

Application of liquid organic fertilizer produced from fish sludge in an aquaponics system: Influences on growth of Nile tilapia (*Oreochromis niloticus*) and peppermint (*Mentha × piperita* L.)

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ARTICLE INFO

Keywords:

Fish sludge
Aerobic digestion
Nutrient mineralization
PSB
Aquaponics
Nile tilapia
Peppermint

ABSTRACT

In aquaponics systems, liquid effluent from fish culture is not enough to meet the nutritional requirements of plants. In this study, an aquaponics system coupled with an aerobic digestion bioreactor (ADBR) for the digestion of the Nile tilapia (*Oreochromis niloticus*) sludge was designed in triplicate. The aerobic digestion of fish sludge was performed using biofertilizer containing phosphate-solubilizing bacteria (PSB) (*Pseudomonas putida* strain P13 and *Pantoea agglomerans* strain P5; 10^7 CFU/mL for each bacterium) at a levels of 0.1 % for 14 days in the ADBR. Nile tilapia (*O. niloticus*) fish and peppermint (*Mentha × piperita* L.) plant were cultivated in two aquaponics (AP) systems for six weeks without and with adding liquid organic fertilizer (LOF) produced from the aerobic digestion of fish sludge as control and AP-LOF treatment, respectively. The use of PSB for aerobic digestion of the fish sludge led to increasing the PO_4^{3-} , K, Fe, Zn, Cu, and Mn concentrations compared to the control ($p < 0.05$). The electrical conductivity (EC) of the LOF increased significantly with adding PSB compared to the control ($p < 0.05$). The final body weight of Nile tilapia increased by 81.82 % and 104.17 % in the AP and AP-LOF treatments after six weeks, respectively. The food conversion ratio (FCR) improved in the AP-LOF treatment compared to the AP treatment ($p < 0.05$). The total ammonia nitrogen (TAN) and nitrate concentrations decreased in aquaponic water with adding the LOF compared to the AP treatment ($p < 0.05$). The biomass and morphological traits of peppermint, including fresh weight of plant, and aerial parts, shoot height, number of leaves, and nodes per plant increased in the AP-LOF treatment compared to the AP treatment ($p < 0.05$). The P, K, Na, Ca, and Fe contents of peppermint cultivated in the AP-LOF treatment were higher than in the AP treatment ($p < 0.05$). In conclusion, the use of PSB in the ADBR unit of aquaponics system improved the nutrient recovery from Nile tilapia sludge and adding liquid organic fertilizer to aquaponic water had a positive effect on the growth performance of Nile tilapia and peppermint plant.

1. Introduction

The world population is estimated to reach 9.7 billion people in 2050, and meeting their need for healthy food is a major global challenge (FAO, 2018; United Nations (UN), 2019). In the face of global natural resource scarcity, developing technologies that produce nutritious food with minimal consumption of natural resources is needed (Ickowitz et al., 2019; Boyd et al., 2020). Examples of these types of technologies are sustainable aquaculture systems such as recirculating aquaculture systems (RAS), and biofloc technology (BFT) that can contribute to the maximum production of aquatic animals per volume

with less use of resources and minimum adverse environmental impact (van Rijn, 2013; Khanjani and Sharifinia, 2020).

Aquaponics system combines the RAS and hydroponics for simultaneous production of aquatic animals and vegetables (Lennard and Aquaponics, 2019). In an aquaponics system, the symbiosis of fish, plants, and microorganisms establishes a complex system in which these organisms must be merged into one working system (Suhl et al., 2016). In aquaponics, fish excreta and feed residue are removed by mechanical filters and toxic ammonia compounds are transformed to nitrate in the biofilter, which is absorbed by plants (Deswati et al., 2020; Pinho et al., 2021). This system has many advantages, including high efficiency in

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<https://doi.org/10.1016/j.aquaeng.2025.102541>

Received 4 February 2025; Received in revised form 4 April 2025; Accepted 6 April 2025

Available online 11 April 2025

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use of water and feed, nutrient reuse, reduced discharge of nutrient-rich effluents into the environment, the variety of products, and reduced cost of fertilizers (Gott et al., 2019; Yep and Zheng, 2019). The dissolved nutrients in the aquaculture effluent cannot meet the nutrient requirements of the plants in an aquaponics system. Several studies have reported that the nutrients provided by aquaculture effluents are insufficient for plant growth (Nicoletto et al., 2018; Yep and Zheng, 2021; Tsoumalakou et al., 2022). Bittsanszky et al. (2016) stated that the nutrient concentrations for plants in aquaponics are significantly lower compared to hydroponics. Essential nutrients for plants, including phosphorous (P), calcium (Ca), potassium (K), iron (Fe), manganese (Mn), and molybdenum (Mo) are frequently at sub-optimal levels in water of aquaponics systems (Rakocy, 2012; Delaide et al., 2017). Deficiency of micronutrients in water led to higher root-to-shoot ratio of lettuce (*Lactuca sativa*) in aquaponics system (Nozzi et al., 2018). Adding nutrient supplements to water, which increases the cost, is necessary to meet the nutrient needs of plants and increase crop productivity in coupled aquaponics systems (Delaide et al., 2016; Nicoletto et al., 2018). Therefore, providing nutrients for plant growth through cost-effective methods is emphasized.

The fish feces and un-consumed feed are filtered by mechanical filters and discharged from aquaponics systems as sludge. It is estimated that daily sludge discharge can reach approximately 5–20 % of total water volume in a recirculating aquaculture system (Timmons and Ebeling, 2007). The fish sludge is known as nutritious animal waste and contains most macronutrients and micronutrients required by plants such as nitrogen, phosphorous, iron, and copper (Khiari et al., 2019; Strauch et al., 2018). Khiari et al. (2019) reported that solid waste from tilapia farming contains 4.5 % N, 0.24 % K, 5.79 % Ca, 0.21 % Mg, 2.35 % P, 29 % Fe, 0.09 % Zn, 0.44 % S, and 0.017 % Cu (based on dry matter). In traditional aquaponics systems, nutrients are only recovered from liquid effluent and fish sludge is removed from the system as waste (Khiari et al., 2019). Strauch et al. (2018) reported that reuse of nutrients from solid waste collected from commercial African catfish RAS can reduce the use of mineral fertilizer in aquaponics system. Therefore, bioconversion of fish sludge into liquid fertilizer through nutrient mineralization can be considered a promising approach for plant nutrition in aquaponics systems. Nutrient mineralization is the decomposition or oxidation of organic matters into soluble and bioavailable nutrients for plants via activities of microorganisms under aerobic and anaerobic conditions (Delaide et al., 2018; Zhang et al., 2021). Aerobic digestion shows higher nutrient recovery and less nutrient loss than anaerobic digestion (Zhang et al., 2021). Monsees et al. (2017) and Delaide et al. (2018) have also demonstrated that aerobic digestion of fish sludge significantly increased macronutrient and micronutrient concentrations compared to anaerobic digestion. Other advantages of aerobic digestion include more environmentally friendly, simpler, and safer operation, lower chemical oxygen demand (COD), and less production of volatile fatty acids (VFAs), and secondary metabolites (Delaide et al., 2018; Zhang et al., 2021).

Aquaculture sludge is rich in phosphorus, with a higher P content than domestic sludge (Timmons and Ebeling, 2007), but this form of phosphorus is not readily available to plants (Abbo, 2020). Other studies have also reported a deficiency in total P in aquaponics systems, possibly due to phosphorus precipitation as a solid form (Yang and Kim, 2020; Nuswantoro et al., 2023). Phosphorous might bind with calcium, iron and aluminum, forming insoluble complexes, which precipitate in the water (Armandeh et al., 2022). Phosphorus is a vital nutrient at all developmental stage of plant from germination to maturity. Phosphate deficiency slows plant growth due to decrease in photosynthesis or increase in energy investment (Malhotra et al., 2018). Many microorganisms, including bacteria, fungi, cyanobacteria, and actinomycetes have potential of the mineralization of organic phosphorus and solubilization of inorganic phosphorus that is unavailable to plants (Doilom et al., 2020; Rawat et al., 2021). Phosphate-solubilizing bacteria (PSB) such as the genera *Bacillus*, *Pseudomonas*, *Enterobacter*, *Nitrosomonas*,

Enterobacter, *Erwinia*, and *Pantoea* have several mechanisms to increase phosphorus availability, including the release of extracellular enzymes, the substrate degradation, and the release of mineral-dissolving compounds (Sharma et al., 2013; Shrivastava et al., 2018). Armandeh et al. (2022) isolated PSB belonging to the genera *Acinetobacter* and *Pseudomonas* with the ability to release phosphorus from fish earthen ponds. The results of another study showed that PSB could solubilize phosphorus with efficiency of 40 % from the scales of tilapia fish (Santanaa et al., 2019). Abbo (2020) reported that aerobic digestion of Nile tilapia (*Oreochromis niloticus*) sludge collected from a recirculating aquaculture system (RAS) with fungus *Trichoderma harzanium* enhanced conversion of organic phosphorus into orthophosphate.

To the best knowledge of the authors, little research has been done on aerobic digestion of fish sludge using PSB and effects of obtained supernatant after mineralization as liquid organic fertilizer on plant growth in an aquaponics system. In this study, an aerobic digestion bioreactor (ADBR) was designed and installed in an aquaponics system. The Nile tilapia (*Oreochromis niloticus*) sludge was converted to liquid organic fertilizer (LOF) using PSB in the ADBR. Then, the effects of adding LOF on the growth performance of Nile tilapia (*O. niloticus*), the yield of peppermint (*Mentha × piperita* L.), and the water quality in an aquaponics system were studied.

2. Materials and methods

2.1. Chemicals

Bio-fertilizer containing phosphate-solubilizing bacteria was purchased from Green Biotech Co. (Tehran, Iran). Ammonium chloride (NH₄Cl), sodium nitrite (NaNO₂), and sodium hydrogen carbonate (NaHCO₃) salts were purchased from Merck Co. (Germany). Nitrogen-Ammonia Reagent set and NitraVer®5 Nitrate Reagent Powder Pillows were obtained from Hach Co. (Colorado, USA).

2.2. Experimental treatments

A completely randomized design was conducted to investigate the effects of adding liquid organic fertilizer (LOF) produced from aerobic digestion of fish sludge using phosphate-solubilizing bacteria to water on the growth performance of Nile tilapia (*O. niloticus*) and yield of peppermint (*Mentha × piperita* L.) in an aquaponics system. In total, two treatments were tested: (1) Nile tilapia and peppermint cultivation in a coupled aquaponics system (AP) with adding LOF, referred to AP-LOF treatment. (2) Nile tilapia and peppermint cultivation in AP without adding LOF, referred to AP treatment (control).

2.3. Aquaponics system design

Three pilot-scale aquaponics (AP) systems coupled with aerobic digestion bioreactors (ADBRs) were installed in a greenhouse at Ferdowsi University of Mashhad (Mashhad, Iran). Each AP-ADBR system consisted of a rectangular polyethylene fish tank (150 L), a radial flow settler (RFS; 50 L), a moving bed bioreactor (MBBR; 50 L) containing plastic media (25 mm × 12 mm) with a specific surface area of 1000 m²/m³, an aerobic digestion bioreactor (ADBR; 15 L), hydroponic unit, a water submersible pump (RS-4000 model, RS Electrical, India), water pipes, and controllable valves. Three pilot-scale aquaponics systems were also installed without ADBR. Water of all systems was aerated via a central air blower (STREAM HG-750SB model, China) and air stones installed in the fish tank, MBBR, and ADBR. The ADBR was equipped with a small water submersible pump (Sea Star Q-800 model, China) for constant circulation, and two air stones for water aeration. The three ADBRs were incubated in a water bath (1 × 0.5 × 0.4 m polyethylene tank) equipped with a 300 W aquarium heater (RS Electrical, India) to adjust water temperature. The lids of all bioreactors were tightly closed to prevent evaporation. Each hydroponic unit consisted of three parallel

nutrient film technique (NFT) channels. Each PVC channel with 120 cm length and 9 cm diameter with six holes for placing net pots was equipped with inlet and outlet valves to control water flow (Fig. 1).

In each coupled aquaponics system, water from fish tank was continuously pumped (1.5 L/min) through a submersible pump to the bottom of the RFS, where feed residues and fish feces settled. Using gravity, the water flowed from upper outlet of the RFS to the MBBR, and then was transferred to the hydroponic unit through the upper outlet. The water flow rate to the three NFT channels was adjusted via controlled valves. After passing through the NFT channels, the water returned to the fish tank by gravity.

2.4. Aerobic digestion of fish sludge

The effect of phosphate-solubilizing bacteria (PSB) on the aerobic digestion process of the Nile tilapia (*O. niloticus*) sludge was investigated for 14 days. The collected fish sludge (feces + feed residues) from the RFS unit was dewatered through a laboratory filter press to increase the dry solid content, and stored at $-20\text{ }^{\circ}\text{C}$ until needed. At first, the dewatered fish sludge was sterilized by autoclaving at $121\text{ }^{\circ}\text{C}$ for 20 min. Each aerobic digestion bioreactor (ADBR) was filled with 10 L of dechlorinated Tap water and dewatered sludge at a rate of 1% (w/v) and mixed well. The biofertilizer containing PSB (*Pseudomonas putida* strain P13 and *Pantoea agglomerans* strain P5) by counting 10^7 of each bacterium per mL (CFU mL $^{-1}$) was added at a level of 0.1% (v/v). The pH and electrical conductivity (EC) of the biofertilizer were 7 and 0.5 ds m $^{-1}$, respectively. The biofertilizer level of 0.1% and digestion period of 14 days were chosen based on a series of pre-tests on aerobic digestion of the Nile tilapia sludge under *in vitro* conditions. Three ADBR units without adding PSB were considered as control. The water-sludge mixture was continuously mixed, aerated and its temperature was adjusted at $30\text{ }^{\circ}\text{C}$ during 14 days of the experiment. The pH was monitored daily and maintained at 6.62 ± 0.036 by adding a small amount of HCL 37%. Water evaporation of each bioreactor was compensated by adding dechlorinated Tap water having the same pH as the mixture. Samples were collected weekly in 100 mL bottles from the supernatant of the bioreactor after stopping the aeration and water circulation. Samples were centrifuged (10,000 \times g) at room temperature for 5 min, and the supernatant was used to measure water quality parameters. The pH and EC were measured using a pH meter (HI 2211 model) and a conductivity meter (JENWAY 4510), respectively. The phosphate

(PO_4^{3-}), total ammonia nitrogen (TAN), and nitrate (NO_3^-) concentrations were determined based on the Hach Molybdovanadate, Hach Ammonia-Nessler, and Hach Cadmium Reduction methods using spectrophotometer (DR 5000TM model) at wavelength of 430 nm, 425 nm, and 500 nm, respectively. The macro and microelements, including K, Na, Ca, Mg, Fe, Zn, Cu, and Mn were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Acros, Germany) after filtration of samples using a Millipore Vacuum Filtration unit equipped with a Whatman filter paper (0.45 μm) and dilution to 25 mL with double distilled water. The water-sludge mixture in each ADBR unit was thoroughly mixed and 100 mL of it was sampled in a bottle. The TSS concentration was measured based on the standard method (APHA, 2017) using a Millipore Vacuum Filtration unit equipped with a Whatman filter paper (0.45 μm). The water-sludge mixture in each ADBR was filtered using a bag filter (5 L; 68 μm), and the liquid phase was separated and used as liquid organic fertilizer (LOF) in the aquaponics systems.

2.5. Fish and plant culture

Juvenile Nile tilapia (*O. niloticus*) (n = 180; with initial weight of 25–30 g) were obtained from a tilapia farm located in Shahrud City, Semnan province, Iran and held in six 150-L tanks (30/ tank) for two weeks to acclimatize to laboratory condition. A commercial tilapia feed (Raman Co., Aquatic Animals Feed Producer., Iran) (Table 1) with pellet size of 3–3.5 mm was used to feed the fish three times (8:00; 12:00 and 16:00) daily at a rate of 2.5% of body weight during acclimatization and experimental periods. The six aquaponics (AP) systems were launched simultaneously, and ammonium chloride (NH_4Cl ; 5 mg L $^{-1}$) and sodium nitrite (NaNO_2 ; 5 mg L $^{-1}$) salts were added to the MBBR unit of each

Table 1
Proximate composition of tilapia commercial feed.

Chemical composition	Content (%)
Dry matter	94.78
Crude protein	32
Crude lipid	13
Crude fiber	3
Total phosphorus	4.1
Ash	12

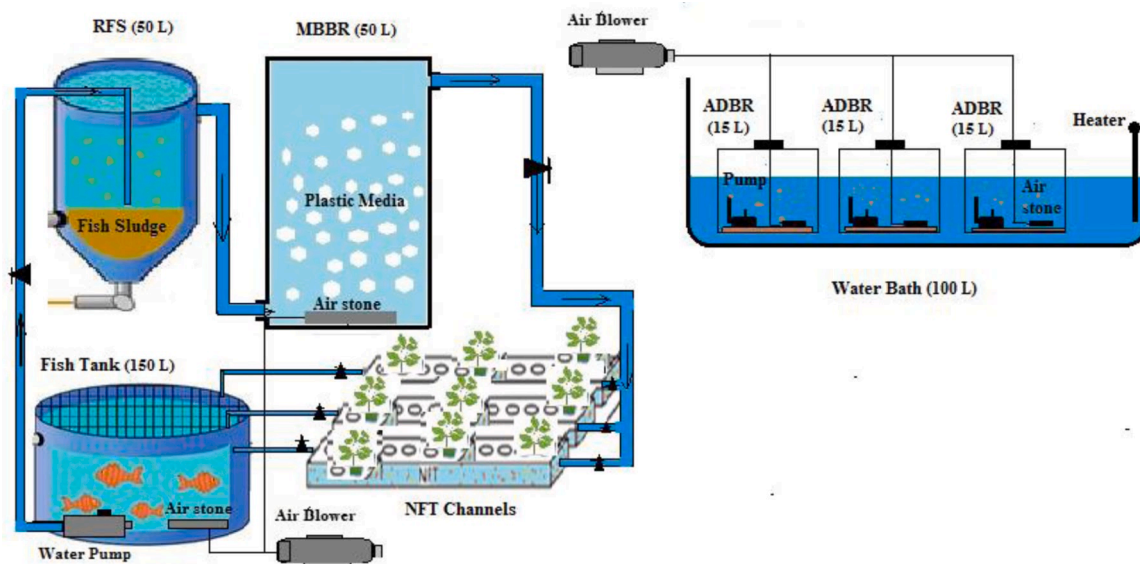


Fig. 1. Schematic figure of the coupled aquaponics system for cultivation of Nile tilapia (*Oreochromis niloticus*) fish and peppermint (*Mentha \times piperita* L.) plant. RFS: radial flow settler. MBBR: moving bed bioreactor. NFT: nutrient film technique. ADBR: aerobic digestion bioreactor.

system two times weekly (Pulkkinen et al., 2018) for two weeks to increase the growth of chemoautotrophic bacteria involved in the nitrification process. After two weeks, 10 Nile tilapia fish were stocked in the fish tank of each AP system and fed with commercial tilapia feed for two weeks to increase water-soluble nutrients for plant cultivation. Then, 30 Nile tilapia fish with an average weight of 29.75 ± 0.89 g were randomly replaced in the fish tank of each AP system and fed for 6 weeks. The sludge that settled at the bottom of the RFS was collected once every three days, dewatered, and stored at -20 °C until used in the ADBR unit.

Eighteen peppermint (*Mentha × piperita* L.) plants with an average weight of 2.42 ± 0.03 g were transplanted in the Net Pots containing cocopeat-perlite mixture (30:70) and transferred to the three NFT channels of each AP system simultaneously with fish stocking. The plants were cultivated for six weeks and 1.5 L of the liquid organic fertilizer (LOF) produced from aerobic digestion of Nile tilapia sludge using PSB added to the NFT channels of three of the six AP systems every three days. The water of each fish tank was replaced with aerated freshwater at a rate of 10 % every day.

2.6. Growth performance

2.6.1. Fish

All fish of each fish tank were counted and individually weighted after anesthesia with clove oil (20 mg L^{-1}) at the beginning and end of the experiment. The following formulae were used to calculate the survival rate and growth indices:

$$\text{Survival rate (\%)} = (\text{Final number of fish} / \text{Initial number of fish}) \times 100$$

$$\text{Weight gain rate (WGR; \%)} = [(\text{Final weight of fish (g)} - \text{Initial weight of fish (g)}) / \text{Initial weight of fish (g)}] \times 100$$

$$\text{Specific growth rate (SGR; \% body weight day}^{-1}\text{)} = [\text{Ln (Final body weight (g))} - \text{Ln (Initial body weight (g))}] / \text{days} \times 100$$

$$\text{Average daily gain (ADG, g)} = (\text{Final body weight (g)} - \text{Initial body weight (g)}) / \text{days}$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed consumed (g)} / \text{Body weight gain (g)}$$

$$\text{Condition factor (CF; g/cm}^3\text{)} = [\text{Final body weight (g)} / \text{Final length}^3 \text{(cm)}] \times 100$$

2.6.2. Plant

The peppermint plants were harvested in two phases. In the first phase, number of leaves, nodes and buds per plant, and height of shoot (cm) were determined three weeks after planting. Then, 10 cm of the shoot of each plant was kept, and the rest was harvested and the fresh weight of harvested aerial parts (shoot and leaves) (g) was measured. In the second phase, all plants were harvested six weeks after planting at the end of the experiment. In addition to the mentioned growth parameters, fresh weight of plant (g), height (cm) and weight (g) of root per plant were also determined.

2.7. Proximate and elemental analysis

At the end of the experiment, three fish were randomly sampled from each fish tank, euthanized, their skin surface was rinsed with distilled water, muscle between gills and dorsal fin was cut and stored at -20 °C until use. The dry matter and ash contents of dewatered sludge, fish muscle, aerial parts, and root of harvested plants were analyzed based on the standard method (AOAC, 2005). The dry matter content was measured by drying the sample in an oven at 105 °C for 24 h. The ash content was determined by incineration at 550 °C for six hours using a

muffle furnace (Exciton Co., 1200 °C model, Iran). The protein content of sludge was determined using kjeldahl system (Buchi Labortechnik AG, Flawil, Switzerland; $N \times 6.25$). To measure the elemental contents of samples, 0.5 g of dried sample was putted in a 50 mL round bottom balloon and 9 mL of HCL, 3 mL of HNO_3 , and 2 mL of H_2O_2 were added. The mixture was maintained at room temperature for 24 h and then heated at 95 °C for 2 h. The digested sample was filtrated using a Millipore Vacuum Filtration unit equipped with a Whatman filter paper (0.45 μm) and dilution to 25 mL with double distilled water. The macro and micro-element concentrations were determined using an inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Acros, Germany). The total nitrogen and carbon contents of the dried sludge were estimated based on the dry combustion method.

2.8. Water quality measurement

During the experiment, temperature and dissolved oxygen were monitored daily in all fish tanks using a portable multi-meter (AZ-8603 model). Temperature and dissolved oxygen were maintained at 26 – 28.5 °C and 6.1 – 6.5 mg L^{-1} , respectively. The water sample was collected in 200 mL bottle from each fish tank every two weeks to measure pH, EC, total suspended solids (TSS), TAN, NO_3^- , PO_4^{3-} , macro, and microelements (K, Na, Ca, Mg, Fe, Zn, Cu, and Mn) according to methods described in 2.4.

2.9. Statistical analysis

Data were presented as mean \pm standard deviation (SD) and statistically analyzed via SPSS 19 software (Chicago, USA). All experimental treatments and analyses were performed in triplicate. The Kolmogorov-Smirnov test was employed to assess the normality distribution of the data. The significant difference between means of two independent treatments was investigated using Independent-Samples T Test. The Significant differences between the means at two times and more than two different times were examined using Paired-Samples T Test and ANOVA with repeated measures test, respectively. Significant differences between means were determined at $p < 0.05$.

3. Results

3.1. Characterization of fish sludge

The proximate composition and elemental analysis of collected Nile tilapia (*O. niloticus*) sludge are presented in Table 2.

Table 2
Proximate composition and elemental analysis of Nile tilapia (*Oreochromis niloticus*) sludge (dry-weight basis, mean \pm SD, n = 3).

Proximate composition of sludge (%)	
Dry matter	61.64 ± 2.93
Crude protein	9.60 ± 1.18
Ash	47.12 ± 4.93
Nitrogen	1.19 ± 0.22
Carbon	11.77 ± 2.72
Elemental analysis of sludge (mg/kg dry weight)	
K	2.50 ± 0.53
Na	0.55 ± 0.14
P	59.70 ± 3.10
Ca	7.95 ± 0.30
Mg	3.37 ± 0.33
Fe	0.836 ± 0.04
Zn	0.267 ± 0.01
Cu	0.191 ± 0.03
Mn	0.034 ± 0.003

3.2. Nutrient concentrations during aerobic digestion of sludge

The fluctuations in water-soluble nutrient concentrations during aerobic digestion of Nile tilapia (*O. niloticus*) sludge without and with adding PSB are shown in Fig. 2(a-c). The phosphate (PO_4^{3-}) concentration was higher in the PSB treatment than in the control on days 7 and 14 ($p < 0.05$). The phosphate concentration increased from 0 to day 7 ($p < 0.05$), while its concentration showed no significant change until day 14 in the PSB treatment ($p > 0.05$). In the control treatment, the phosphate concentration showed no significant change during 14 days ($p < 0.05$) (Fig. 2a). No significant difference was observed in the total

ammonia nitrogen (TAN) concentration between the two treatments on different days of the aerobic digestion ($p > 0.05$). In both treatments, the TAN concentration decreased significantly during aerobic digestion ($p < 0.05$). The lowest TAN concentration was observed on day 7 ($p < 0.05$) (Fig. 2b). There was no significant difference in nitrate (NO_3^-) concentration between the two treatments ($p > 0.05$). The nitrate concentration increased in the first week, but decreased in the second week of aerobic digestion in both treatments ($p < 0.05$) (Fig. 2c). The electrical conductivity (EC) and total suspended solids (TSS) values were higher in the PSB treatment than in the control on days 7 and 14 ($p < 0.05$). After two weeks of aerobic digestion, the EC and TSS values

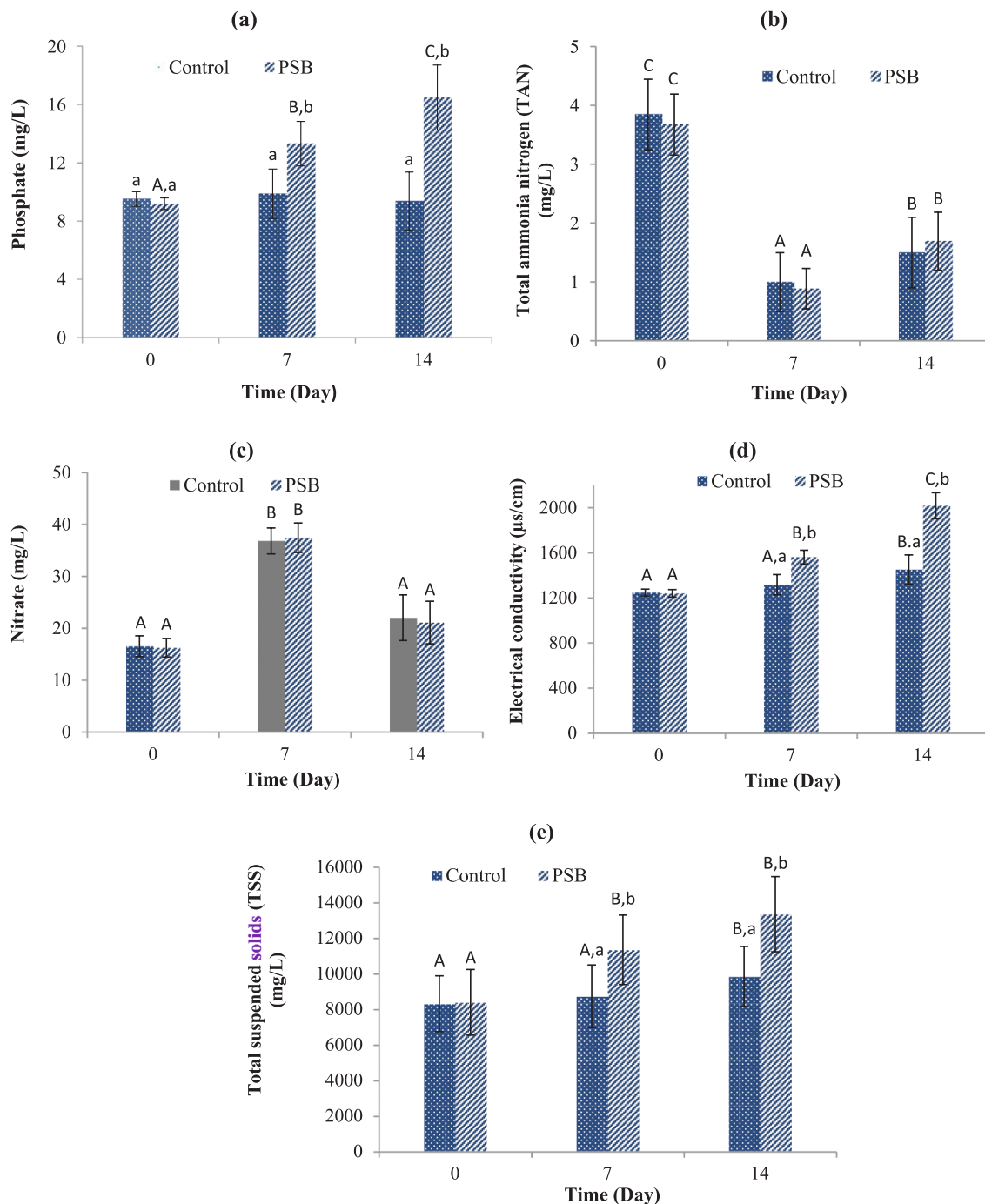


Fig. 2. Variations of phosphate (PO_4^{3-}) (a), total ammonia nitrogen (TAN) (b), nitrate (NO_3^-) (c), electrical conductivity (EC) (d), and total suspended solids (TSS) (e) concentrations at different days of aerobic digestion treatments of Nile tilapia (*Oreochromis niloticus*) sludge without and with phosphate-solubilizing bacteria (PSB) for 14 days (mean \pm SD; n = 3). Bars with different lowercase letters in each time show significant difference among two treatments (Independent-Samples T Test, $p < 0.05$). Bars with different uppercase letters in each treatment are significantly different (ANOVA with repeated measures, $p < 0.05$).

increased significantly from day 0 to day 14 ($p < 0.05$) (Fig. 2e, d).

The comparison of macronutrient and micronutrient concentrations at different days of aerobic digestion of Nile tilapia (*O. niloticus*) sludge without and with PSB for 14 days is shown in Table 3. The K, Fe, Zn, Cu, and Mn concentrations were higher in the PSB treatment than in the control on day 14 ($p < 0.05$). In both treatments, the concentrations of all elements except Mg increased significantly from day 0 to day 14 ($p < 0.05$).

The dry matter and ash contents of Nile tilapia (*O. niloticus*) sludge before and after aerobic digestion with and without PSB for 14 days are shown in Table 4. The dry matter content of the sludge decreased after aerobic digestion compared to before aerobic digestion ($p < 0.05$). The lowest dry matter content was observed in the PSB treatment ($p < 0.05$). In the PSB treatment, the ash content of sludge decreased after aerobic digestion compared to before aerobic digestion ($p < 0.05$).

The soluble nutrient concentrations of liquid organic fertilizer (LOF) produced from aerobic digestion of fish sludge with adding PSB is shown in Table 5. The pH and EC of the LOF were recorded as 6.62 ± 0.025 and 2.01 ± 0.11 ms/cm

3.3. Growth performance of fish

Table 6 shows the growth performance and survival rate indices of Nile tilapia (*O. niloticus*) reared in the two treatments of aquaponics systems for six weeks. The final weight, final length, specific growth rate (SGR), weight gain rate (WGR), and average daily gain (ADG) indices increased significantly in the AP-LOF treatment compared to the AP treatment ($p < 0.05$). The food conversion ratio (FCR) in the AP-LOF treatment was significantly lower than in the AP treatment ($p < 0.05$). No significant differences in survival rate and condition factor (CF) of fish were observed between the two experimental treatments ($p > 0.05$).

The dry matter, ash, macro and microelements contents of muscle showed no significant differences among Nile tilapia reared in the two treatments of aquaponics systems ($p > 0.05$) (Table 7).

3.4. Plant growth

The growth parameters of peppermint (*M. piperita* L.) at the beginning of the experiment and three weeks after cultivation in the two treatments of aquaponics systems are shown in Table 8. The shoot height, number of leaves, and nodes per plant increased significantly on day 21 compared to day 0 ($p < 0.05$). The highest values of these parameters were observed in the AP-LOF treatment ($p < 0.05$). The fresh weight of harvested aerial parts and number of buds per plant in the AP-LOF treatment were significantly higher than in the AP treatment on day 21 ($p < 0.05$).

Comparison of growth performance of peppermint plants cultivated in the two treatments of aquaponics systems for six weeks is presented in Table 9. The fresh weight of plant, aerial parts, shoot height, number of leaves, and nodes per plant in the AP-LOF treatment were higher than in the AP treatment ($p < 0.05$). There were no significant differences in the fresh weight of root, height of root, and number of buds per plant

Table 3

Comparison of macro and microelement concentrations at different days of aerobic digestion of Nile tilapia (*Oreochromis niloticus*) sludge without and with phosphate-solubilizing bacteria (PSB) for 14 days (mean \pm SD; n = 3).

Treatments	Macroelement (mg L ⁻¹)				Microelement (mg L ⁻¹)				
	Initial of aerobic digestion (Day 0)								
	K	Na	Ca	Mg	Fe	Zn	Cu	Mn	
Control	24.98 \pm 7.13 ^a	128.32 \pm 5.14 ^a	123.18 \pm 16.35 ^a	37.68 \pm 4.56 ^a	0.0014 \pm 0.0007 ^a	0.0018 \pm 0.0007 ^a	0.0013 \pm 0.0004 ^a	0.0017 \pm 0.0004 ^a	
PSB	25.34 \pm 6.19 ^a	127.01 \pm 3.35 ^a	121.74 \pm 14.06 ^a	38.23 \pm 2.19 ^a	0.0015 \pm 0.0006 ^a	0.0017 \pm 0.0005 ^a	0.0015 \pm 0.0005 ^a	0.0016 \pm 0.0003 ^a	
	End of aerobic digestion (Day 14)								
Control	39.82 \pm 4.62 ^b	159.50 \pm 10.06 ^b	192.25 \pm 9.33 ^b	38.74 \pm 1.68 ^a	0.12 \pm 0.04 ^b	0.051 \pm 0.012 ^b	0.014 \pm 0.002 ^b	0.10 \pm 0.012 ^b	
PSB	68.64 \pm 5.01 ^c	164.88 \pm 6.36 ^b	199.03 \pm 6.53 ^b	42.22 \pm 0.93 ^a	0.307 \pm 0.03 ^c	0.14 \pm 0.023 ^c	0.023 \pm 0.004 ^c	0.19 \pm 0.002 ^c	

Values shown with different letters in each column are significantly different (ANOVA with repeated measures, $p < 0.05$).

Table 4

Dry matter and ash contents of Nile tilapia (*Oreochromis niloticus*) sludge before and after aerobic digestion without and with phosphate-solubilizing bacteria (PSB) for 14 days (mean \pm SD; n = 3).

Parameters	Before aerobic digestion	After aerobic digestion	
		Control	PSB
Dry matter (%)	61.64 \pm 2.93 ^c	44.40 \pm 3.78 ^b	38.06 \pm 1.74 ^a
Ash (%)	47.12 \pm 4.93 ^b	43.13 \pm 3.81 ^{ab}	37.58 \pm 3.03 ^a

Values assigned with different letters in the same raw are significantly different (ANOVA with repeated measures, $p < 0.05$).

cultivated in the two experimental treatments ($p > 0.05$).

The dry weight and ash content of aerial parts and root of peppermint in the two treatments of aquaponic system on different days of the cultivation period are shown in Fig. 3. The dry weight of aerial parts of plants was higher in the AP-LOF treatment than in the AP treatment on days 21 and 42 ($p < 0.05$). The dry weight of aerial parts in the AP-LOF treatment increased from day 0 to day 21 ($p < 0.05$), but this value did not alter significantly until day 42 ($p > 0.05$). In the AP treatment, this value showed no significant difference during cultivation period ($p > 0.05$) (Fig. 3a). The ash content of aerial parts was higher in the AP-LOF treatment than in the AP treatment on day 42 ($p < 0.05$). In both treatments, the ash content of aerial parts increased from day 0 to day 42 ($p < 0.05$) (Fig. 3b). On day 42, the dry weight and ash content of the root were higher in the AP-LOF treatment than in the AP treatment ($p < 0.05$). The dry weight of root in the AP-LOF treatment increased from day 0 to day 42 ($p < 0.05$). In both treatment, the ash content of root increased significantly during 42 days of the cultivation period ($p < 0.05$) (Fig. 3c, d).

Table 10 shows the macro and microelement contents of peppermint cultivated for six weeks in the two treatments of aquaponics systems. The K, Na, P, Ca, and Fe contents of plant were higher in the AP-LOF treatment than in the AP treatment ($p < 0.05$). No significant differences in the Mg, Zn, Cu, and Mn contents of plant were observed between the two treatments ($p > 0.05$).

3.5. Water quality

Variations of water quality parameters in the two treatments of aquaponics systems on different days of the experiment are shown in Fig. 4. The TAN and nitrate concentrations were significantly lower in the AP-LOF treatment than in the AP treatment on days 28 and 42 ($p < 0.05$). In both treatments, the nitrate concentration decreased from day 0 to day 42 ($p < 0.05$) (Fig. 4a, b). There was no significant difference in phosphate concentration among the two treatments during different experiment days ($p > 0.05$). The phosphate concentration decreased from day 0 to day 14 ($p < 0.05$), but its concentration show no significant change until day 42 in both treatments ($p > 0.05$) (Fig. 4c). No significant difference was observed in the electrical conductivity (EC) of water between the two treatments on different days of the experiment ($p > 0.05$). In both treatments, the EC concentration

Table 5

Soluble nutrient concentrations of liquid organic fertilizer produced from aerobic digestion of Nile tilapia (*Oreochromis niloticus*) sludge using phosphate-solubilizing bacteria (PSB) for 14 days (mean \pm SD; n = 3).

Nutrients (mg/L)	PO ₄ ³⁻	TAN	NO ₃ ⁻	K	Na	Ca	Mg	Fe	Zn	Cu	Mn
	16.5	1.66	20.25	68.64	164.88	199.03	42.22	0.30	0.14	0.023	0.18
	\pm 2.2	\pm 0.51	\pm 3.80	\pm 5.01	\pm 6.36	\pm 6.53	\pm 0.93	\pm 0.03	\pm 0.04	\pm 0.004	\pm 0.002

Table 6

Growth performance and survival rate (%) of Nile tilapia (*Oreochromis niloticus*) after six weeks of rearing in aquaponics systems without and with adding liquid organic fertilizer (LOF) (mean \pm SD; n = 30).

Parameters	Experimental treatments	
	AP (control)	AP-LOF
Initial weight (g)	30.25 \pm 0.78 ^a	29.26 \pm 0.97 ^a
Initial length (cm)	12.27 \pm 0.24 ^a	12.21 \pm 0.08 ^a
Final weight (g)	55 \pm 2.77 ^a	59.74 \pm 0.98 ^b
Final length (cm)	16.41 \pm 0.15 ^a	16.8 \pm 0.12 ^b
SGR (%BW day ⁻¹)	1.42 \pm 0.18 ^a	1.7 \pm 0.05 ^b
WGR (%)	81.94 \pm 9.81 ^a	104.26 \pm 4.27 ^b
ADG (g)	0.58 \pm 0.08 ^a	0.72 \pm 0.01 ^b
CF (g cm ⁻³)	1.24 \pm 0.02 ^a	1.26 \pm 0.03 ^a
FCR	1.74 \pm 0.29 ^b	1.42 \pm 0.23 ^a
Survival rate (%)	98.33 \pm 2.35 ^a	100 \pm 0.00 ^a

AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer. SGR: specific growth rate; WGR: weight gain rate; ADG: average daily gain; CF: condition factor; FCR: food conversion ratio. Values with dissimilar letters in same lines are significantly different (Independent-Samples T Test, $p < 0.05$).

Table 7

Proximate composition and macro and microelement contents of Nile tilapia (*Oreochromis niloticus*) muscle reared for six weeks in aquaponics systems without and with adding liquid organic fertilizer (LOF) (mean \pm SD; n = 3).

	Experimental treatments	
	AP (control)	AP-LOF
Muscle proximate composition (%)		
Dry matter	20.24 \pm 0.75	20.52 \pm 0.24
Ash	4.33 \pm 1.32	5.88 \pm 1.34
Macro and microelement contents of muscle (mg/kg dry weight)		
K	134.60 \pm 9.38	129.98 \pm 4.08
Na	69.45 \pm 0.61	75.99 \pm 7.22
P	204.98 \pm 9.64	198.93 \pm 8.03
Ca	61.56 \pm 10.59	74.52 \pm 5.27
Mg	28.45 \pm 0.92	27.14 \pm 2.44
Fe	0.48 \pm 0.067	0.49 \pm 0.136
Zn	0.51 \pm 0.002	0.56 \pm 0.062
Cu	0.033 \pm 0.009	0.051 \pm 0.004
Mn	0.034 \pm 0.010	0.032 \pm 0.008

AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer. Values with dissimilar letters in same lines are significantly different (Independent-Samples T Test, $p < 0.05$).

increased from day 0 to day 42 ($p < 0.05$) (Fig. 4d). The pH of water was lower in the AP-LOF treatment than in the AP treatment on days 14, 28, and 42 ($p < 0.05$). In the AP-LOF treatment, the pH decreased from day 28 to day 42 ($p < 0.05$) (Fig. 4e). The total suspended solids (TSS) concentration show no significant difference among the two treatments during different days of the experiment ($p > 0.05$) (Fig. 4f).

The elemental analysis of water in the two treatments of aquaponics systems during the experimental period is presented in Fig. 5. The K, Na, Ca, Mg, Fe, and Zn concentrations had no significant differences among the two treatments on different days of the experiment ($p > 0.05$) (Fig. 5a-f). On days 28 and 42, the Cu concentration was higher in the AP-LOF treatment than in the AP treatment ($p < 0.05$) (Fig. 5g). The Mn concentration increased in the AP-LOF treatment compared to the AP treatment on days 14, 28, and 42 ($p < 0.05$) (Fig. 5h). In the AP

Table 8

Growth performance of peppermint (*Mentha \times piperita* L.) cultivated for 21 days in aquaponics systems without and with adding liquid organic fertilizer (LOF) (mean \pm SD; n = 18).

Parameter	Initial (Day 0)		Day 21	
	AP (control)	AP-LOF	AP (control)	AP-LOF
Fresh weight (g plant ⁻¹)	2.45	2.40	-	-
	\pm 0.04	\pm 0.02		
Root fresh weight (g plant ⁻¹)	0.68	0.71	-	-
	\pm 0.03	\pm 0.07		
Aerial part fresh weight (g plant ⁻¹)	1.77	1.68	-	-
	\pm 0.06	\pm 0.08		
Root height (cm)	10.21	9.63	-	-
	\pm 0.71	\pm 0.73		
Shoot height (cm)	13.90	14.70	32.75	35.72
	\pm 0.06 ^a	\pm 0.16 ^a	\pm 0.25 ^b	\pm 0.69 ^c
Number of leaves/plant	9.46	8.86	35.5	45.11
	\pm 0.26 ^a	\pm 0.17 ^a	\pm 2.16 ^b	\pm 3.35 ^c
Number of nodes/plant	4.66	4.84	22.41	22.83
	\pm 0.57 ^a	\pm 0.10 ^a	\pm 1.25 ^b	\pm 3.60 ^b
Number of buds/plant	-	-	0.91	2.05
			\pm 0.08 ^a	\pm 0.48 ^b
Harvested aerial parts fresh weight (g plant ⁻¹)	-	-	2.21	3.55
			\pm 0.016 ^a	\pm 0.12 ^b

AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer. Values shown with different letters in each row are significantly different (ANOVA with repeated measures; Independent-Samples T Test, $p < 0.05$).

Table 9

Growth performance of peppermint (*Mentha \times piperita* L.) cultivated for six weeks in aquaponics systems without and with adding liquid organic fertilizer (LOF) (mean \pm SD; n = 18).

Parameters	Experimental treatments	
	AP (control)	AP-LOF
Fresh weight (g plant ⁻¹)	4.13 \pm 0.42 ^a	5.37 \pm 0.08 ^b
Root fresh weight (g plant ⁻¹)	1.21 \pm 0.27 ^a	1.28 \pm 0.08 ^a
Aerial parts fresh weight (g plant ⁻¹)	2.92 \pm 0.15 ^a	4.09 \pm 0.07 ^b
Root height (cm)	13.58 \pm 1.16 ^a	15.08 \pm 1.12 ^a
Shoot height (cm)	20.95 \pm 1.20 ^a	24.11 \pm 0.82 ^b
Number of leaves/plant	69.16 \pm 5.66 ^a	81.55 \pm 4.29 ^b
Number of nodes/plant	37.68 \pm 3.75 ^a	44.49 \pm 3.25 ^b
Number of buds/plant	3.76 \pm 0.43 ^a	4.77 \pm 0.34 ^a

AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer. Values shown with different letters in each row are significantly different (Independent-Samples T Test, $p < 0.05$).

treatment, the all macro and microelement concentrations decreased significantly from day 0 to day 42 ($p < 0.05$). In the AP-LOF treatment, Na, Ca, Mg, and Zn concentrations decreased from day 0 to day 42, while the Mn concentration increased during the experimental period ($p < 0.05$). The K, Fe, and Cu concentrations showed no significant differences between different days of the experiment in the AP-LOF treatment ($p > 0.05$).

4. Discussion

In many aquaponics systems, only liquid effluent is utilized, while

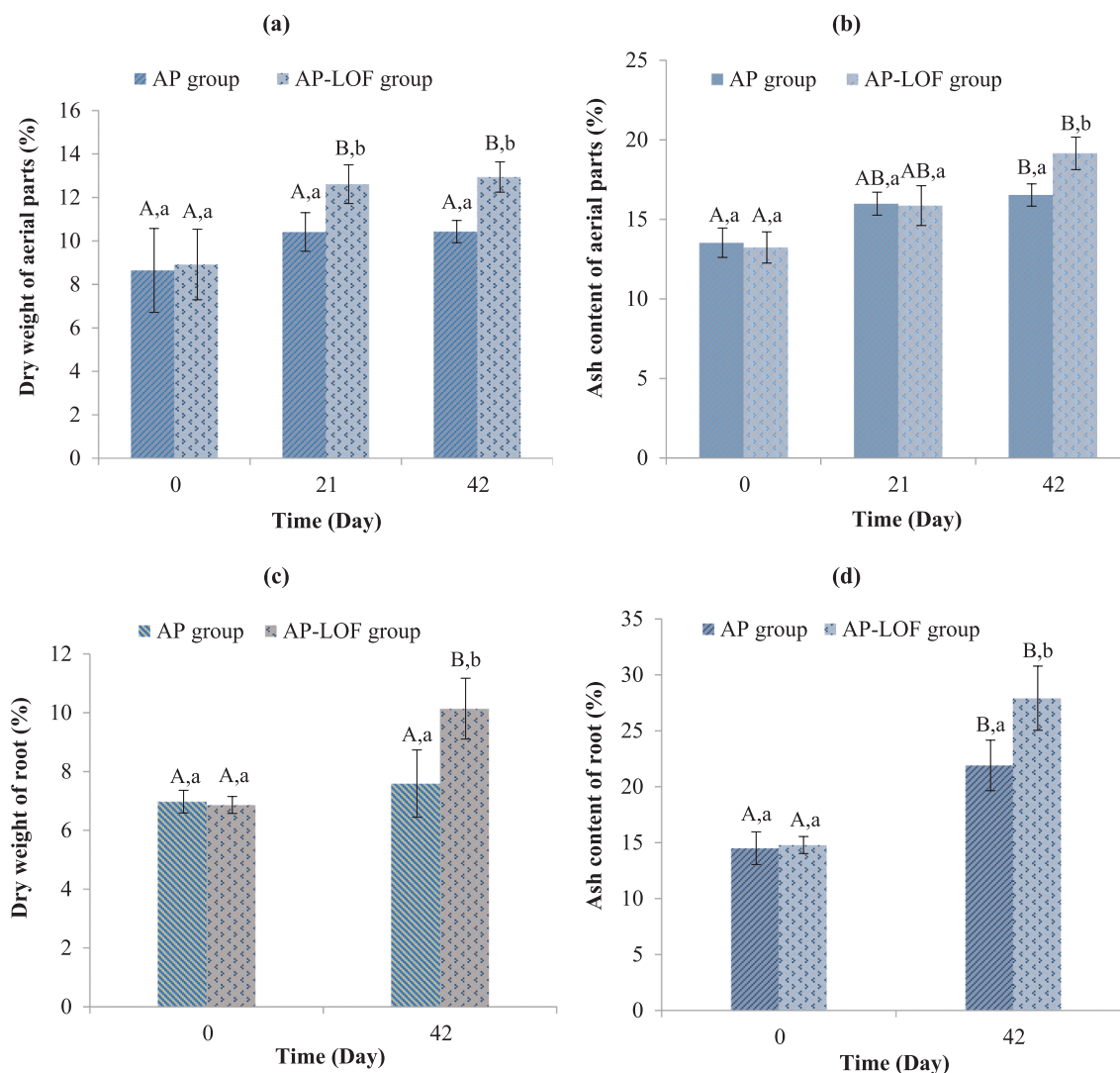


Fig. 3. Comparison of dry weight (%) and ash content (%) of aerial parts (a, b) and root (c, d) of peppermint (*Mentha × piperita* L.) cultivated in aquaponics systems without and with adding liquid organic fertilizer (LOF) at different days of the experiment (mean ± SD; n = 18). Bars with different lowercase letters in each time show significant difference among aquaponics system treatments (Independent-Samples T Test, $p < 0.05$). Bars assigned with different uppercase letters in each aquaponics system treatment are significantly different (ANOVA with repeated measures; Paired Samples-T test, $p < 0.05$). AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer.

Table 10
Macro and microelement contents (mg/kg dry weight-plant) of peppermint (*Mentha × piperita* L.) cultivated for six weeks in aquaponics systems without and with adding liquid organic fertilizer (LOF) (mean ± SD; n = 18).

Element	Experimental treatments	
	AP (control)	AP-LOF
K	169.80 ± 31.96 ^a	281.10 ± 31.38 ^b
Na	302.86 ± 27.5 ^a	401.24 ± 29.94 ^b
P	84.76 ± 5.55 ^a	100.60 ± 7.17 ^b
Ca	334.11 ± 52.59 ^a	484.95 ± 55.40 ^b
Mg	109.10 ± 3.15	119.12 ± 7.59
Fe	30.58 ± 3.14 ^a	46.47 ± 7.12 ^b
Zn	7.55 ± 0.58	7.95 ± 0.42
Cu	0.68 ± 0.07	0.73 ± 0.09
Mn	7.44 ± 0.20	8.40 ± 0.71

AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer. Values assigned with different letters in each row are significantly different (Independent-Samples T Test, $p < 0.05$).

fish sludge collected by a mechanical filter unit is removed from system as solid waste. Nutrient recovery from fish sludge not only reduces the discharge of organic contaminants into the environment, but can also be used as liquid fertilizer (Khiari et al., 2019). Fish sludge contains large amounts of nutrients that are essential for plant growth (Zhang et al., 2021). Khiari et al. (2019) reported that the sludge of Nile tilapia (*O. niloticus*) contains 4.5 % of the total nitrogen (TN) and 27.7 % of the ash based on dry matter. In the present study, the TN and ash contents of Nile tilapia sludge were measured as $1.19 ± 0.22 %$ and $47.12 ± 4.93 %$, respectively. The amounts of macronutrients like P, K, Na, Ca, Mg, and micronutrients such as Fe, Zn, Cu, and Mn in the sludge of different fish species vary (Strauch et al., 2018; Goddek et al., 2019), may be due to differences in the amount, chemical form, and bioavailability of nutrients in feed used for fish feeding, as well as depending on the sludge collected from freshwater or saltwater fish farming systems (Sele et al., 2024). The elemental analysis indicated that the Nile tilapia sludge like domestic animal sludge can be used as solid manure for plant cultivation. However, the bioavailability of fish sludge nutrients is low for plants in aquaponics systems. Therefore, mineralization of organic nutrients and solubilization of inorganic nutrients via digestion process

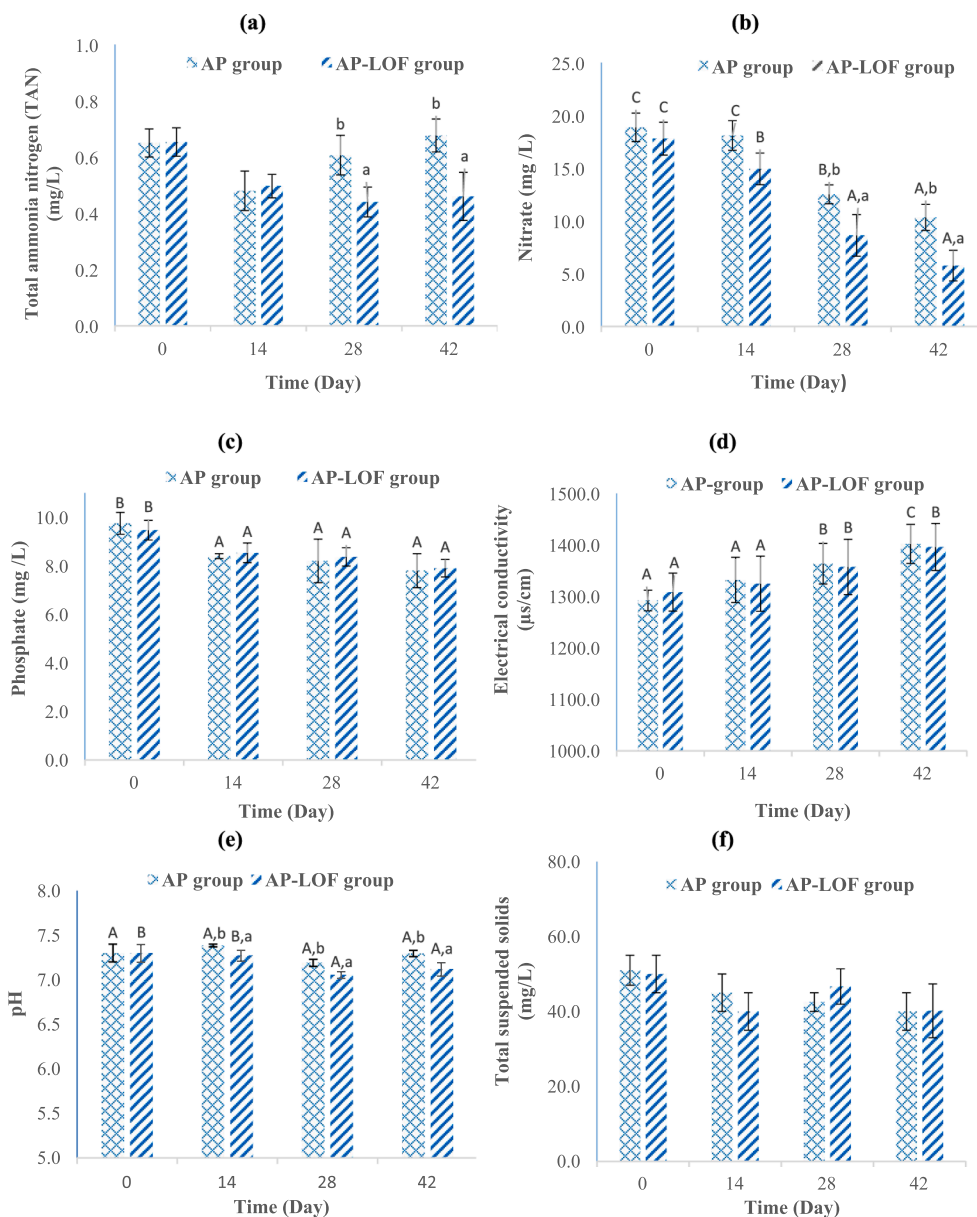


Fig. 4. Fluctuations in total ammonia nitrogen (TAN) (a), nitrate (NO_3^-) (b), phosphate (PO_4^{3-}) (c), electrical conductivity (EC) (d), pH (e) and total suspended solids (TSS) (f) concentrations of aquaponic water without and with adding liquid organic fertilizer (LOF) during different days of the experiment (mean \pm SD; $n = 3$). Bars with different lowercase letters in each time show significant difference among aquaponics system treatments (Independent-Samples T Test, $p < 0.05$). Bars assigned with different uppercase letters in each aquaponics system treatment are significantly different (ANOVA with repeated measures, $p < 0.05$). AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer.

can be considered.

The phosphate concentration in the ADBR increased by 44.56 % and 79.34 % after aerobic digestion of the Nile tilapia sludge using *Pseudomonas putida* strain P13 and *Pantoea agglomerans* strain P5 as phosphate-solubilizing bacteria (PSB) for 7 days and 14 days, respectively. The PSB released phosphate from insoluble phosphorus compounds by secreting organic acids and phosphatase enzyme (Sharma and Sharma, 2002). Qian et al. (2019) also reported that the release and transformation of phosphorus to orthophosphate was significantly enhanced by adding *Pseudomonas putida* as a PSB to biochar-P derived from sewage sludge. The aerobic digestion of the sludge without and with the PSB for 7 days resulted in a decrease in the TAN concentration by 311.12 %, and 285 % and an increase in the nitrate concentration by 130.86 %, and 123.03 %, respectively. These results can be attributed to the occurrence of the nitrification process in the aerobic digestion bioreactor (Uludag-Demirer et al., 2008). Reducing pH below neutrality enhances nitrogen

mineralization due to the reduction of nitrogen losses caused by ammonia volatilization (Sánchez-Monedero et al., 2001). In the present study, the pH of the ADBRs was kept in the range of 6.58–6.67 for 14 days. Ruiz et al. (2003) reported that the nitrification process inhibited at pH lower than 6.45 and higher than 8.95.

In the second week of the aerobic digestion of sludge, the TAN concentration increased by 50 % and 88.88 %, while the nitrate concentration decreased by 40.21 % and 43.58 % in the control and PSB treatments, respectively. Nitrogen in nitrate can be converted into ammonium through assimilatory or dissimilatory nitrate reduction to ammonium by autotrophic and heterotrophic microorganisms (Huang et al., 2020; Zhang et al., 2022; Hu et al., 2023). Huang et al. (2023) found that four species of *Pseudomonas* spp. produced ammonium through dissimilatory nitrate reduction to ammonium (DNRA) under aerobic conditions.

The potassium (K) concentration in the ADBR after 14 days increased

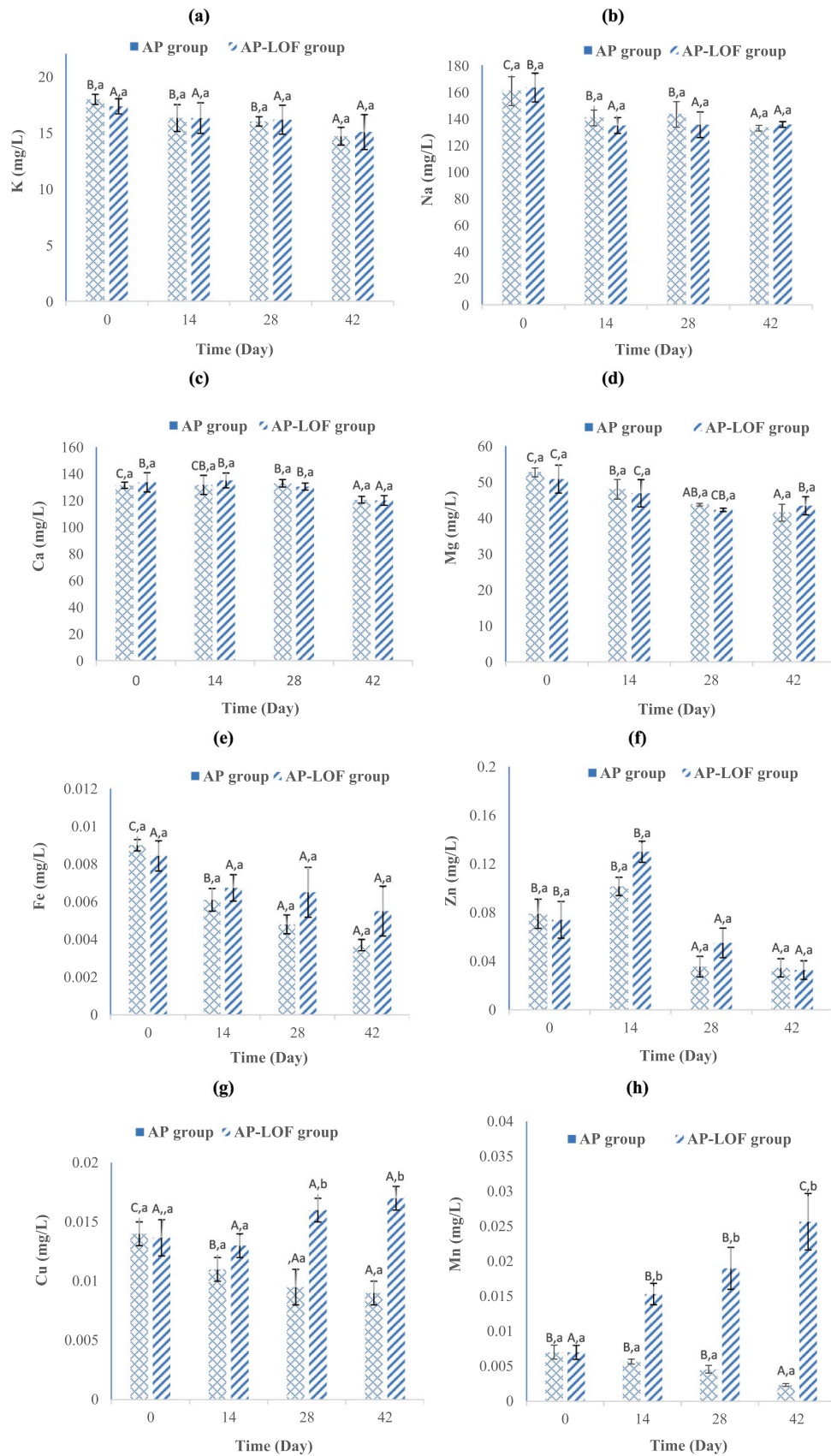


Fig. 5. Variations of potassium (K) (a), sodium (Na) (b), calcium (Ca) (c), magnesium (Mg) (d), iron (Fe) (e), zinc (Zn) (f), copper (Cu) (g), and manganese (Mn) (h) concentrations of aquaponic water without and with adding liquid organic fertilizer (LOF) during different days of the experiment (mean \pm SD; n = 3). Bars with different lowercase letters in each time show significant difference among aquaponic system treatments (Independent-Samples T Test, $p < 0.05$). Bars assigned with

different uppercase letters in each aquaponic system treatment are significantly different (ANOVA with repeated measures, $p < 0.05$). AP: aquaponic; AP-LOF: aquaponic-liquid organic fertilizer.

by 59.40 % and 170.87 % in the control and PSB treatments, respectively. Monsees et al. (2017) reported that aerobic digestion of Nile tilapia (*O. niloticus*) sludge for 14 days resulted in a 31 % increase in potassium concentration. In the study by Ezziddine et al. (2020), the K concentration increased 1.7 fold and 2.1 fold after aerobic digestion of fish sludge collected from an aquaponics system for three and four weeks, respectively. In the current study, the use of PSB at a level of 0.1 % had a positive effect on increasing K concentration. Sarikhani (2016) found that the release of K from K-containing minerals by PSB in the presence of insoluble phosphate increased by 66 %, indicating the ability of PSB to release K, probably due to the secretion of organic acid or chelants from the bacteria.

The aerobic digestion of the Nile tilapia sludge for 14 days resulted in a 24.29 % and 29.81 % increase in sodium concentration in control and PSB treatments, respectively. In treatments, 56.07 % and 63.38 % increase in calcium (Ca) concentration was found. Adding PSB to the ADBR had no significant effect on Na, Ca and Mg concentrations. Ezziddine et al. (2020) reported that the concentrations of Ca and Mg increased 3 and 2 times, after three weeks of aerobic digestion of fish sludge, respectively. In the present study, the magnesium (Mg) concentration did not increase after two weeks of aerobic digestion. Similarly, no change in Mg concentration was observed within 14 days of aerobic digestion of Nile tilapia sludge (Monsees et al., 2017).

The concentrations of Fe, Zn, Cu, and Mn increased by 85.71 %, 28.33 %, 10.76 %, and 58.82 % after two weeks of aerobic digestion in the control treatment, and by 204.66 %, 82.35 %, 15.33 %, and 118.75 % in the PSB treatment, respectively. The concentrations of Fe, Zn, Cu, and Mn in the PSB treatment were 2.55, 2.74, 1.64, and 1.9 times higher than in the control. The results show that aerobic digestion has an excellent potential for solubilizing micronutrients from Nile tilapia sludge, and the PSB treatment can significantly improve nutrient solubilization, as evidenced by the significant reduction in the ash content of sludge after aerobic digestion compared to before digestion in the PSB treatment. The increases in Fe, Zn, Cu, and Mn concentrations might be attributed to the solubilization of phosphate bound to these micronutrients by PSB (Han et al., 2022). Phosphorus exist in minerals form such as CaP, FeP, AlP, ZnP, and MnP in soil and phosphate-solubilizing microorganisms release phosphorus from the complex minerals through secretion of exopolysaccharides (EPS) (Devi et al., 2024). In general, the average concentrations of Ca, Zn, and Cu in liquid organic fertilizer produced from aerobic digestion of Nile tilapia sludge are 1.24, 3.6, and 1.15 times those of Hoagland nutrient solution (Zhang et al., 2020), respectively. The average concentrations of Mg, K, P, Fe, and Mn reached 86.87 %, 29.20 %, 53.22 %, 12 %, and 28 % of Hoagland nutrient solution, respectively.

The growth performance parameters, including final weight, final length, weight gain rate (WGR), specific growth rate (SGR), average daily gain (ADG), and food conversion ratio (FCR) improved significantly for the Nile tilapia (*O. niloticus*) cultured in the aquaponics (AP) system with adding liquid organic fertilizer (LOF) compared to those cultured in the AP without adding LOF. These results may be due to the improvement in water quality in the AP-LOF treatment, so the TAN and nitrate concentrations in this treatment were significantly lower than the AP treatment on days 28 and 42 of the cultivation period. Ogah et al. (2020) also attributed the improvement in the growth performance of barb hybrid (*Hypsibarbus wetmorei* ♂ × *Barbonymus gonionotus* ♀) fingerlings co-cultured with peppermint (*Mentha × piperita* L.) in an aquaponics system to improved water quality. Exposure of fish to sub-lethal ammonia concentrations can reduce growth rate, increase FCR, and reduce disease resistance (Kuttchantran, 2013). Lu et al. (2023) also reported that culture of juvenile Nile tilapia (*O. niloticus*) in water with TAN concentration of 0.88 mg/L for eight weeks decreased

final weight, body weight gain, SGR%, feed efficiency, and survival rate. The decrease in growth performance parameters of fish exposed to ammonia can be attributed to a reduction in appetite and consequently a decrease in food consumption by the fish (Zeitoun et al., 2016). The improvement in growth performance of Nile tilapia (*O. niloticus*) in the AP-LOF treatment could also be due to the increased concentration of macronutrients and micronutrients in the aquaponic water. Ru et al. (2017) reported that the addition of macro and micronutrients to water of aquaponics system improved Nile tilapia (*O. niloticus*) production, fish feed consumption, and feed conversion ratio (FCR) by 52.9 %, 31.5 %, and 14.3 %, respectively, compared to the control. In the present study, adding the LOF improved the WGR, SGR%, and FCR by 27.24 %, 19.72 %, and 18.40 %, respectively. Fish can absorb the mineral nutrients directly from the water in addition to their diet. Most minerals are carried within bloodstream to the tissues of the kidneys, liver, heart, and brain within minutes after absorption, and then are transported to the muscles and skin. The mineral content of fish tissues may vary and depends on various factors such as species and health status of the fish (Terech-Majewska et al., 2016). Similar to the results of a study by Ru et al. (2017), our study showed that adding the LOF to aquaponic water had no significant effect on the accumulation of macronutrients and micronutrients in Nile tilapia (*O. niloticus*) muscle, as the ash content of the muscle was the same in both treatments.

The nitrate concentration of aquaponic water decreased from 18.9 mg/L to 10.3 mg/L and from 17.8 mg/L to 5.8 mg/L during six weeks in the AP and AP-LOF treatments, respectively. The peppermint plant has a high ability to take up nitrogen compounds from water (DAFF, 2012). The higher reduction in TAN and nitrate concentrations in the AP-LOF treatment could be due to the better performance of nitrifying bacteria in oxidizing ammonium to nitrate and higher nitrate uptake by the peppermint plants from the water, respectively. The growth and function of nitrifying bacteria depend on the mineral nutrients of magnesium, calcium, iron, copper, and molybdenum (Hem et al., 1994). Adding the LOF containing macronutrients and micronutrients probably had a positive effect on the growth and performance of nitrifying bacteria, although this issue requires further study. In general, the simultaneous function of nitrifying bacteria and plants in an aquaponics system improves water quality for fish growth (Amin et al., 2023).

Aromatic herbaceous plants such as peppermint (*Mentha piperita* L.), spearmint (*Mentha spicata* L.), and basil (*Ocimum basilicum* L.) are widely cultivated worldwide for their flavoring, aromatic, and medicinal properties. Cultivation of various mint species in aquaponics systems has received attention due to their low nutritional requirements and good growth potential (Somerville et al., 2014). The results of the current study showed that the morphological traits of peppermint (*M. piperita* L.), including height of shoot, number of leaves, and nodes per plant in the first harvesting (day 21) increased by 135.61 %, 317.54 %, and 380.90 % in the AP treatment, and by 143 %, 409.11 %, and 371.70 % in the AP-LOF treatment compared to the initial of the cultivation period, respectively. On day 21, the fresh weight of harvested aerial parts in the AP-LOF treatment increased by 60.63 % compared to the AP treatment. Salama et al. (2020) found that the nutrient composition of aquaponic water was insufficient to meet the nutritional requirements of mint (*Mentha longifolia* L.) cultivated with Nile tilapia (*O. niloticus*), and adding chemical nutrient solution containing macronutrients and micronutrients led to higher fresh and dry yields of mint than the aquaponic solution, as well as N, K, and P contents of mint plants. Nozzi et al. (2018) found that the highest yield of lettuce (*Lactuca sativa*)

was achieved in aquaponic system received nutrients from fish feed and supplements of the macronutrients, P and K compared to system that only received nutrients derived from fish feed. Adding nutrients as

chemical solutions to aquaponic water increases costs (Goddek et al., 2015). In the current study, adding liquid organic fertilizer obtained from fish sludge using PSB improved the fresh weight of the plant and aerial parts, shoot height, number of leaves, and nodes per plant by 30 %, 40 %, 15 %, 17.91 %, and 18 % compared to the control at the end of the cultivation period, respectively. The PSB improves phosphate supply to plant through solubilizing inorganic and organic phosphate and, therefore, improves plant growth (Datta et al., 2012; Sivasakthivelan et al., 2021). Phosphorous is essential for proper growth of plant and its deficiency cannot be compensated for by any other elements (Sivasakthivelan et al., 2021). Additionally, the PSB enhances the plant growth by chelating heavy metals (Liang et al., 2020), altering the concentrations of various plant hormones (Neha, 2022), and producing antibiotics (Vassilev et al., 2006).

The concentration of macronutrients and micronutrients of aquaponic water decreased significantly during six weeks, and also the ash content of the aerial parts and root of the peppermint increased in the AP treatment, indicating uptake of these nutrients by peppermint plants from water. In the AP-LOF treatment, despite adding three liters of the LOF to aquaponic water every week, the concentration of macronutrients (phosphate, potassium, sodium, calcium, and magnesium) in water decreased or remained constant during six weeks. In addition, the ash content of the aerial parts and root of the peppermint increased, which could indicate the uptake of the macronutrients by the plant. The trend of changes in the concentration of micronutrients of Fe and Cu was constant, while the Zn and Cu concentrations showed a decreasing and increasing trend, respectively. The pH of aquaponic water affects the bioavailability and uptake of nutrients by plants (Somerville et al., 2014). Adding the LOF with a pH of 6.62 decreased the pH of water on days 28 and 42 of the experiment. Lowering the pH of water probably led to more absorption of mineral nutrients by the peppermint, so that the P, K, Na, Ca, and Fe contents of the peppermint cultivated in the AP-LOF treatment were higher than in the AP treatment. Similar to our results, Souza et al. (2010) found that the bioavailability of micronutrients of Mn, Zn, and Cu was significantly reduced at pH above 7.

In summary, the use of the PSB in the ADBR unit of aquaponic system improved the nutrient recovery from the Nile tilapia sludge. Adding the LOF to water of aquaponics system enhanced the growth performance of the Nile tilapia and peppermint plant. Therefore, the administration of liquid organic fertilizer produced from fish sludge can be considered as a promising approach to compensate the nutrient deficiency of plants in aquaponics systems. However, the long-term sustainability and scalability of this approach should be considered.

5. Conclusion

There is no sufficient information on improving nutrient recovery from fish sludge in the scientific literature. In the present study, an aerobic digestion bioreactor (ADBR) was designed for the digestion and recovery of nutrients from the Nile tilapia sludge using phosphate-solubilizing bacteria (PSB). The results revealed that use of the PSB at a level of 0.1 % for aerobic digestion of sludge for two weeks improved the release and solubilization of macronutrients of phosphate, and potassium and micronutrients of iron, zinc, copper, and manganese. Practically, installing the ADBR in aquaponics systems for bioconversion of collected fish sludge into liquid organic fertilizer (LOF) can reduce the use of chemical fertilizers and also prevent the discharge of sludge into the environment. In this study, adding the LOF produced from aerobic digestion of fish sludge using the PSB to water was investigated as an effective approach to enhance the productivity of both fish and plants in an aquaponics system. The addition of LOF to aquaponic water enhanced the growth performance of the Nile tilapia (*O. niloticus*) and peppermint (*M. piperita* L.) plant. Adding the LOF increased the P, K, Na, Ca, and Fe contents of the peppermint. Water quality parameters, including the TAN and nitrate also improved with adding the LOF. Despite these positive results, this study encountered constraints such as

the relatively short duration of the experiment and the use of a single plant species. Further studies should focus on improving the efficiency of aerobic digestion of fish sludge and better understanding the impacts of the LOF on growth performance of fish and microbial communities in aquaponics systems. Additionally, the effects of adding LOF to an aquaponics system on the growth performance of other species of fish and plants over a long cultivation period should be investigated.

Ethics statement

Fish care and handling were carried out according to the guidelines of Ferdowsi University of Mashhad (FUM) (IR.U.M.REC.1401.208).

Funding statement

This research received support from Ferdowsi University of Mashhad (FUM) (No. 3/59121).

CRediT authorship contribution statement

Sarkheil Mehrdad: Writing – original draft, Supervision, Methodology, Conceptualization. **Zahedi Saeed:** Writing – review & editing, Data curation, Conceptualization. **Arouei Hossein:** Writing – review & editing, Methodology, Data curation. **Madady Mohammad Hossein:** Writing – review & editing, Project administration, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to acknowledge the staff of the Faculty of Natural Resources and Environment of Ferdowsi University of Mashhad (FUM) for supporting and providing research facilities. This work received financial support from Ferdowsi University of Mashhad (FUM) (No. 3/59121).

Author's statement

The authors gratefully thank to the editor and referees for their constructive comments and recommendations which definitely help to improve the readability and quality of the paper. All the comments that need to be incorporated to the revised manuscript have been done and the others that only need to be clarified for the referees are explained here only. Detailed responses to the comments and recommendations are as follows. Please note that we replied to all the comments immediately below the comments.

Consent to participate

Not applicable

Consent for publication

Not applicable

Code availability

Not applicable

Data availability

Data will be made available on request.

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