresh biomass was 42% higher when applying the biofertilizer (100% NPK provided with microalgae biomass) than the negative control (without fertilizer) and 6% higher than a recommended dose of NPK from an inorganic fertilizer. Wuang et al. (2016) compared a wastewater grown Spirulina fertilizer with a commercial one in three different crops. Their results suggest that the efficiency of the fertilizer is species-dependent. For instance, in bayam red (*Ameranthus gangeticus*), the treatment combining Spirulina biomass with an inorganic fertilizer increased the dry weight by 108% in comparison to Spirulina fertilizer alone; whereas in arugula (*Eruca sativa)*, the combination of both fertilizers only increased the dry weight by 10% in comparison with the Spirulina fertilizer.

Previous research indicated that microalgae slowly provide plant-available nutrients during their growth (Castro et al., 2017). The slow release also helps reducing N-loses trough leachate and improve N uptake efficiency (Sharma et al., 2021). Moreover, it is well known that microalgae are capable of producing growth-promoting substances (Kapoore et al., 2021). Gatamaneni Loganathan et al. (2021) discussed that the better results in plant growth could be attributed to the presence of phytohormones, like auxins, gibberellic acid and cytokinins, besides other micronutrients and secondary metabolites from microalgae. However, the effects of the microalgae biofertilizer over plant parameters may depend on the microalgae composition (Renuka et al., 2017). In the case of wastewater grown microalgae, biomass composition will depend on the species and the characteristics of wastewater, which is highly variable.

#### 3.3.2. Roots length

Fig. 3 shows the effect of each treatment on roots at harvest (after five weeks of growth). While the IF treatment presented a longer root system, the IF + MF and MF treatments produced shorter and broader roots and were mainly located rigth where microalgae biomass was applied. Previous studies did not report any significant effect on roots due to microalgae biofertilizers application either. Sharma et al. (2021) observed that the recoverable root length and root weight/plant of spinach were not significantly different with microalgae biofertilizer

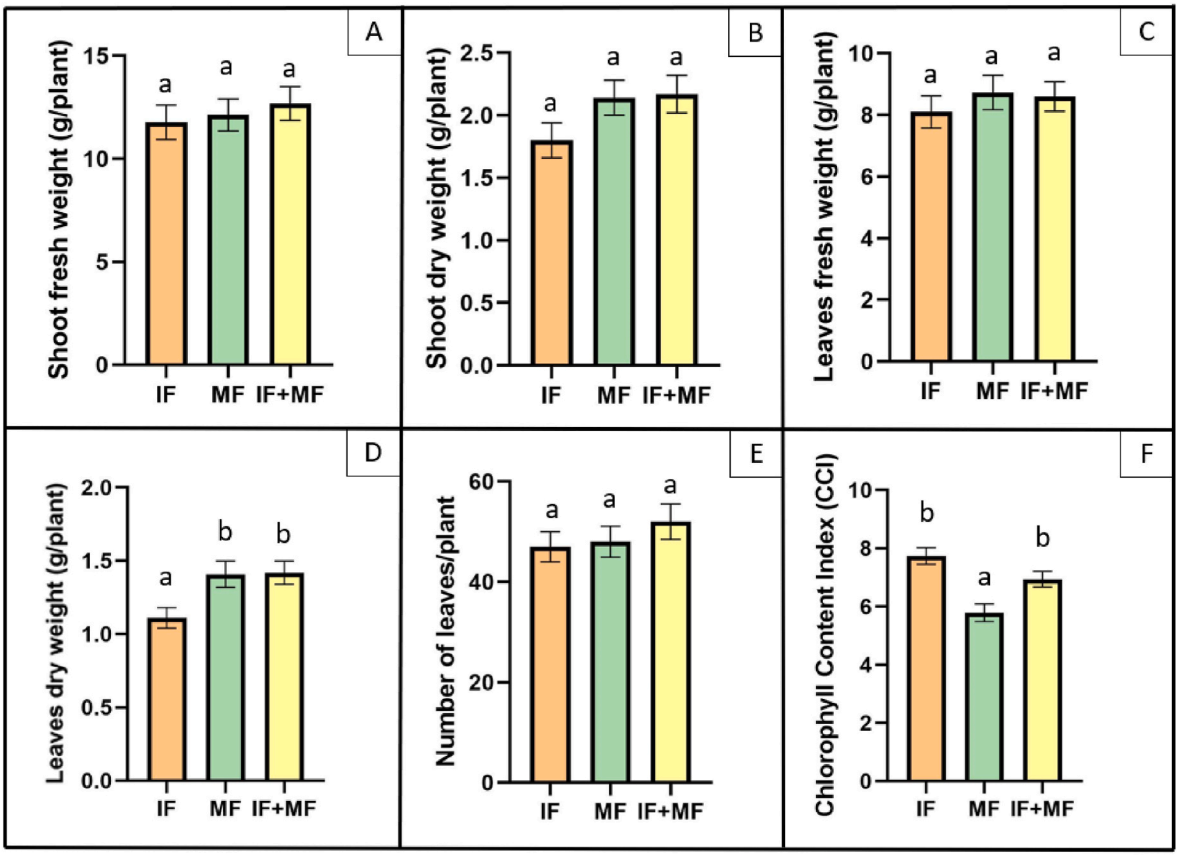


**Fig. 2.** Shoot fresh weight (A), shoot dry weigh (B), leaves fresh weight (C), leaves dry weight (D), number of leaves (E) and leaf chlorophyll content (F) (n = 30) of basil plants grown with different fertilizer treatments: inorganic fertilizer (IF), inorganic fertilizer + microalgae fertilizer (IF + MF) and microalgae fertilizer (MF). Error bars correspond to the standard error of the mean. Different letters indicate significant differences (α < 0.05) according to Tukey's post hoc test.


**Fig. 3.** Qualitative comparison of the basil roots among treatments: IF (inorganic fertilizer), IF + MF (inorganic fertilizer + microalgae fertilizer), MF (microalgae fertilizer).

and inorganic fertilizer. Wuang et al. (2016) observed both in Bayam Red (*Ameranthus gangeticus)* and Pak Choy (*Brassica rapa* ssp. *chinensis)* that the roots length was not significantly different when comparing Spirulina biofertilizer with a inorganic fertilizer. Conversely, Joshi et al. (2015) reviewed several studies on vermicompost application as organic fertilizer and concluded that this material improved plant growth, including root length and root number.

### 3.3.3. Leaf chlorophyll content

The leaf chlorophyll content index varied between 6 and 8 CCI. The CCI was significantly lower (α < 0.05) in the MF treatment (6) than in the IF + MF (7) and IF (8) treatments (Fig. 2F). Hristozkova et al. (2018) also observed a reduction in the leaf chlorophyll content (6.1% lower than the control) when *Synechocystis* was applied in basil crop. On the other hand, Renuka et al. (2017) reported higher leaf chlorophyll content (53% increase in comparison with the positive control) in wheat crop when wastewater grown microalgae were added as biofertilizer. However, these microalgae were combined with inorganic fertilizer, which provided 100% the dose of P and K and 75% the dose of N, meaning that microalgae only contributed with 25% the dose of N.

In general, the lack of chlorophyll can suggest a limitation in the absorption of inorganic N, which may be due to the fact that microalgae mainly provide organic N, which has to undergo to a mineralization process in order to be taken up by plants (Jimenez et al., 2020; Suleiman et al., 2020). However, as microalgae are unicellular microorganisms, the mineralization process is quicker in comparison to other organic fertilizers (Sharma et al., 2021).

### 3.3.4. Leaf nutrients content

Macronutrients (N, P, K, Mg, Ca and S) and micronutrients (Fe and Na) were measured in the leaf tissue in order to understand the potential effect of the microalgae biofertilizer on the nutritional value of basil plants. The nutrients analyses revealed that there were no significant differences among treatments regarding some of the nutrients, namely P, Ca and Na (Fig. 4B, E, G). However, the IF treatment had significantly (p

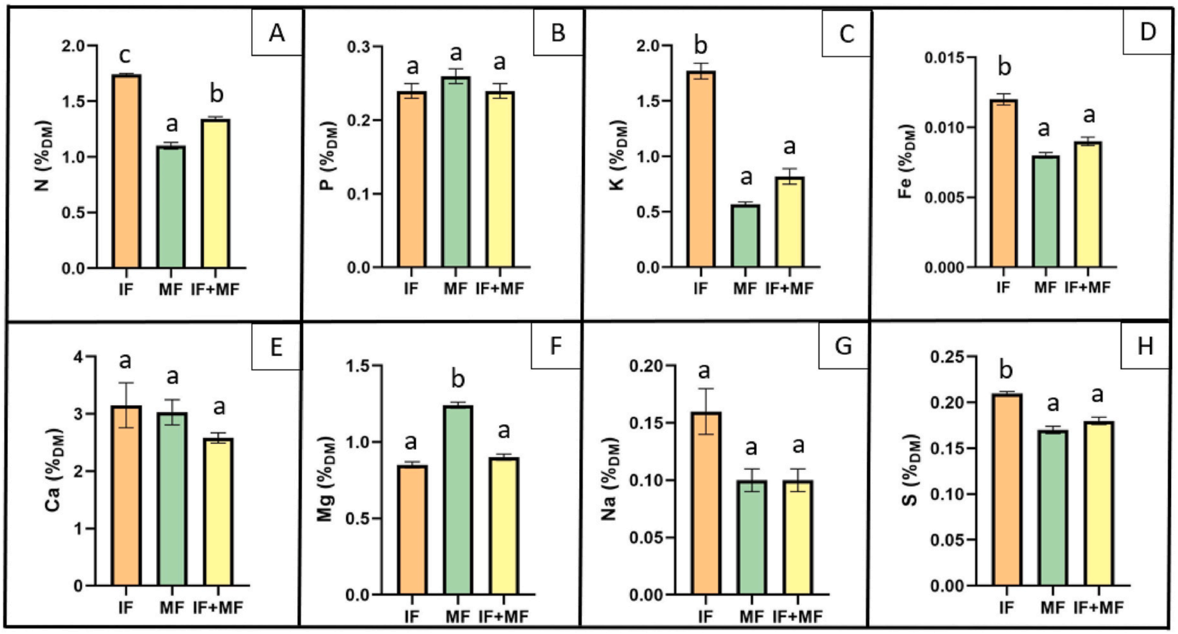


**Fig. 4.** Leaf nutrient content in basil plants grown with different fertilizer treatments: IF (inorganic fertilizer), IF + MF (inorganic fertilizer + microalgae fertilizer), MF (microalgae fertilizer). Error bars correspond to the standard error of the mean. Different letters indicate significant differences according to Tukey's post hoc test ($\alpha < 0.05$).

$< 0.05$) higher N content, with an increase of 23 and 37% as compared to the IF + MF and MF treatments, respectively (Fig. 4A). As for K, the IF treatment presented a significantly ($p < 0.05$) higher content than the IF + MF and MF treatments, with an increase of 54 and 68%, respectively (Fig. 4C). Also for S, the IF treatment was significantly ($p < 0.05$) higher than the IF + MF and MF treatments, by 12 and 15%, respectively (Fig. 4H). As for Fe, the IF treatment presented a significantly ($p < 0.05$) higher content than the IF + MF and MF treatments, with an increase of 23 and 33%, respectively (Fig. 4D). Conversely, the MF presented significantly ($p < 0.05$) higher Mg content than the IF and IF + MF treatments, with an increase of 31 and 27%, respectively (Fig. 4F). On the whole, the positive control (IF) increased the N, K, S and Fe content in leaves, while the microalgae fertilizer (MF) increased the Mg content, and similar results were obtained for P, Ca and Na. As far as the IF + MF treatment is concerned, the N content was 18% higher than in the MF treatment (Fig. 4A). The lowest N and Fe content in leaves is consistent with the lowest chlorophyll content in the MF treatment (Fig. 2F). Indeed, low chlorophyll content can be attributed to a deficiency in N and Fe content (Imsande, 1998).

In the present study, there were no significant differences in the P content among treatments, whereas the N content was lower in the IF + MF and MF treatments than in the positive control (IF). The results suggest that the experimental period (five weeks) was not long enough for releasing and transforming all the N accumulated in microalgae biomass into plant-available forms, confirming the slow release nature of the microalgae fertilizer. Indeed, the mineralization rate of microalgal biomass under the study conditions seems to be lower than that obtained by Rupawalla et al. (2021). Mulbry et al. (2005) observed that 41% of total algal N was plant-available after 63 days, which seems to fit better with the results from the present study. Considering the amount of N in leaves (IF: 19.3 mg N/g plant; IF + MF: 19.1 mg N/g plant; MF: 15.5 mg N/g plant), in the microalgae treatment it was only 20% lower than in the positive control (IF) which indicates that part of the microalgae N was mineralized. Rupawalla et al. (2021) reported that P was rapidly released during the first days, being 18% higher in the algae treatment in respect to the synthetic fertilizer during the first five days. However, the algae treatment only released 25% of the total P after 28 days. Algal N and P seem therefore to have different release behaviour, which could explain why basil plants accumulated similar P and different N contents with microalgae and the positive control. Prospective studies should quantify the mineralization rate of microalgal biomass so as to define the optimal dose of microalgae and inorganic fertilizers in view of field trials.

In agreement with the results of the present study, Lorentz et al. (2020) also obtained lower N and K content and higher P and Ca content in marandu grass (*Uruchloa brizantha cv. Marandu*) fertilized with microalgae (a combination of *Scenedesmus* and *Chlorella*) when compared to a inorganic fertilizer; while there was no difference regarding S. Conversely, Coppens et al. (2016) found that the N content was higher, and the K and Mg content lower, in leaf samples of tomato plant (*Solanum lycopersicum)* grown with the algal fertilizer than with commercial organic fertilizer treatment.

However, combining the inorganic and microalgae fertilizer (IF + MF treatment) seems the best approach to counter balance the nutrients deficiency observed in the MF treatment, providing higher leaf dry weight than the IF treatment and higher chlorophyll content than MF treatment. According to Antille et al. (2013), organo-mineral fertilisers can be defined as the ones obtained by blending, chemical reaction, granulation, or dissolution in water of inorganic fertilisers with organic fertilisers or soil improvers. Wastewater grown microalgae seem to accumulate K in low concentration (Table 3), which may not meet crop requirements. Indeed, in this study, the K content in leaves was lower in the microalgae fertilizer in relation to the inorganic treatment (Fig. 4), just as other researchers reported (Coppens et al., 2016; Lorentz et al., 2020). Microalgae biomass could be therefore combined with potash, phosphates and liquid ammonia in order to balance the nutrients content.

According to the results of the present study, similar basil growth was obtained by replacing 50% of the inorganic fertilizer by microalgae biomass grown in wastewater, which means that the cost of basil production could be reduced. Prospective field studies would allow for evaluating the economic impact of reducing inorganic fertilizers application. Moreover, the benefits of using microalgae as fertilizer should not only be seen from an economic perspective, but also from the environmental point of view (Arashiro et al., 2022). In fact, this is a sustainable solution using a residue to solve or at least reduce the problems associated with the production of mineral fertilizers (high energy costs) and their use (soil and water pollution due to N-loses trough leachate).

## 4. Conclusion

This study shows the potential use of microalgal biomass grown in domestic wastewater as a biofertilizer. Plant growth parameters (number of leaves, fresh and dry shoot weight, and fresh leaf weight) did not show any significant differences among treatments; while the dry leaf weight was higher in the treatments using microalgae. Nevertheless, plants grown with the microalgae biofertilizer showed a lower leaf chlorophyll content, whereas the combination of microalgae and inorganic fertilizer presented similar values as the inorganic one. The leaf nutrient content showed that P, Ca and Na were not significantly different among treatments; Mg was higher and N, K, S and Fe were lower in the microalgae treatment. The combination of both fertilizers seems a suitable alternative to replace 50% of the inorganic fertilizer while recovering nutrients from wastewater through microalgae biomass in a circular bioeconomy approach. Further research would be needed to determine the rate of mineralization of microalgal biomass (i.e. nutrient availability) and optimize the mixture of microalgal biomass with inorganic nutrients.

## Author contributions

Ana Álvarez-González: Formal analysis, Investigation, Data curation, Writing – original draft. Enrica Uggetti: Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. Lydia Serrano: Methodology, Validation, Writing – review & editing. Gil Gorchs: Methodology, Validation, Writing – review & editing. Ivet Ferrer: Conceptualization, Writing – review & editing, Funding acquisition. Rubén Díez-Montero: Conceptualization, Investigation, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.116224.

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