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Reclamation Potential of Onsite Wastewater Post-Treatment with Microalgae: Chemical Elements Perspective

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Abstract: Algae-based wastewater treatment is a promising technology with various applications for excess biomass such as biofertilizer production or valuable elements extraction. The benefits of the technology have been discussed for larger wastewater treatment plants (WWTPs), but the use of microalgae in decentralized wastewater treatment has been barely reported. The current study screens the possible resource recovery potential of onsite technology, which adds algae-based post-treatment to the conventional biological treatment of domestic wastewater. The effluent from the onsite sequencing batch reactor (SBR) of a household was further processed in laboratory conditions using an SBR technology with two local monocultures of algae—*Klebsormidium nitens* (Kützing) Lokhorst and *Tetradesmus obliquus* (Turpin) M. J. Wynne. The decant and the generated algal biomass were analyzed in terms of their element content. The post-treated effluent has a slightly better quality for irrigation purposes than the effluent of the onsite treatment facility—up to 1.6 times increased concentration for macro-elements and up to 1.9 times for micro elements. However, the generated algal biomass shows promising potential for re-use as a fertilizing agent since it contains valuable macro- and micro-elements and the heavy (hazardous) metal content is considerably lower than the limiting values in the current European and national legislations. The *K. nitens* strain may attract interest since it accumulates valuable metals such as chromium (36 mg/kgDS), nickel (83 mg/kgDS), and silver (0.7 mg/kgDS) that can be derived from the biomass and turn the technology to a circular one.

Keywords: algae; circular technology; decentralized sewerage systems; fertilizer; irrigation; nature-based solutions; onsite wastewater treatment; resource recovery

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1. Introduction

Water scarcity and valuable materials depletion are global threats, addressed in many research studies and policies. They had an even more pronounced appearance in 2022 with the aggravation of the world political situation and the manifestation of global warming. For example, recent drought events in Europe in August 2022 led to devastation of crops and fish population while negatively affecting, power plants, barge traffic, and industry due to parched waterways [1].

Wastewater is among the studied solutions for the mitigation of these threats. Wastewater is recognized as a source of both the needs of water and valuable materials [2]. While many promising achievements for reclaiming wastewater and extracting valuable materials at the municipality level have been reported, insufficient attention has been paid to areas with decentralized wastewater collection and treatment [3]. Typically, the decentralized management of wastewater includes an onsite treatment/collection facility, which periodically is emptied as shown in Figure 1.

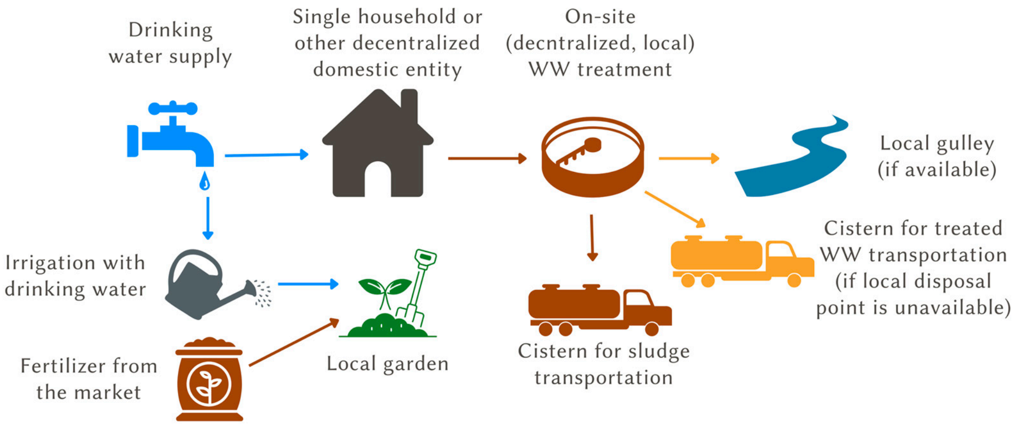

Figure 1. Typical decentralized onsite wastewater (WW) treatment and disposal scheme.

Since it is presumed that the decentralized systems are located mostly in areas with larger plots, there is a high potential for the use of generated reclaimed wastewater for irrigation or fertilization needs. However, in many cases, onsite treatment facilities do not show a stable treatment process. This is mainly due to the pattern of water use in households. The generation of influent wastewater with non-constant quantity and quality leads to unstable water quality effluent [4,5]. A possible polishing step could be algae-based technology, which has been gaining increasing interest in the field of wastewater treatment, in particular at the municipal and urban levels [6–8]. Algae use was reported also for decentralized systems in urban environments at administrative buildings, university campuses, etc., as a main treatment process or as a method for a reduction of aeration expenditures [7,9,10]. However, publications on algae use as a final polishing step (post-treatment after the activated sludge process) at a decentralized level were not found in the studied literature.

The possibility of the application of an algae-based post-treatment system after an activated sludge bioreactor at an onsite domestic level encompasses three main potential benefits: (i) local use of treated wastewater (water scarcity mitigation); (ii) local use of the generated algal biomass as a fertilizer; (iii) potential for valuable elements (materials) recovery. The state of the art of these applications is briefly summarized below.

- Use of reclaimed wastewater for irrigation

The UN's SDG6 considers the reuse of treated wastewater as a reliable solution to accommodate available water for all [11–13]. The use of treated wastewater for crop irrigation provides not only water to plants and crops but also simultaneously provides valuable soil fertilization due to its nutrient content [14]. Many countries have already been widely using treated wastewater, such as Israel with its 50% reclaimed water for agricultural needs [15], or Singapore, where 40% of the water demand is from reclaimed sources [16,17].

At a local onsite scale, water use for individual agricultural gardens in villages causes an increased overall expenditure for the populated place [18,19]. At the same time, these suburban areas are usually not connected to the centralized collecting systems. Thus, the local reuse of the onsite generated and treated effluent for irrigation is an appropriate solution, which solves the problems of discharge of the treated wastewater and overuse of drinking water for irrigation purposes.

Even though wastewater reuse at the household level is an opportunity, it possesses a risk mainly due to the potential bacteriological and microorganic pollution [17]. Analyses of the treated wastewater and verification of its harmless use as a source for irrigation should be carried out strictly and in line with the local laws, directives, and environmental impact assessments. Appropriate onsite treatment technologies facilitate the process of obtaining safe reclaimed water.

A possible method for improvement of the treated wastewater quality is through the application of microalgae as a tertiary step of treatment. The subject is still novel and insufficient knowledge has yet been gathered. Some recent papers report the local reuse of the decant (algae-treated wastewater) for irrigation to utilize the remaining nutrients in it, enhancing the effect of the local circular solution [20]. De Morais and co-authors applied algae-treated effluent for seed production and irrigation of naturally restricted use areas (hedges, containment areas, terraced meadows). Uggetti and co-authors report on the use of reclaimed algae-treated wastewater for the irrigation of a small area of agricultural land (around 250 m^2) in Spain [21]. Both teams, however, considered an additional treatment for total suspended solids (TSS), disinfection, and pH control (de Morais et al., 2022) or solar disinfection (Uggetti et al., 2018) [20,21]. Being a new application, the algae-post treatment method in a decentralized solution needs a great deal of research work. The current paper aims at making a step forward in that direction.

- Use of algae as fertilizer

Algae have demonstrated their potential for application as a bio-fertilizer due to their ability to accumulate high amounts of the main macro- and micronutrients [22–24]. For example, an improvement in the content of N, P, and K in the soil and the enhanced development of the roots, shoots, and grains of wheat plants were achieved when unicellular and filamentous algae were used for soil enrichment [25,26]. Other studies also suggest that the presence of microalgae from wastewater treatment in non-sterile soil improves P release when they are mixed with phosphorus-solubilizing organisms [26,27].

In parallel with the beneficial content, the excess algae from wastewater treatment can retain trace concentrations of essential and non-essential heavy metals for their vital processes via active and passive uptake, sorption, biosorption, ion exchange, and detoxification [28–30]. On one hand, the accumulation of heavy metals by algae can be beneficial since it can reduce toxicity in the treated wastewater and, respectively, in the waterbodies [22,28,29]. On the other hand, it raises concerns about the phyco-remediation of its excess biomass, generated in the wastewater treatment process, for soil fertilization and plant production since it can transfer the heavy metal toxicity from the treated wastewater to the soil in which it is used [22,24]. Studies have been made on the topic of using large-scale urban biologically treated wastewater coupled with algae post-treatment. However, no data was found on a decentralized coupling of the two technologies (with all of their specifics) and the local application of the algal biomass as a fertilizing agent. This is one of the novel aims of the current paper.

- Use of algae as a source for extraction of valuable materials

With the recent advancements in "green" technologies, the demand for raw materials that are used in the electrical power sector has significantly increased. Studies have forecast that these needs will lead to future scarcity of copper in the US and the world in general by 2050 [31,32]. The rapidly expanding battery and microchip industry alongside the environmental complications with lithium and cobalt natural extraction, led to the inclusion of both elements among the Critical Raw Materials in the list of the European Commission for 2020 [33]. Another element that is still not on that list but is closely monitored, in view of developments related to growth in demand for battery storage, is nickel [33].

The process of phyco-remediation of valuable metals is a promising method for their extraction from wastewater, reducing the amounts of the pure metals derived from the natural mine sites [22,34]. The effectiveness of their uptake by algae is mainly dependent on the pH and temperature of the medium, the used strain, and the contact time in the reactor [22,24,34]. An additional benefit of microalgal cultivation is the production of precious metals. Recent studies suggest that the cultivated algal biomass can be used for the synthesis and extraction of precious metals like silver and gold which is triggering the scientific and business world for research advancements in that field [35–37].

Our study was designed to answer the research question of whether the existing onsite (decentralized) treatment technologies can be improved to address the circular economy principles by producing high-quality effluent for local re-use purposes as well as for extraction of valuable materials. Similar studies have not been found in the scientific literature. In our experiment one of the best available technologies for onsite (decentralized) wastewater treatment (based on biological sequencing batch process) was coupled with one of the most promising green technologies (algae-based technologies) and this is considered the novelty of this research. The experimental set-up consists of an onsite operating treatment facility in a household (biological treatment) and laboratory algal photo-bioreactors (post-treatment). Three aspects were studied from a chemical elements perspective: (i) the irrigating potential; (ii) the potential to use the algae as fertilizer; (iii) the potential to use the algae as a source for extraction of valuable materials. Research reports in this regard have also not been found in the literature for the studied technology.

2. Materials and Methods

2.1. Experimental Setup

The process flow diagram is shown in Figure 2. Algae post-treatment was simulated in laboratory conditions, but wastewater was taken from an operating decentralized (single household) onsite treatment facility.

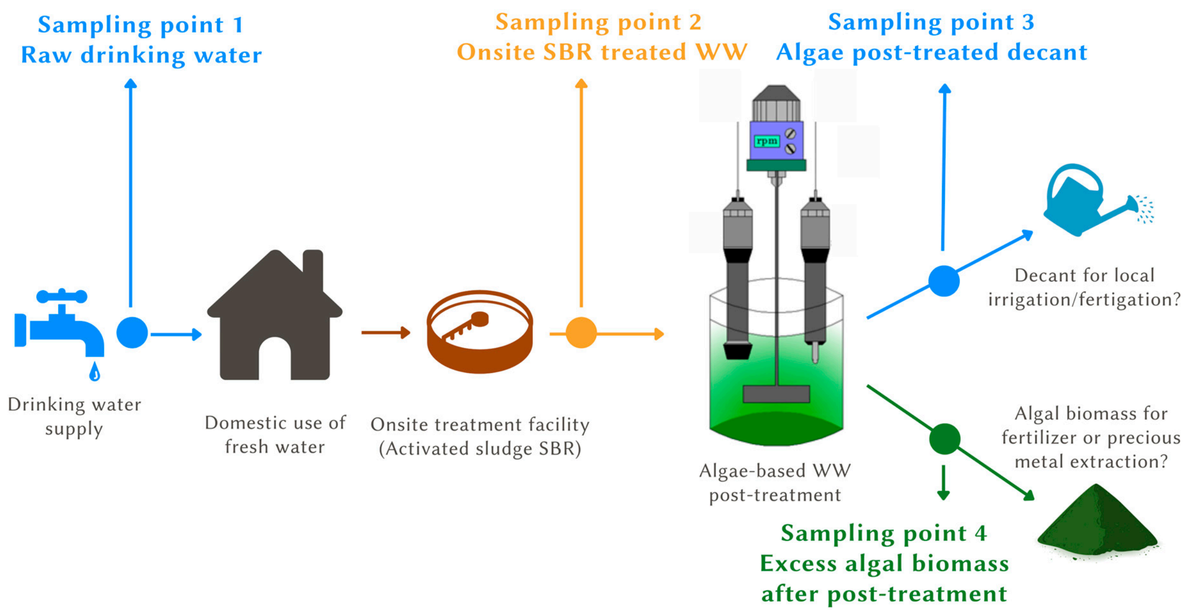

Figure 2. The process flow diagram and sampling points of the experiment (WW-wastewater).

- Onsite (decentralized) treatment facility (activated sludge SBR)

The onsite treatment facility (capacity for 10 people) is located in a household in Bistritsa, Southwest Bulgaria. The treatment process consists of an equalizing tank and an activated sludge sequencing batch reactor (SBR) without phosphorus and nitrogen removal. Feeding of wastewater for the algae laboratory reactors was taken at the outlet of the SBR system while its decanting cycle was in progress so that the microalgal post-treatment process would simulate a final polishing step. Treated wastewater from the outlet of the local treatment facility was filtered through 25 µm pore-size paper filters before feeding into the algae reactor to remove any remaining coarse particles and to partially reduce the concentration of total suspended solids (TSS).

- Laboratory reactors

Two identical laboratory scale, open photo-sequencing batch reactors (PSBRs) were maintained throughout the experiment (Figure 3).

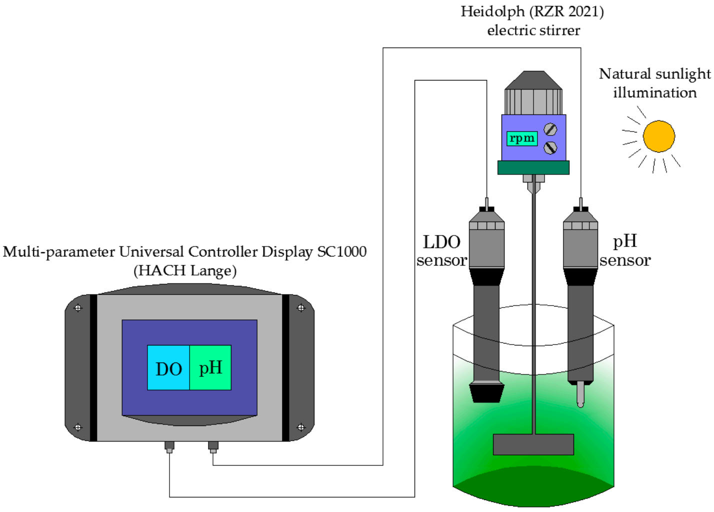

Figure 3. Laboratory scale open PSBR, used in the experiment (**left**—scheme; **right**—photo of the set-up).

Both systems, reactor 1 and 2, operated with natural sunlight intensity, i.e., without an artificial light source. The approximate photoperiod was 14 h of solar illumination and 10 h of dark mode. The temperature of the water varied in the range of 19 °C to 33 °C. The components of each of the reactors were: (i) glass cylinders with D = 170 mm and H = 270 mm (used volume of approx. 4.5 L); (ii) Heidolph RZR 2021 (manufactured by Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) electric stirrers (propeller size—B = 120 mm and H = 50 mm), set to 30–40 rpm; (iii) Multi-parameter Universal Controller Display SC1000 (manufactured by HACH Lange, Berlin, Germany) with a Luminescent Dissolved Oxygen (LDO) Sensor, a 1200-S pH Sensor, and a temperature sensor, recording the three parameter values hourly.

2.2. Algal Strains and Their Adaptation

The experiment used suspended growth algal systems with monocultures of two morphologically different strains from two different phyla of the green evolutionary line—the filamentous streptophyte *Klebsormidium nitens* (Kützing) Lokhorst (reactor 1) and the coenobial coccal chlorophyte *Tetradesmus obliquus* (Turpin) M. J. Wynne (reactor 2). Both strains originated from Bulgaria, but *K. nitens* was purposively introduced by us in the primary experimental studies, whereas *T. obliquus* appeared occasionally, successfully co-exhibited with it, and was isolated for use in this study.

These strains were used in previous experiments and showed potential worth for further exploration as explained below.

(1) *Klebsormidium nitens* (Kützing) Lokhorst

This strain was isolated from the high-alpine soils of Rila National Park (Bulgaria) and was cultivated as ACUS00207 in the Collection of Living Algae ACUS of the Sofia University "St. Kliment Ohridski" on standard Bold-Basal medium [38,39]—Figure 3. *K. nitens* is a green non-branched, uniseriate filamentous alga from the phylum Streptophyta, which grows in different types of habitats with a preference for the aero-terrestrial mode of life [40]. It has a large parietal trough-like plastid with one pyrenoid and reproduces by several means: vegetatively most often by the disintegration of the filaments, which ensures very fast replication, by division of cells in two daughter cells, or formation of peculiar enlarged resting cells (akinetes) or, rarer, asexually by zoospores [39,40]. Its potential for treating municipal wastewater was demonstrated in a study by Valchev et al., 2021 [41].

(2) *Tetradesmus obliquus* (Turpin) M. J. Wynne

This strain was found as invasive in one of the reactors during the experiment. It was purified to a monoculture and included in the Collection of Living Algae ACUS of the Sofia University "St. Kliment Ohridski" as ACUS00220. Similar, to all other strains in ACUS, it is cultivated on the standard Bold-Basal medium [38]. The isolated strain represents a widespread freshwater green microalga from the phylum Chlorophyta [42], which is generally found as 4-celled coenobia, but during cultivation could disintegrate into single cells—Figure 4. Each cell has a large parietal pyrenoid with well-pronounced pyrenoid and reproduces mainly asexually through non-motile spores, which are mini-copies of the mother cell (autospores) and ensure fast replication (e.g., Komárek and Fott 1983; Stoyneva-Gärtner and Uzunov 2017 [43,44]). *Tetradesmus obliquus* was relatively recently derived from the genus *Scenedesmus* [45], which is one of the most widely used algae in wastewater treatments due to its possibility to grow both autotrophically and mixotrophically with a broad range of tolerance to different types of domestic and synthetic waste (e.g., Acevedo et al., 2017; Di Caprio et al., 2018; Ye et al., 2020; Sánchez-Zurano et al., 2021; Fereira et al., 2022 [46–50]).

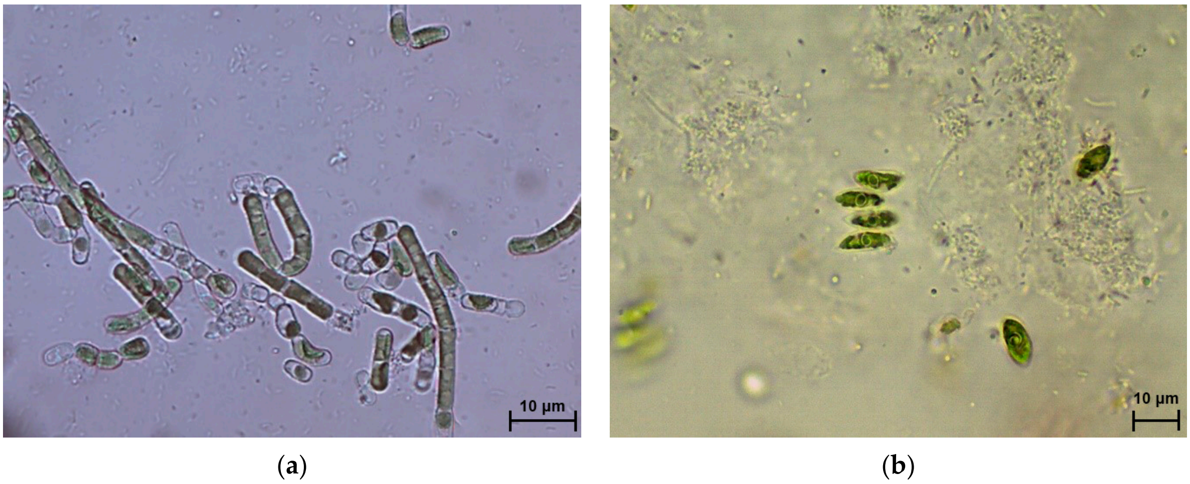

Figure 4. (**a**) *K. nitens* strain ACUS00207 at magnification 40×; (**b**) *T. obliquus* strain ACUS00220 at magnification 100×.

- **Algae adaptation**

After the desired amount of biomass was achieved in the pre-experimental phase, each of the strains was added to the respective PSBR with the real wastewater. The algal cultures were adapted to the new conditions of the laboratory set-up (wastewater, temperature, photoperiod, working mode, etc.). The adaptation period was considered finished once the results from each reactor started to become consistent and a stable monoculture system was established.

The adaptation periods for the two strains were approximately two weeks for each of the reactors. During the adaptation period, reactors were periodically (once every 2 days) supplied with a "fresh" amount of wastewater from the onsite treatment facility to compensate for the intensive evaporation.

- **Algae monitoring during the experiment**

During the experiment, water samples were checked regularly (two to three times a month) on non-permanent slides using a conventional light Motic BA400 microscope. During the work, microphotographs were taken on the same microscope by a Moticam 2 camera supplied by the Image Plus Program.

2.3. Experimental Sequence

After the adaptation period, the actual experimental cycles (Figure 5) of the PSBR wastewater treatment process took place. The sequencing batch mode was selected to match the algae post-treatment with the biological onsite treatment facility (working in sequencing batch mode as explained above).

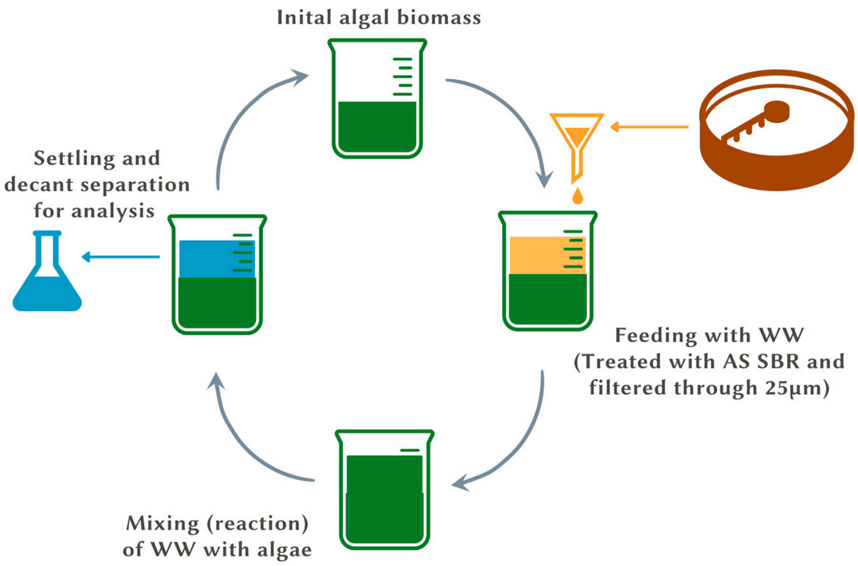

Figure 5. Algae PSBR cycle sequence.

The main phases of the PSBR working mode of each reactor consisted of the following.

Phase 1: Filling—pre-treated wastewater was filtered and fed to the algal biomass in each of the reactors.

Phase 2: Mixing and reac-tion—the suspension (wastewater and algae) was homogenized with a vertical electric stirrer. The dynamics of the TP and TN concentrations were followed during this phase with periodic measurements.

Phase 3: Settling and decanting—at the end of the reaction phase, at a pH of approx. 8–9 (autoflocculation occurred at these values), the electric stirrer was stopped, and the algal biomass was left for 30 min of settling. After that, the decant was separated and removed from the reactor while the residual settled biomass was left in the reactor for the next cycle of treatment.

The hydraulic retention time (HRT) for each cycle was approximately 24 h for most of the experiment. In some cycles, the HRT was extended up to 5 days due to influent wastewater quality variation, caused by a significant increase of the onsite treatment facility inlet load from additional residents of the local household.

2.4. Chemical Analyses

Samples were taken from the sampling points shown in Figure 2 and analyzed with ICP-MS. Three independent samples were taken from each sampling point. For sampling point 4, samples were taken at the beginning of the first cycle and at the end of the final cycle in order to analyze the accumulation of the elements in the biomass.

For sample preparation, HNO_3 (67–69%, Fisher Chemicals, TraceMetal Grade) and H_2O_2 (30% Fisher Chemicals, Trace Analysis Grade), and double-deionized water (MilliQ) were used for samples and standards preparation.

The samples containing wastewater and algae were stored in a freezer for 24 h. After thawing, a green precipitate and a clear solution were obtained. The solution was decanted, and the wet algae residue was placed in a water bath at 80 °C for 2 h and afterwards dried to constant weight in an oven. Three parallel samples of each dry algae residue were weighed on an analytical balance (appr. weight 0.05 g) and transferred to a glass vessel. For complete digestion of the samples, a mixture of 5 mL conc. HNO_3 and 2 mL H_2O_2 was added and the samples were heated on a hotplate at 200 °C. The heating continued until clear solutions were obtained and the volume of the sample was reduced to about 1 mL. The digested solutions were then transferred to polypropylene tubes and diluted to 20 mL with double-deionized water.

To check the influence of organic matter in the wastewater samples, they were analyzed without sample preparation and after acid digestion. An aliquot of 10 mL of each wastewater sample was transferred to a glass vessel, mixed with 8 mL HNO_3 and 2 mL H_2O_2, and heated on a hotplate at 200 °C. The heating continued until transparent solutions were obtained and the volume was reduced to less than 1 mL. Next, the samples were quantitatively transferred to polypropylene tubes by rinsing several times with double deionized water (MilliQ) and diluted to 10 mL. Three parallel samples were prepared from each wastewater sample. No influence of the matrix was established during the analysis. The drinking water samples were analyzed without any pretreatment.

The analyses of the samples were carried out using ICP-MS (Perkin-Elmer SCIEX Elan DRC-e) with a cross-flow nebulizer. External calibration by multi-element standard solution was performed. The concentrations of the elements were determined using the isotopes as follows: ^{31}P, ^{39}K, $^{42,44}Ca$, $^{24,25,26}Mg$, ^{32}S, determined as ^{48}SO in DRC mode, using oxygen as a reaction gas [51], ^{11}B, $^{63,65}Cu$, $^{54,57}Fe$, ^{55}Mn, $^{60,62}Ni$, $^{64,66}Zn$, ^{52}Cr, $^{107,109}Ag$, ^{59}Co, ^{7}Li, $^{204,206,208}Pb$, $^{110,112,114}Cd$, and ^{75}As. The determination of the macro-elements P, K, Ca, Mg, Si, and Fe was performed in cell-based mode by optimization and application of an individual dynamic bandpass tuning parameter (RPa) for each isotope, as described in Lyubomirova et al., 2020 [52]. Working standard solutions for calibration were prepared from single-element standard solutions (Fluka) with initial concentrations of 1000 mg/L.

The estimation of accuracy was performed by the analysis of wastewater CRM (landfill leachate—trace metals LGC6177), two surface water standard reference materials: SPS-SW2 (Reference Material for Measurement of Elements in Surface Waters, Spectrapure Standards, Norway), and NWTM-23.5 (Environmental matrix reference material, a trace element-fortified sample, Environment, and Climate Change, Canada) and plant CRM NIST 1547 (peach leaves, National Institute of Standards and Technology, USA).

During each cycle, the total nitrogen (TN) concentration in the reactor was also measured regularly to ensure the medium had enough of the element to support algal life. Chemical oxygen demand (COD) was also measured throughout the experiment to monitor the balance of the organic content in the water medium. The TN and the COD concentrations were measured using HACH Lange cuvette tests (approved by ISO 15705) and spectrophotometric method analysis. Before each TN measurement, the sample was filtered through a 0.45 μm glass fiber filter.

Out of 68 measured chemical elements, only those of higher interest (i.e., valuable or posing environmental/health risk) were analyzed in the study and are discussed below. They are presented in Table 1.

The dynamics of the elements in their pathway during the performed treatment were analyzed as follows:

- from drinking water (tap) to effluent of the onsite treatment facility (sampling point 1 to sampling point 2, Figure 2)
- from the effluent of the onsite treatment facility to the algae post-treated effluent (sampling point 2 to sampling point 3, Figure 2)
- from the effluent of the onsite treatment facility to the algal biomass (sampling point 2 to sampling point 4, Figure 2).

Drinking water sampling was chosen because it is one of the sources for the elements presented in the final treatment products and it is easier to be monitored than the other generated wastewater flows.

- The analysis is used as a basis to discuss the three possible values of the treated effluent and accumulated biomass:
- irrigation with the algae post-treated effluent

- use of microalgae as fertilizer
- use of microalgae to extract valuable elements.

Table 1. Chemical elements analyzed and the reason for inclusion in the study.

Element	Macronutrient	Micronutrient	Valuable Metals	Heavy (Hazardous) Metals: Environmental or Health Risk
Phosphorus	✓			
Potassium	✓			
Calcium	✓			
Magnesium	✓			
Sulfur	✓			
Boron		✓		
Copper		✓	✓	✓
Iron		✓		
Manganese		✓		
Molybdenum		✓		
Nickel		✓	✓	✓
Zinc		✓		
Chromium			✓	✓
Silver			✓	
Cobalt			✓	✓
Lithium			✓	
Lead				✓
Cadmium				✓
Arsenic				✓

3. Results and Discussion

3.1. Process Monitoring

Since pH, dissolved oxygen (DO), temperature, nitrogen, phosphorus, and carbon concentrations in the water medium are important factors for algal growth and algal intake of the different elements, they were monitored throughout the entire experiment. The concentration ranges of each parameter are presented in Table 2.

Table 2. Inlet and media parameters of the algae reactors.

Parameter	Unit	Measured Range
pH	-	6.1 ÷ 11.6
DO	mg/L	4.2 ÷ 21.1
Temperature	°C	19 ÷ 33
TN	mg/L	4.6 ÷ 58.2
TP	mg/L	4.8 ÷ 11.6
COD	mg/L	47.8 ÷ 84.4

The DO and pH in the reactor show high variations (Table 2). This is a common observation for these types of bioreactors since the values of these two parameters are strongly affected by the illumination and the photoperiod of the bioreactor [41]. The values of DO and pH affect the P removal in the algal reactor due to a process known as "alkaline precipitation" as well as metals removal and accumulation by the algal biomass, as discussed in the introduction.

The other parameters that influence the metal uptake and accumulation by algae—TN, TP, and COD—also vary in a significantly broad range of concentrations at the inlet of the algal reactor (Table 2). This is a result of the variation of the effluent quality of the household treatment facility (Figure 2). As discussed in the introduction, the performance of the individual decentralized WW treatment facilities is unstable due to the water use pattern of the households. The experiments were performed with real effluent taken on different days, respectively having different quality.

The correlations between all parameters shown in Table 2 and the elements accumulated in the algae biomass are not discussed in this paper. The focus is on the elements' fate and accumulation alone.

3.2. Accumulation of Elements at Onsite SBR Effluent

The measurements carried out at two sampling points—at the tap and at the outlet of the onsite treatment facility (Figure 2, sampling points 1 and 2)—are discussed in this section. Two sampling campaigns were carried out—in autumn (October 2021) and in spring (March 2022).

The results for the beneficial elements ratios between the SBR-treated effluent and tap water are shown in the next two figures (Figures 6 and 7) and their values are presented in Table 3. The measured concentrations in sampling point 2 were divided by the corresponding measured concentration in sampling point 1. In this way, the transformation was traced (accumulation or dissimilation—depending on the ratio, respectively above or below the red line of the value 1 in the figures).

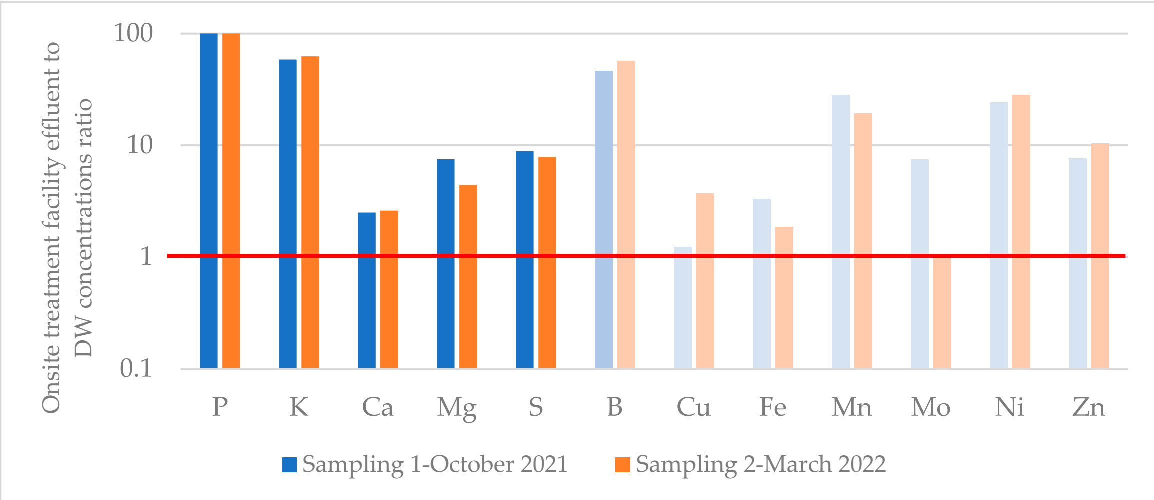

Figure 6. Accumulated soil-related macro- and micronutrients in the onsite treated effluent (DW—drinking water).

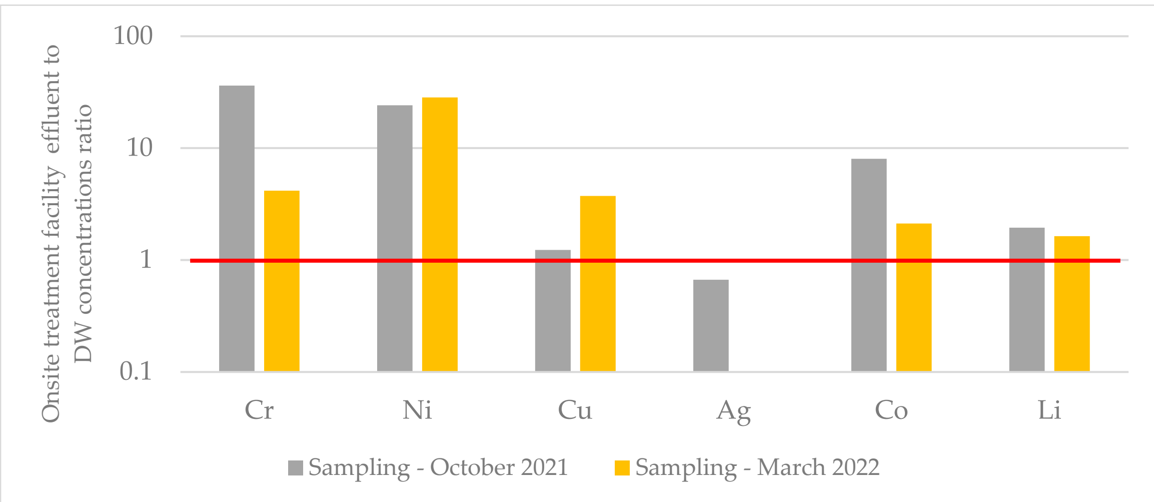

Figure 7. Accumulated valuable elements in the onsite treated effluent.

Table 3. Comparison of the measured macro- and micro-elements, measured in October 2021 and March 2022.

		Sampling Point 1		Sampling Point 2	
Element	**Unit**	**Drinking Water (October 2021)**	**Drinking Water (March 2022)**	**SBR-Treated Effluent (October 2021)**	**SBR-Treated Effluent (March 2022)**
Mg	μg/L	271 ± 8	477 ± 12	2032 ± 33	2108 ± 41
P	μg/L	<0.01	<0.01	7931 ± 167	8157 ± 180
K	μg/L	413 ± 9	352 ± 8	24,100 ± 550	22,000 ± 480
Ca	μg/L	2826 ± 51	2822 ± 48	7079 ± 106	7346 ± 125
S	μg/L	1290 ± 40	1040 ± 35	11,440 ± 430	8170 ± 285
Fe	μg/L	20 ± 1	67 ± 2	67 ± 2	124 ± 4
Mn	μg/L	0.40 ± 0.02	0.75 ± 0.04	11.3 ± 0.5	14.4 ± 0.6
Zn	μg/L	5.54 ± 0.08	7.90 ± 0.09	42.2 ± 0.6	82.0 ± 0.9
B	μg/L	1.9 ± 0.1	0.55 ± 0.03	87 ± 3	32 ± 2
Cu	μg/L	4.7 ± 0.2	4.4 ± 0.2	5.8 ± 0.3	16.4 ± 0.7
Mo	μg/L	1.47 ± 0.06	0.95 ± 0.04	10.9 ± 0.3	0.94 ± 0.03
Ni	μg/L	0.18 ± 0.02	0.09 ± 0.01	4.2 ± 0.2	2.4 ± 0.1
Cr	μg/L	0.39 ± 0.02	0.61 ± 0.04	14.3 ± 0.6	2.5 ± 0.1
Ag	μg/L	0.049 ± 0.004	0.075 ± 0.006	0.033 ± 0.003	<0.01
Co	μg/L	0.016 ± 0.002	0.034 ± 0.003	0.13 ± 0.01	0.072 ± 0.007
Li	μg/L	0.41 ± 0.03	0.53 ± 0.04	0.80 ± 0.06	0.86 ± 0.07

Based on these two sampling campaigns, the following might be concluded: (i) there is a relatively stable accumulation trend in these two measuring campaigns; (ii) all macro- and micronutrients increase in the treated effluent; (iii) the highest increase (more than 10 times) was measured for P, K, B, Mn, and Ni (Figure 6); (iv) valuable elements were accumulated in the treated effluent, so it is worth studying them further to assess the cost-efficiency of their extraction (Figure 7).

Along with the beneficial elements, heavy (hazardous) metals were accumulated as well. The SBR treated effluent to tap water ratios of accumulation are presented in Figure 8 and the values from the two sampling points—1 and 2—are shown in Table 3.

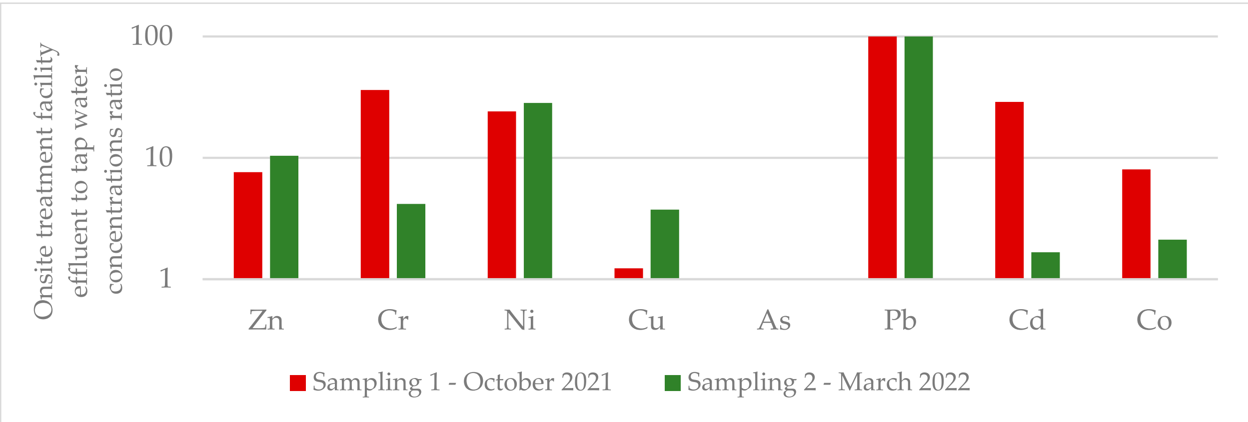

Figure 8. Accumulated heavy (hazardous) metals in the onsite treated effluent.

Although the ratios look extremely high (above 10) for many of the measured heavy metals, it should be noted that their initial concentrations in the drinking water were very low, ranging from <LOD to 0.61 μg/L. Thus, heavy metals in the effluent are not considered to be problematic. Discussion concerning heavy metals is presented in the next section.

3.3. Possible Circular Value 1: Irrigation with the Post-Treated Effluent

3.3.1. Micro- and Macronutrients

The dynamics of the elements were traced further in the algae post-treatment step. Their accumulation in the final effluent (sampling point 3, Figure 2) was compared to the effluent of the onsite treatment facility (sampling point 2, Figure 2). The ratio of the measured concentrations was calculated. Two groups of elements were analyzed—macro- (saturated colors in Figure 9) and micronutrients (unsaturated colors in Figure 9) and heavy metals (Table 4).

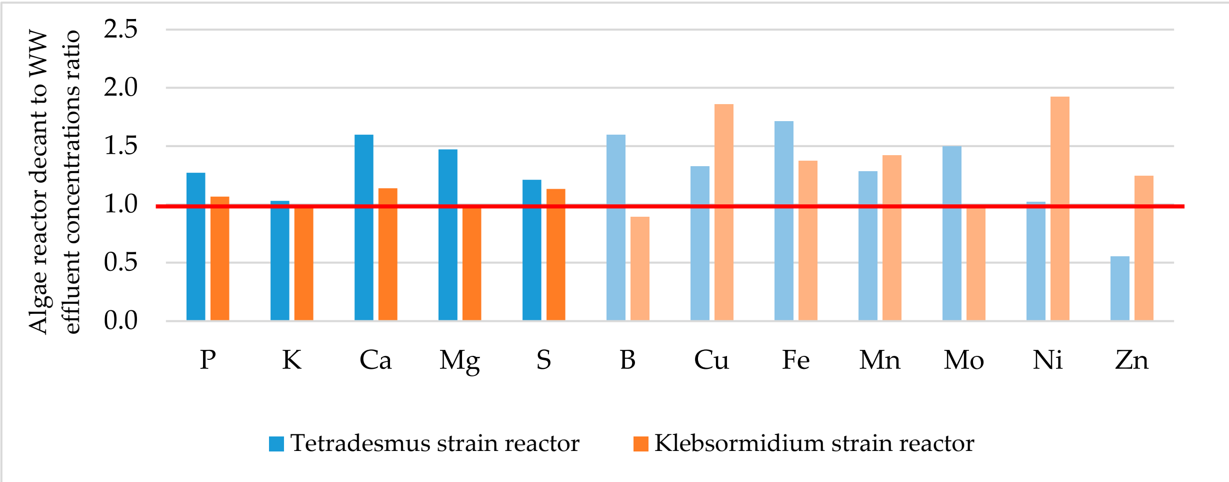

Figure 9. Accumulated macro- (saturated colors on the figure) and micronutrients (unsaturated colors on the figure) in the algae post-treated effluent; WW—wastewater (onsite treatment facility outlet).

While macro- and micronutrient accumulation in treated wastewater (sampling point 2, Figure 2) versus drinking water (sampling point 1, Figure 2) shown in Figure 6 is significant (ratio above 10), the effect of algae post-treatment (Figure 9) is negligible (ratio of around 1 to 2). Therefore, it might be concluded that from the point of view of providing nutrients for the soil, post-treatment with algae does not have a significant effect. No considerable difference was registered between the reactors with *K. nitens* and *T. obliquus.*

3.3.2. Heavy (Hazardous) Metals

The recent Regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for water reuse does not provide limit values for heavy metals content when reclaimed water is used for irrigation [53]. It appears that the establishment of such values is a great challenge. From one side, many of the heavy metals are biologically beneficial in small quantities, and from the other side plant uptake of heavy metals is highly dependent on soil conditions [54]. Probably due to this complexity the EU regulation encourages risk assessment to be performed in any particular case as necessary (Regulation (EU) 2020/741) [53].

To have a rough understanding of the concentration measured in this study, the values obtained were compared with the values provided in the Portuguese standard NP4434 on water reuse for irrigation (4).

Table 4. Comparison of the measured concentrations of heavy metals in October 2021 with the Portuguese standard on water reuse for irrigation.

		Sampling Point 1	Sampling Point 2	Sampling Point 3		Portuguese Standard NP4434 on Water Reuse for Irrigation * [55]	
Element	**Unit**	**Drinking Water**	**Onsite Treated Effluent**	***T. obliquus* Strain Reactor**	***K. nitens* Strain Reactor**	**Recommended Value**	**Max Allowed Value**
Zn	μg/L	5.54 ± 0.08	42.2 ± 0.6	140 ± 2	52.6 ± 0.9	2000	10,000
Cr	μg/L	0.39 ± 0.02	14.3 ± 0.6	2.38 ± 0.09	9.98 ± 0.04	100	20,000
Ni	μg/L	0.18 ± 0.02	4.2 ± 0.2	6.65 ± 0.08	8.12 ± 0.09	500	2000
Cu	μg/L	4.7 ± 0.2	5.8 ± 0.3	25.4 ± 0.5	10.8 ± 0.3	200	5000
As	μg/L	<0.01	<0.01	0.56 ± 0.04	<0.01	<0.01	10,000
Pb	μg/L	<0.01	0.27 ± 0.02	0.48 ± 0.06	0.40 ± 0.05	5000	20,000
Cd	μg/L	0.0010 ± 0.0003	0.023 ± 0.002	0.072 ± 0.005	0.050 ± 0.003	10	50
Co	μg/L	0.016 ± 0.002	0.13 ± 0.01	0.21 ± 0.03	0.09 ± 0.01	50	10,000

* do Monte, 2007; Portuguese standard NP4434, 2008 [55,56].

The data show that the heavy (hazardous) metal concentrations in the study were much below the recommended values of the Portuguese standard on water reuse for irrigation. Although it might be necessary to perform a risk assessment prior to the particular application, at this very early research stage, the results do not show the necessity for concern regarding heavy metal content in the reclaimed wastewater.

Although *T. obliquus* has been well-studied for its great biotechnological potential and high content of proteins, carbohydrates, lipids, and bioactive compounds [57,58], there is only a little data on the content of its inorganic compounds. A very recent study on *T. obliquus* strain RDLR01 biosorption of heavy metals from a tannery effluent after 15 days of treatment demonstrated their reduction in the following descending line of percentage: Cr—99.1%, Co—98.2%, Ni—97.4%, Cd—97.4%, Pb—94.3%, Zn—98.3% and Cu—96.3% [59]. The phylogeny, morphology, physiology, and ecology of the genus *Klebsormidium*, as one of the potential progenitors of land plants, has been well-studied (for details see Stoyneva-Gärtner et al., 2019 [39]) but according to our best knowledge, there are no data on heavy metal accumulation in *K. nitens* particularly. In algal mats of the close species *Klebsormidium klebsii* (G. M. Smith) P. C. Silva, K. R. Mattox et W. H. Blackwell, in both field and cultured conditions the bioconcentration of Al (mg/kg DW) was the highest, and in the field studies it was followed by accumulation of Fe > Mn > Zn [60]. Some tolerance to Cu, Zn, Ni, and Mn in other strains, i.e., *Klebsormidium flaccidum* (Kützing) P. C. Silva, K. R. Mattox, and W. H. Blackwell, *K. rivulare* (Kützing) Morison and Sheath, and *Klebsormidium* sp. was demonstrated in the studies by Say et al., 1977, Say and Whitton 1981; Takamura et al., 1989; Stevens et al., 2001; Skowrońsky et al., 2002; Gaysina et al., 2009 [61–66]. Although there is lack of data on the species used in our study, it is generally known that algae can hyper-accumulate different metals, including the non-nutrient Pb and Cd, but also the nutritional Cu, Zn, and Fe, with the highest accumulation ratio of Fe, followed by Pb, Cu, Cd, and Zn [67–72]. An important statement was made after the experiments conducted by Oberholster et al., 2014, who demonstrated that certain algal species have preferences in elemental bioaccumulation since the accumulated amount of selected metals in the biomass was not strongly dependent on their concentration in the wastewater. This, together with the variability of environmental and cultural conditions probably explains the fact that published data on metal bioaccumulation in algae are highly variable and therefore difficult to compare (for details see Oberholster et al., 2014 [60]).

3.4. Possible Circular Value 2: Using the Microalgae as Fertilizer

3.4.1. Valuable Elements for the Soil

The measured main macro- and microelements for soil fertilization in both strains (Sampling point 4, Figure 2) after approximately two months of reactor operation are presented in Table 5.

Table 5. Macro- and microelement content per kg of algal dry weight from both reactors at the end of the experiment.

				Other Studies	
Element	**Unit**	***K. nitens* Strain**	***T. obliquus* Strain**	***Klebsormidium* sp. NIVA-CHL142 (Indoor Inoculum)**	**Reference [73] Range Batch 1–3**
Mg	g/kg DW	2.39 ± 0.07	6.62 ± 0.09	2.35 ± 0.02	1.7–3
P	g/kg DW	6.83 ± 0.08	16.1 ± 0.5	8.31 ± 0.05	1.8–2.3
K	g/kg DW	4.09 ± 0.06	78 ± 2	11.2 ± 0.1	6.1–7
Ca	g/kg DW	4.06 ± 0.12	313 ± 12	2.74 ± 0.07	41–84
S	g/kg DW	9.8 ± 0.4	7.7 ± 0.3	-	-
Fe	g/kg DW	1.96 ± 0.05	0.83 ± 0.04	0.34 ± 1.69	1–2.7
Mn	mg/kg DW	190 ± 8	272 ± 9	34.0 ± 0.18	176–357
Zn	mg/kg DW	182 ± 6	1145 ± 42	56.9 ± 0.06	121–194
Ni	mg/kg DW	83 ± 2	33.5 ± 0.9	<2	3.1–5
Cu	mg/kg DW	46.3 ± 0.6	88 ± 4	23.1 ± 1.69	18–45
Mo	mg/kg DW	2.27 ± 0.08	1.00 ± 0.04	-	-
B	mg/kg DW	28 ± 1	<0.05	-	-

Note: DW—dry weight.

Both algal strains accumulate all of the main macro- and micro-elements for plant growth except for boron which was not detected in the *T. obliquus* biomass. In terms of macro-elements, the biomass from the *T. obliquus* strain acquired higher amounts of Mg, P, K, and Ca, while the *K. nitents* biomass accumulated more S in the wastewater treatment process. The microelement content of the *T. obliquus* algal dry weight is higher in Mn, Zn, and Cu, whereas *K. nitents* amassed more Fe, Ni, Mo, and B. Therefore, the conducted experiment for the specific local conditions suggests that from the elemental point of view the excess biomass of *T. obliquus*, generated after the onsite algae-based wastewater post-treatment process would be more appropriate for application in soils and with plants that require P, Mg, K, Ca, Mn, Cu, and Zn enrichment. On the other hand, the *K. nitents* biomass would be more suitable for S, Fe, Ni, Mo, and B fertilization. In this respect, here it is worthy to recall that the possibility to apply the inoculum of peculiar vegetative resting cells (akinetes) from different species of the genus *Klebsormidium* as soil biofertilizer has been already proposed [37]. Currently, a bulk biomass of filamentous algae containing *Klebsormidium* sp. NIVA-CHL142 was proposed as a feedstock for new green fertilizer production in a win-win strategy in the circular economy for enterprises that include wastewater treatment plants [71]. Among the important advantages of the algal biomass from our study, is the fact that both studied strains have never been registered as toxin producers, which ensures the safety of the biofertilizer for soils and plants (e.g., Gärtner et al., 2021 [74]).

3.4.2. Heavy (Hazardous) Metals

The elements that induce soil toxicity according to the European and the Bulgarian national legislative documents for the use of WWTP sludge as fertilizer are As, Cd, Hg, Cr, Ni, Pb, Zn, Cu, and Co. It is required that they are periodically monitored. Their permitted content in the dry weight of the final product is determined in the Sewage Sludge Directive 86/278/EEC and the National Ordinance 339/2004 for the use of sludge in agriculture [75,76].

All of the regulated elements were measured in the generated algae biomass during the conducted experiment. Considering that the current experiment uses wastewater generated in a household and there is no industrial contribution, no major load of heavy metals is expected in the influent and subsequently in the generated biomass. However, the reactors operated in a batch mode and each cycle led to the addition of a new portion of raw treated wastewater and respectively a new portion of heavy metals. The largest amount of accumulated elements in the algal biomass is achieved at the end of the final cycle after approximately two months of operation. The heavy metal content per kg of algal dry weight from the two identical reactors at the end of the final cycle in comparison with the permitted legislative contents is presented in Table 6.

Table 6. Heavy (hazardous) metal content per kg of algal DW from both reactors compared to the legislative values.

		Algae Biomass in the Reactor		Limit Value	
Element	**Unit**	***T. obliquus* Strain**	***K. nitens* Strain**	**Sewage Sludge Directive 86/278/EEC [75]**	**National Ordinance 339/2004 for the Use of Sludge in Agriculture [76]**
As	mg/kg DW	0.56 ± 0.04	<0.002	-	25.00
Cd	mg/kg DW	0.33 ± 0.02	0.37 ± 0.02	20.00	30.00
Hg	mg/kg DW	0.28 ± 0.02	<0.002	16.00	16.00
Cr	mg/kg DW	5.1 ± 0.3	36 ± 2	-	500.00
Ni	mg/kg DW	33.5 ± 0.9	83 ± 2	300.00	350.00
Pb	mg/kg DW	8.3 ± 0.4	30 ± 2	750.00	800.00
Zn	mg/kg DW	1145 ± 42	182 ± 6	2500.00	3000.00
Cu	mg/kg DW	88 ± 4	46.3 ± 0.6	1000.00	1600.00
Co	mg/kg DW	0.99 ± 0.07	0.87 ± 0.06	-	-

Overall, both algal strains register low heavy (hazardous) metal contents. Notably higher contents of Cr, Ni, and Pb in the algal dry weight are recorded in the biomass from the reactor with the filamentous strain *K. nitens* while the Zn and Cu levels were significantly higher in the biomass from the PSBR with the coenobial *T. obliquus*.

However, all of the legislatively regulated heavy (hazardous) metals have residual concentrations in the generated algal biomass from both strains but none of them exceed the permitted limit. Furthermore, the heavy metal content in the algal dry weight is 2 to 96 times lower than the allowed content even after two months of operation and progressive accumulation in the biomass. Hence, in terms of heavy metal content, the generated algal biomass in the performed laboratory wastewater post-treatment of onsite treated effluent did not show any risk and promises to be safe for use as a soil enrichment fertilizer.

3.5. Possible Circular Value 3: Using the Microalgae to Extract Valuable Elements

All of the critical metals from the list of the European Commission are analyzed below [33]. In addition, the precious metal silver (Ag) was included.

The element content in the algal biomass was measured: (i) at the beginning of the experiment (after the cultivation and before starting the trials with wastewater-treated effluent) and (ii) after approximately two months of operation of the photobioreactors. The final to initial ratios of each element in the biomass from the respective reactor are presented in Figure 10 in a logarithmic ordinate.

The analyzed data (Figure 10) suggest that no critical or precious metals were accumulated in the biomass of the coenobial *T. obliquus*. On the other hand, the biomass from the filamentous algal strain *K. nitens* accumulated amounts of Cr, Ni, and Ag. The highest accumulation shows Ni as 44.6 times higher in the final biomass sample, for Cr it was 5.95 times higher, and for Ag it was 2.13 times higher. Such significant accumulation of these elements in *K. nitens* in the process of local onsite wastewater treatment, without any major heavy metal contributors (industries, factories, etc.) to the influent draws attention to this specific algal strain for its high potential in future phyco-remediation and circular economy development. Since further extraction and purification of these elements are expensive and time-consuming steps for the production of raw element materials, more extensive research is needed to verify this potential and prove its practical application for wastewater valuable elements utilization.

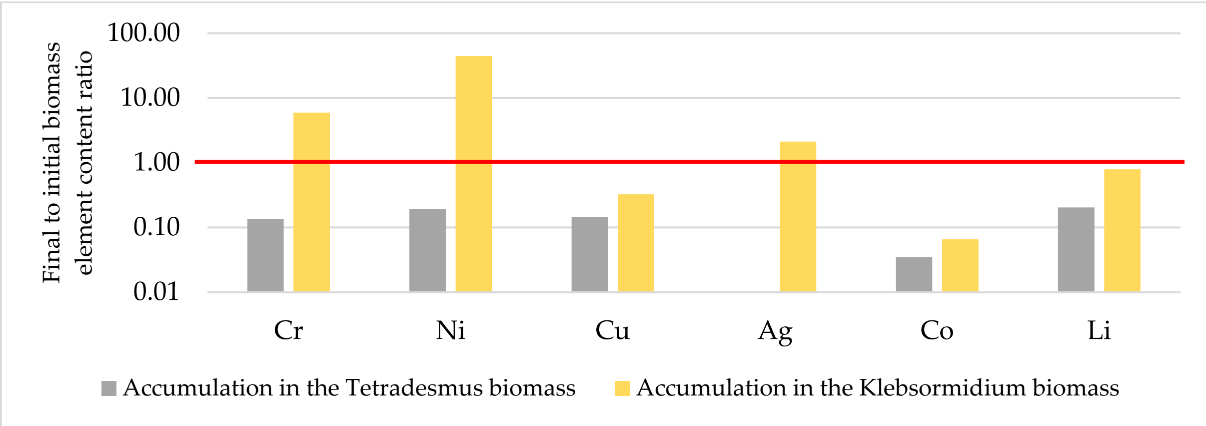

Figure 10. Final to initial ratios of valuable elements in the algal biomass of both reactors.

4. Conclusions

The investigated technology for onsite algae-based wastewater post-treatment after an activated sludge SBR provided two final products—reclaimed wastewater and algal biomass. Both of them have the potential for reuse from a chemical element perspective. The treated effluent is suitable for local irrigation needs, while the algal biomass could be used either as a fertilizer for soil enrichment or as a carrier of valuable elements that could be potentially extracted. Both final products have the following specifics:

- Algae-based post-treatment of the biologically treated wastewater could be classified as neutral in terms of improvement of the final effluent for agricultural irrigation application. A barely noticeable increase of the main macro- and micronutrients and heavy (hazardous) metals is observed.
- The generated algal biomass in the polishing step is a potentially appropriate fertilizing agent for soils since it contains valuable macro- and micro-elements needed for plant and crop growth. The heavy (hazardous) metal content of the algal dry weight is considerably lower than the limiting values in the current European and national legislations.
- The *K. nitens* strain accumulates valuable metals such as chromium, nickel, and silver that can be derived from the biomass. Therefore, the *K. nitens* algae-based post-treatment has the potential of being a local "factory" for the extraction of valuable metals and it can attract interest in its development.

The current conclusions are based on a two-month experiment in laboratory conditions with the use of real treated wastewater from a single onsite treatment facility. Further research at a larger scale is needed in order to validate the achieved results. Future studies to investigate the technology from this paper should focus on the use of treated wastewater from more local onsite treatment facilities. The performed experiments should be carried out for a longer period in order to follow the full mass balances and dynamics of the elements both in the treated effluent and in the generated biomass.

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