タイトル Title	Aquaponics using saline groundwater: Effect of adding microelements to fish wastewater on the growth of Swiss chard (Beta vulgaris L. spp. cicla)
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掲載誌・巻号・ページ Citation	Agricultural Water Management , 227 : 105851
刊行日 Issue Date	2020-01-20
資源タイプ Resource Type	学術雑誌論文 / Journal Article
版区分 Resource Version	著者版 / Author
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DOI	10.1016/j.agwat.2019.105851
URL	https://repository.lib.tottori-u.ac.jp/7312

Aquaponics Using Saline Groundwater:

Effect of adding microelements to fish wastewater on the growth of Swiss chard (*Beta vulgaris* L. spp. *cicla*)

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Abstract

Saline soil and saline groundwater reduce agricultural productivity on drylands. We are developing a new aquaponics system to improve food productivity on such lands while effectively utilizing saline groundwater. In this study, cultivation of Swiss chard (*Beta vulgaris* L. spp. *cicla* cv. Seiyou Shirokuki) was carried out using fish wastewater with a high salt concentration (1150 mg L^{-1} NaCl). The levels of microelements (e.g., Fe, Mn, Zn, and Cu) in the fish wastewater were very low, so we added microelements at 100% (W100), 50% (W50), 25% (W25), and 0% (W0) of the levels in the standard hydroponics solution to the fish wastewater and investigated the effects on growth of Swiss chard. At the first harvest, yields in all wastewater treatments were as high or higher than in the control. At the second harvest, yields in W100, W50, and W25 were not significantly different from the control, while in W0 the yield was significantly lower and chlorosis was evident. At the third harvest, the yield in all wastewater treatments was less than in the control, and chlorosis symptoms were observed in W25 and W0. Since leaf Mn and Zn concentrations in W25 and W0 had decreased to below the critical values for those microelements, Mn and Zn deficiency might have contributed to the observed chlorosis and yield loss. For the cultivation of Swiss chard with fish wastewater, sufficient yield (i.e., comparable to or better than the control) without chlorosis was obtained when microelements were added at 50% of the level of the control solution. In addition, since sufficient yield was obtained even in W0 at the first harvest, it is suggested that longer-term cultivation and higher yield could be achieved by applying 50% microelements after the first harvest.

Keywords: Salinity, Wastewater, Yield, Microelement, Productivity, Dryland

1. Introduction

According to the United Nations, the world's population is estimated to reach approximately nine billion in the year 2050 (Pitman and Läuchli, 2002). In order to secure the food consumption needs of this increased population, food production urgently needs to be developed and expanded. Although the technology required for increasing food production has progressed remarkably, the production volume per land area has almost plateaued. In addition, it is reported that the dryland area will increase by 23% by the year 2100, and will account for 56% of the total land area of the earth (Huang et al., 2016). It is believed that 78% of this expansion of dryland will occur in developing countries, which could exacerbate rising poverty levels and land degradation in dryland areas (Huang et al., 2016). As a result, there is a need for innovative and sustainable food production technologies in drylands that occupy a large land area. Agriculture in drylands can be problematic, as those places often do not have adequate water resources. In an effort to increase food production in those conditions, farmers often unintentionally use excessive amounts of saline water to irrigate their land, and/or overly fertilize low fertility soils, both of which causes salt accumulation in the soil and results in declining crop yields (Saysel and Barlas, 2001). On the other hand, drylands are rich in solar radiation, which promotes photosynthesis in plants and increases agricultural productivity. Thus, it is possible that drylands can become highly productive for agriculture if water resources are secured and irrigation is carried out properly.

In the 1960s, aquaponics (a combined system of aquatic animal production and hydroponic plant growth) was introduced as a new food production system to achieve effective water use (Enduta et al., 2011). In aquaponics, freshwater fish are cultured in an aquaculture system, then the plant is hydroponically cultivated using fish wastewater from that system, which contains various nutrients derived from fish feed and manure. This removes nutrients from the fish wastewater while simultaneously purifying the water.

In general, aquaponics uses low saline or non-saline water, so it is possible to cultivate high cash crops hydroponically, including glycophytes (salt sensitive) such as lettuce and herbs. Those plants, however, cannot be grown in conditions where the salt concentration of groundwater is high, such as drylands. Therefore, we have been aiming to develop a new non-circulate "aquaculture–hydroponics–field cultivation type" aquaponic system that uses saline groundwater and is thus suitable for dryland conditions.

In a standard aquaponic system, when the salt concentration in the wastewater is increased by sodium (Na) contained in fish feed and manure, the yield of hydroponic crops is reduced, and a large amount of water must be discarded (Hambrey consulting, 2013). An advantage of aquaculture is that it does not consume water (water will be lost only by evaporation). In our aquaponics system, Na and other nutrients from fish wastewater are removed by hydroponically grown plants, and that purified water can be used effectively for conventional field farming without wasting it. There have been many reports of aquaponics focusing on aquaculture, but few studies have focused on the nutrient dynamics of plants (Goddek et al., 2015). The aquaponics technology we aim to develop is diverse, such as aquaculture, hydroponics, and field cultivation. In this study, we aim to contribute to the development of aquaponics technology in drylands, as well as broadening the knowledge on the nutrient dynamics of hydroponic culture, especially in aquaponics using saline water.

The electrical conductivity of groundwater in Los Planes, Baja California Sur, Mexico, which is categorized as dryland, is approximately 3 to 5 dS m^{-1} and its sodium (Na) concentration is as high as 690 to 1150 mg L^{-1} (unpublished results). In areas with such high sodium levels, it is difficult to cultivate agricultural plants, most of which are categorized as glycophytes. Instead, salt-tolerant or salt-loving plants are often cultivated. Salt-loving plants are those whose growth is promoted by Na in the medium (Yamada et al., 2016a, 2016b) and include Swiss chard (*Beta vulgaris* L. spp. *cicla* cv. Seiyou Shirokuki), table beet (*Beta vulgaris*

L. spp. *vulgaris* cv. Detroit Dark Red), and dwarf glasswort (*Salicornia bigelovii* Torr.). These plant species not only utilize Na for growth but also absorb and accumulate high concentrations of Na in the plant body (Yamada et al., 2016a). Therefore, even high-Na groundwater could potentially produce high yield in an aquaponic system if salt-loving plants are cultivated. In this study, we elucidated the cultivation conditions for growing Swiss chard in our aquaponic system. Swiss chard is a plant of the Amaranthaceae family and is widely produced and consumed throughout the world, including in Mexico. It has been found that sodium can be used for the growth of Swiss chard as an alternative to potassium (Kaburagi et al., 2015; Yamada et al, 2016b). Therefore, a medium that is lacking potassium but is rich in sodium, which is provided by fish wastewater with high saline content, may be also suitable for the cultivation of Swiss chard.

When fish wastewater is used directly for hydroponics in an aquaponic system, the mineral concentrations in the water must be sufficient for plant cultivation. Fish wastewater contains sufficient N for plant growth, which is derived from fish feed and manure. Our previous studies have confirmed that 280 mg L^{-1} of NO_3–N does not inhibit the growth of Swiss chard (unpublished results). On the other hand, fish wastewater is often lacking essential microelements for plants (Graber and Junge, 2009; Roosta and Hamidpour, 2011). Analysis of mineral concentrations in the fish wastewater used in this study revealed the similar pattern. The concentration of phosphorous (P) was 1/2 that of the standard hydroponics nutrient solution, zinc (Zn) was 1/5, manganese (Mn) was 1/20, and iron (Fe) was about 1/90 (Table 1).

Table 1 Comparison of mineral concentration in the standard hydroponics nutrient solution and the fish wastewater

Mineral	Standard nutrient solution*	Fish wastewater
(mg L^{-1})		
NO_3–N	42.0	241.9
P	12.4	6.8
K	78.0	89.5
Ca	40.8	359.1
Mg	48.6	40.8
Fe	2.0	0.02
Mn	0.5	0.02
Zn	0.1	0.02
Cu	0.01	0.04

*Kaburagi et al. (2014)

Fe is involved in the formation of chlorophyll, which is necessary for light absorption during photosynthesis (Jeong and Guerinot, 2009). Mn is involved in processes such as photosynthesis, protein synthesis, and redox reactions to remove oxygen radicals (Ducic and Polle, 2005; Shao et al., 2017). However, these microelements are not readily translocated from older leaves to other growing plant parts. Therefore, if the medium is lacking microelements, deficiency symptoms appear in photosynthetically active leaves and plant growth is inhibited (Hell and Stephan, 2003).

In this study, we added different concentrations of microelements to saline fish wastewater and investigated the effects on the growth of Swiss chard. We also evaluated the food safety (NO_3 content) of Swiss chard leaves grown using fish wastewater.

2. Materials and Methods

2.1. Aquaculture

Tilapia (*Oreochromis niloticus*) was stocked and reared in a 1500-L tank designed as a closed recirculating aquaculture system. Tilapia is tolerant to salinity, low oxygen, and high nitrate nitrogen (NO_3–N) in the water and can adapt to a wide range of pH and temperature conditions (Stickney, 2017; Goddek et al., 2015). In this study, groundwater was used for the fish farming and adjusted to 1150 mg L^{-1} of sodium chloride (NaCl) to make the salt concentration similar to that of the groundwater in Baja California, Mexico. Feeding was carried out twice a day. Water was collected every 7 days to analyze NO_3–N concentration. The wastewater was used for hydroponic cultivation when the NO_3–N concentration reached approximately 250 mg L^{-1}. This research was conducted in a greenhouse at Tottori University from March to May 2016.

2.2. Plant cultivation

Seeds of Swiss chard (*Beta vulgaris* L. spp. *cicla* cv. Seiyou Shirokuki) were sown in vermiculite. When the first true leaves appeared, seedlings were transplanted and grown hydroponically in 30–L plastic containers (6 plants per container). Plants were subjected to five different treatments based on either groundwater or fish wastewater (Table 2).

Table 2 Mineral concentrations in the treatment solutions at the start of cultivation

	Macroelements (mg L^{-1})					Microelements (mg L^{-1})			
	NO_3–N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Control	44.1	12.7	80.1	55.5	45.8	1.78	0.44	0.12	0.01
W100	238.0	6.7	93.9	344.8	40.0	1.62	0.46	0.12	0.04
W50	260.6	6.8	93.6	350.8	40.9	0.86	0.25	0.07	0.05
W25	241.5	6.4	93.1	356.5	41.0	0.42	0.13	0.05	0.04
W0	248.0	6.9	100.9	357.5	41.1	0.02	0.02	0.02	0.03

Groundwater was used for the control solution and adjusted to 1150 mg L^{-1} of NaCl as well as the fish wastewater

The treatments were Control (groundwater with standard hydroponics nutrient solution (Kaburagi et al., 2014)); W100 (fish wastewater with 100% of the control microelement level); W50 (fish wastewater with 50% of the control microelement level), W25 (fish wastewater with 25% of the control microelement level), and W0 (fish wastewater without micronutrient supplementation). Each treatment comprised four replicates. The first harvest was carried out 27 days after transplanting. We harvested leaves that were about 20 cm in length (market-transaction size) and left the smaller, younger leaves for the next harvest. The second harvest was carried out 10 days after the first harvest, and the third harvest was 11 days after the second harvest. Leaves from six plants in each bat were weighed to determine fresh weight (FW) and then oven-dried at 70°C for 48 h. The nitrogen concentration in the control solution was 56 mg L^{-1}, which was 1/5 of the wastewater. This was renewed weekly so that nitrogen deficiency would not become a growth-inhibiting factor throughout the cultivation period. The pH of the control solution was adjusted to 5.5. The wastewater treatment solutions were not renewed during the cultivation period, and the pH was adjusted to 5.5 only at the transplanting.

2.3. Chlorophyll

At the time of harvesting, the chlorophyll content of the largest developed leaves of each individual plant was measured with a portable chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan).

2.4. Determination of inorganic ion concentrations

Dried leaves from each harvest were ground, decomposed by sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at 200°C, and diluted with deionized water. Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) concentrations in decomposed leaves and in each treatment solution were quantitatively analyzed at the time of each harvest by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Spectro Ciros CCD, Spectro, Kleve, Germany).

2.5. Determination of NO_3–N and P concentrations

Nitrate (NO_3) and P were extracted from dried leaves by water at 80°C for 2 h. The Cataldo method (Cataldo et al., 1975) was used to measure the NO_3 concentrations in leaves and treatment solutions, and then converted to the concentration on FW basis. The P concentrations in leaves and treatment solutions were determined at the time of each harvest by the molybdenum blue method (Murphy and Riley, 1962) using a spectrophotometer (V-630BIO, JASCO, Tokyo, Japan).

2.6. Statistical analysis

Statistical analyses for each treatment at each harvest time were carried out using version 7.0b of the GraphPad Prism software program (GraphPad Software, Inc., La Jolla, CA, USA). All data were presented as means ± standard error (SE). Significant differences ($p < 0.05$) were assessed by one-way ANOVA, followed by Bonferroni's multiple-comparison test if the ANOVA revealed a significant difference.

3. Results

3.1. Plant growth and total yield

Leaf FW at the first harvest was significantly higher in all of the wastewater treatments than in the control (Fig. 1a). Even at the second harvest, FWs of W100, W50, and W25 were

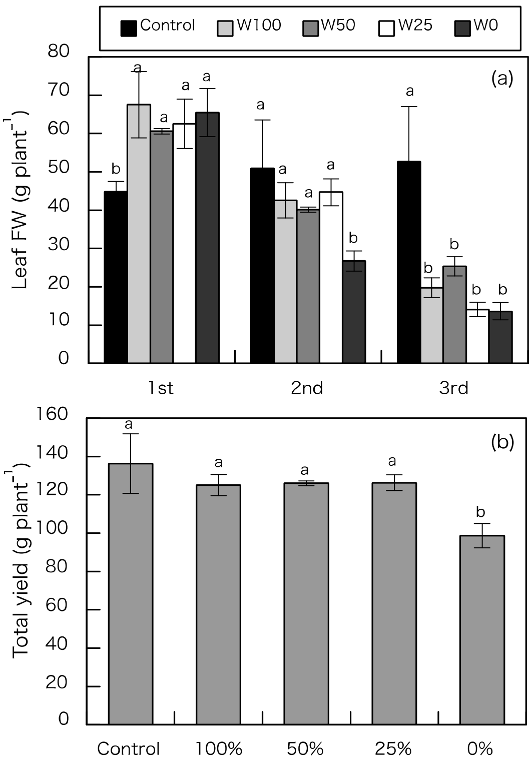

Fig. 1 (a) Leaf fresh weight (FW) from five different treatments at three harvest times. (b) FW-based total yield across all three harvests. Bars labeled with different letters at each harvest time (a) and each treatment (b) differ significantly (one-way ANOVA, P < 0.05; Bonferroni's multiple-comparison test). Values are mean ± SE (n = 4).

not significantly different from the control, but FW of W0 was significantly lower than for the other treatments. At the third harvest, the leaf FWs in all wastewater treatments was less than 50% of the control value. On the other hand, the total yield across all three harvests did not differ significantly between the control and wastewater treatments except for W0, which was significantly lower (70% of the control value; Fig. 1b).

3.2. Chlorophyll in leaves (SPAD value)

The SPAD values of the leaves in W100 and W50 were the same as or higher than the control value at all three harvest times (Fig. 2).

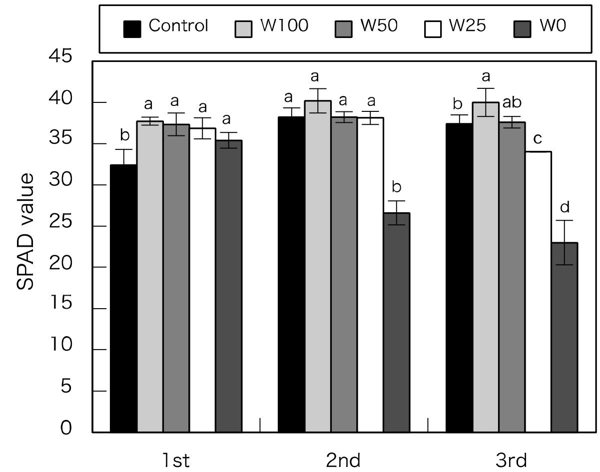

Fig. 2 SPAD values of leaves in five different treatments at three harvest times. Bars labeled with different letters at each harvest time differ significantly (one-way ANOVA, $P < 0.05$; Bonfferoni's multiple-comparison test). Values are mean ± SE ($n = 4$).

In W25, the SPAD value equaled or exceeded the control value until the third harvest, when it was significantly lower than that of the control and the plants showed chlorosis symptoms. In W0, the SPAD value was not significantly different from the control at the first harvest. However, it was significantly lower (70% of the control level) at the second harvest, and 60% of control at the third harvest, both of which exhibited chlorosis.

3.3. Fe, Mn, Zn, and Cu concentrations in leaves

The Fe concentration in leaves was not significantly different between treatments at the first and second harvests. At the third harvest, Fe concentration in leaves of W50, W25 and W0 were significantly lower than the control. However, the Fe concentration was constant in all treatments throughout the three harvest times, even in the W25 and W0 which exhibited chlorosis (Fig. 3).

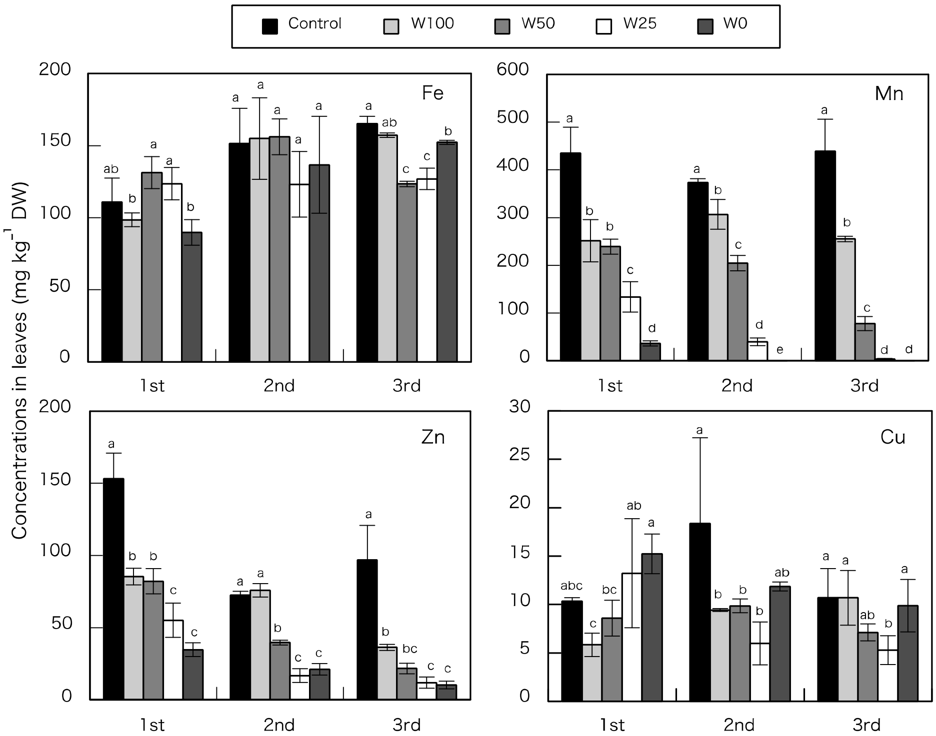

Fig. 3 Fe, Mn, Zn, and Cu concentrations in leaves (DW basis) from five different treatments at three harvest times. Bars labeled with different letters at each harvest time differ significantly (one-way ANOVA, *P* < 0.05; Bonfferoni's multiple-comparison test). Values are mean ± SE (*n* = 4).

At all harvest times, the Mn concentration in leaves from the W50, W25, and W0 decreased as the treatment micronutrient concentration decreased, while the W100 maintained constant concentration. In W0, the Mn concentration in leaves decreased markedly to 1 mg kg^{-1} DW and the chlorosis symptoms were exhibited by the second harvest; the same pattern was observed in W25 at the third harvest. As with Mn, the Zn concentration in leaves decreased as the treatment micronutrient concentration decreased. The Zn concentration in leaves of all wastewater treatments was significantly lower than the control at the third harvest. The Cu concentration in leaves did not differ markedly among harvests or treatments. The Cu concentration in leaves remained constant even in W0 where the decrease in growth was significant.

3.4. NO_3, P, K, Ca, Mg, and Na concentrations in leaves

The NO_3 concentration in leaves (FW basis) was not significantly different between treatments at the first or second harvest times. At the third harvest, NO_3 was significantly lower in W0 than in W100 or W50 (Fig. 4).

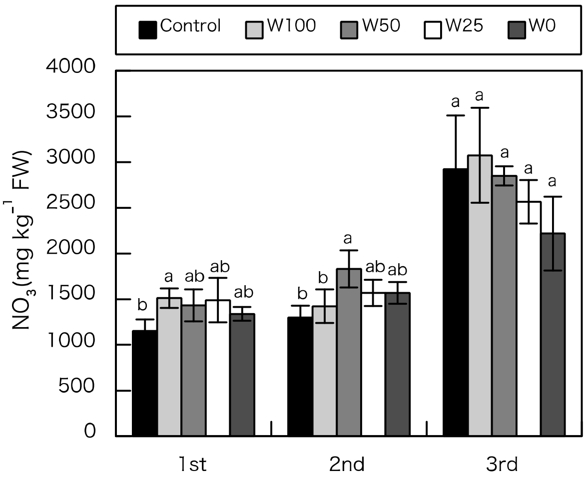

Fig. 4 NO_3 concentrations in leaves (FW basis) from five different treatments at three harvest times. Bars labeled with different letters at each harvest time differ significantly (one-way ANOVA, $P < 0.05$; Bonfferoni's multiple-comparison test). Values are mean ± SE ($n = 4$).

The P concentration in leaves from all wastewater treatments was significantly lower than the control at all three harvest times (Fig. 5). However, the P concentration in leaves from all wastewater treatments at the second and third harvests significantly decreased to 1/2 and 1/3 of the control levels, respectively.

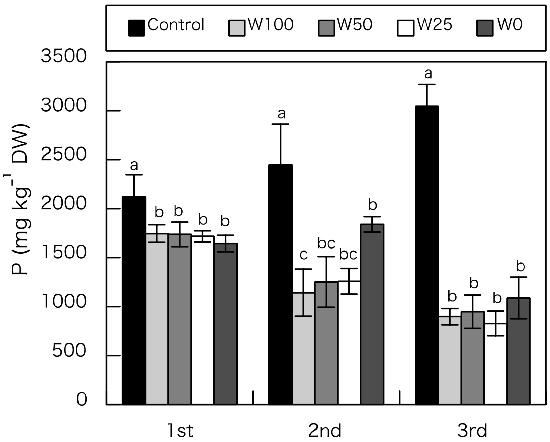

Fig. 5 P concentrations in leaves (DW basis) from five different treatments at three harvest times. Bars labeled with different letters at each harvest time differ significantly (one-way ANOVA, $P < 0.05$; Bonfferoni's multiple-comparison test). Values are mean ± SE ($n = 4$).

Leaf concentrations of K, Ca, Mg, and Na are not mentioned since the trends were unclear and not significant.

4. Discussion

As a water source for aquaponics, fish wastewater contains adequate amounts of NO_3–N derived from fish feed and manure. The fish wastewater used in this study contained 250 mg L^{-1} of NO_3–N at the start of hydroponic cultivation, which is approximately 6 times the concentration in the standard hydroponics solution (Table 2). Nitrogen is an essential element for plant growth, and NO_3 is readily absorbed as a counterion of cations such as K, Ca, and Mg. NO_3 taken up into the plant is synthesized into amino acids and proteins (Tischner, 2000). In this study, at the first harvest, all wastewater treatments showed growth exceeding that of the control (Fig. 1a), likely due to the high levels of NO_3–N. At the third harvest, the FW decreased significantly in all wastewater treatments compared to the control, while the total yields of W100, W50, and W25 across the three harvest times were not significantly different from the control (Fig. 1b). Together, these results demonstrate that the optimum amount of microelement supplementation in fish wastewater is 50% of the control level, which was sufficient to prevent chlorosis and to obtain the same yield as in the control. Furthermore, because yields at the first harvest exceeded the control level, even in W0, we speculate that long-term cultivation with high yield and good plant color could be achieved by the addition of 50% microelements after the first harvest.

In general, the requirement for Fe is very high among all the microelements, and it is essential to chlorophyll biosynthesis. Therefore when Fe is deficient, chlorosis symptoms occur in young leaves. In this study, the initial Fe concentration in fish wastewater (W0) was about 1/90 of that in the control (Table 2). Interestingly, however, the Fe concentration in Swiss chard leaves was not significantly different between treatments at any harvest time, and was sufficiently maintained even in leaves from W25 and W0, which showed chlorotic symptoms at later harvest times (Fig. 3). This result suggested that Swiss chard has either a high efficiency of Fe absorption or a low requirement for Fe. Similarly, there were no significant differences in Cu concentration in leaves between treatments at each harvest date since according to Table 2, there were no substantial differences in Cu concentration among the treatments.

On the other hand, at each harvest time, the Mn and Zn concentrations in leaves remarkably decreased as treatment micronutrient concentration decreased (Fig. 3). The Mn concentration in healthy plant leaves is dozens to hundreds of milligrams per kilogram dry matter, and it is reported that deficiency occurs when the leaf Mn concentration is 10–20 mg kg^{-1} or less (Mengel and Kirkby, 2001). Most Mn contained in the chloroplasts is associated with PSII (Anderson et al., 1964). Ohki (1981) reported that net photosynthesis and chlorophyll content were significantly decreased when Mn was deficient. Also, a clear relationship between the intensity of Mn deficiency symptoms and Mn concentration was found in sugar beet, which is in the same family (Amaranthaceae) as Swiss chard (Farley and Draycott, 1973). In this study, in W0 (at first harvest) and W25 (at second harvest), the SPAD value and the leaf FW did not differ from the control when the leaf Mn concentration was only about 40 mg kg^{-1}. At the third harvest, on the other hand, chlorosis symptoms were observed and the leaf FW was greatly reduced in W0 and W25 when the leaf Mn concentration was 10 mg kg^{-1} or less. Zn plays important roles in plant metabolism such as maintaining the conformation (structure) of various enzymes (Barker and Pilbeam, 2015). Other authors suggest a relationship between Zn and auxin, a plant hormone promoting elongation, which might explain the suppression of plant growth observed when Zn is deficient (Salisbury and Ross, 1992). In general, Zn deficiency in plant leaves appears at 15 mg kg^{-1} or less, which is considered as the critical value (Barker and Pilbeam, 2015; Jones, 2012). In this study, the leaf Zn concentration was less than 15 mg kg^{-1} in W25 and W0 at the third harvest, when the decrease in FW was remarkable. From these results, we conclude that deficiency of Mn and Zn limited the growth of Swiss chard in fish wastewater. Thus, the addition of microelements, especially Mn and Zn, is necessary to maintain yield during long-term cultivation. However, confirming the effects of specific microelements (Mn and Zn) would require additional experiments (e.g., manipulating each microelement separately).

P plays an important role as a major constituent of phosphate compounds such as DNA and biomembranes and is involved in energy generation, enzyme activation and inactivation, etc. (Vance et al., 2003). An adequate amount of P in plant leaves is generally about 2000 to 4000 mg kg^{-1} on a dry-matter basis, and P deficiency occurs at 1000 to 2000 mg kg^{-1} or less (Mengel and Kirkby, 2001). The P concentration in the fish wastewater used in this study was about 1/2 that of the control solution (Table 2). Thus, the P concentration in the leaves of all wastewater treatments reached the deficiency level at the third harvest (Fig. 5). However, with the exception of W0, the P concentration in the leaves of all wastewater treatments was above the deficiency level at the first two harvests, and the leaf FW was comparable to the control (Fig. 1a and Fig. 5). From these results, we considered it is possible to cultivate Swiss chard for at least two harvests using fish wastewater without adding P, which indicates that fish wastewater can be a valuable source of P nutrition. However, the P concentration in all wastewater treatment solutions at the third harvest was below the detection limit (Table 1S) and the P concentration in leaves was also at the deficiency level (Fig. 5). In the case of longer-term cultivation using fish wastewater, addition of P may be necessary.

Due to the depletion of phosphate ore in recent years, P fertilizers are becoming increasingly expensive (Abelson, 1999; Vance et al., 2003). In addition, fertilizers generally account for between five to ten percent of the production costs in conventional hydroponic cultivation, and the fossil fuels used for fertilizer production may have a negative environmental impact (Hochmuth and Hanlon 2010). Therefore, reducing both the cost of fertilizer as well as the amount of fertilizer required for crop cultivation using wastewater in aquaponic systems is necessary for the future of sustainable food production.

The Swiss chard leaves was not negatively impacted by the concentrations of K, Ca and Mg in the fish wastewater at each of the harvest times (data was not shown and mentioned in the results section as the trends were not significant). These nutrients were not additionally applied. This suggests that Swiss chard is suitable for cultivation not only in saline water, but also in less nutrient rich mediums, such as fish wastewater.

The initial NO_3–N concentration in fish wastewater was about 6 times that of the control solution (Table 2). When nitrogen absorbed by the plant body becomes excessive, the leaves become succulent and dark green in color, which can increase disease and pest damage (Jones, 2012). Also, if excess NO_3 is taken into the human body, it is quickly metabolized to NO_2, which oxidizes the iron of hemoglobin in the blood and may hinder oxygen transport (IARC, 2010). In our study, the SPAD values (an indicator of leaf color) in the wastewater treatments were no more than 15% higher than the control in all of the wastewater treatments except W0 (Fig. 2). In addition, the NO_3 concentration in leaves (FW basis) was lower than the upper limit (3500 mg kg^{-1} FW) of the regulated value for the spinach, which is in the same family (Amaranthaceae) as Swiss chard, reported by the UK's Food Standard Agency (Red Tractor Farm Assurance, 2016) (Fig. 4). However, the NO_3 concentration was markedly higher at the third harvest time than at either of the first two, and some values are approaching the 3500 mg kg^{-1} FW limit. This suggests that longer growth times might need to be monitored for further increases in leaf NO_3.

5. Conclusion

We found that sufficient yield of Swiss chard (i.e., comparable to or better than the control) could be obtained by adding 50% of the microelement level of the control solution when utilizing fish wastewater for aquaponics. Also, since sufficient yield of Swiss chard was obtained in all treatments at the first harvest, even with no addition of microelements, it appears that long-term cultivation and high yield could be obtained by supplying 50% microelements after the first harvest. Although the depletion of phosphorus ore has become a problem in recent years, the amount of P originally contained in fish wastewater was enough to obtain sufficient yield of Swiss chard for at least two harvests. However, to enable long-term cultivation, the effectiveness of supplying P as well as microelements, especially Mn and Zn, should be examined in the future. The NO_3 concentration in Swiss chard leaves was below the regulated value for spinach in the UK even though the concentration of NO_3–N in fish wastewater was about 6 times that in the control solution.

Acknowledgement

This research was supported by Japan Science and Technology Agency (JST) / Japan International Cooperation Agency (JICA), Science and Technology Research Partnership for Sustainable Development (SATREPS) program. This research was supported also by the project of maximum use and efficiency model of saline water coupled to a one-way aquaponics-agriculture system (CONACYT–PN–2017–I, grant number 4631, Project supported by FOINS).

References

Abelson, P.H., 1999. A potential phosphate crisis. Science 283, 2015, http://doi.org/10.1126/science.283.5410.2015.

Anderson, J. M., Boardman, N. K., David, D. J., 1964. Trace metal composition of fractions obtained by digitonin fragmentation of spinach chloroplasts. Biochem. Biophys. Res. Commun. 17, 685–689, http://doi.org/10.1016/0006-291X(64)90415-2.

Barker, A. V., Pilbeam, D. J., 2015. Handbook of Plant Nutrition, second ed. CRC Press, Boca Raton, FL, USA.

Cataldo, D. A., Haroon, M., Schrader, L. E., Youngs, V. L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6, 71–80, http://doi.org/10.1080/00103627509366547.

Ducic, T., Polle, A., 2005. Transport and detoxification of manganese and copper in plants. Braz. J. Plant Physiol. 17, 103–112, http://doi.org/10.1590/S1677-04202005000100009.

Enduta, A., Jusoh, A., Ali, N., Wan Nik, W. B., 2011. Nutrient removal from aquaculture wastewater by vegetable production in aquaponics recirculation system. Desalin. Water Treat. 32, 422–430.

Farley, R. F., Draycott, A. P., 1973. Manganese deficiency of sugar beet in organic soil. Plant Soil 38, 235–244, http://doi.org/10.1007/BF00779008.

Graber, A., Junge, R., 2009. Aquaponic systems: Nutrient recycling from fish wastewater by vegetable production. Desalination 246, 147–156, http://doi.org/10.1016/j.desal.2008.03.048.

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H., Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. Sustainability 7, 4199–4224, http://doi.org/10.3390/su7044199.

Hambrey consulting 2013. The relevance of aquaponics to the New Zealand aid programme, particularly in the Pacific, in: Aquaponics research project, commissioned report by Hambrey consulting, https://www.mfat.govt.nz/assets/Aid/Aid-approach-aquaponics-Pacific-report-2013.pdf (accessed 15 October 2018).

Hell, R., Stephan, U. W., 2003. Iron uptake, trafficking and homeostasis in plants. Planta 216, 541–551, http://doi.org/10.1007/s00425-002-0920-4.

Huang, J., Yu, H., Guan, X., Wang, G., Guo, R., 2016. Accelerated dryland expansion under climate change. Nat. Clim. Chang. 6, 166–171, http://doi.org/10.1038/nclimate2837.

Hochmuth, G.J., Hanlon, E.A., 2010. Commercial vegetable fertilization principles. Horticultural sciences department, Gainesville, USA, pp. 1–17.

IARC (International Agency for Research on Cancer) 2010. IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monogr. Eval. Carcinog. Risks Hum. 94, 1–412.

Jeong, J., Guerinot, M.L., 2009. Homing in on iron homeostasis in plants. Trends Plant Sci. 14, 280–285, http://doi.org/10.1016/j.tplants.2009.02.006.

Jones Jr., J. B., 2012. Plant Nutrition and Soil Fertility Manual, second ed. CRC Press, Boca Raton, FL, USA.

Kaburagi, E., Morikawa, Y., Yamada, M., Fujiyama, H., 2014. Sodium enhances nitrate uptake in Swiss chard (*Beta vulgaris* var. *cicla* L.). Soil Sci. Plant Nutr. 60, 651–658, http://doi.org/10.1080/00380768.2014.938595.

Kaburagi, E., Yamada, M., Fujiyama, H., 2015. Sodium, but not potassium enhances root to leaf nitrate translocation in Swiss chard (*Beta vulgaris* var. *cicla* L.). Environ. Exp. Bot. 112, 27–32, http://doi.org/10.1016/j.envexpbot.2014.11.007.

Mengel, K., Kirkby, E. A., 2001. Principles of Plant Nutrition, fifth ed. Kluwer Academic Publishers, Dordrecht, Netherlands.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27, 31–36, https://doi.org/10.1016/S0003-2670(00)88444-5.

Ohki, K., 1981. Manganese critical levels for soybean growth and physiological processes. J. Plant Nutr. 3, 271–284, http://doi.org/10.1080/01904168109362836.

Pitman, M.G., Läuchli, A., 2002. Global impact of salinity and agricultural ecosystems, in: Läuchli, A., Lüttge, U. (Eds.), Salinity: Environment–Plants–Molecules. Springer, Netherlands, pp. 3–20.

Red Tractor Farm Assurance 2016. Crop Module: Spinach, version 3.2, Fresh Produce Standards. https://assurance.redtractor.org.uk/contentfiles/Farmers-6575.pdf?_=635971947694929508 (accessed 15 October 2018).

Roosta, H. R., Hamidpour, M., 2011. Effect of foliar application of some macro- and micro-nutrients on tomato plants in aquaponic and hydroponic systems. Sci. Hortic. 129, 396–402, http://doi.org/10.1016/j.scienta.2011.04.006.

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, fourth ed. Wadsworth Publishing Company, Belmont, CA. USA.

Saysel, A. K., Barlas, Y., 2001. A dynamic model of salinization on irrigated lands. Ecol. Modell. 139, 177–199, http://doi.org/10.1016/S0304-3800(01)00242-3.

Shao, J. F., Yamaji, N., Shen, R. F., Ma, J. F., 2017. The key to Mn homeostasis in plants: Regulation of Mn transporters. Trends Plant Sci. 22, 215–224, http://doi.org/10.1016/j.tplants.2016.12.005.

Stickney, R. R., 2017. Tilapia feeding habits and environmental tolerances, in: Perschbacher, P. W., Stickney, R. R. (Eds.), Tilapia in Intensive Co-culture. Wiley-Blackwell, West Sussex, UK, pp. 25–35.

Tischner, R., 2000. Nitrate uptake and reduction in higher and lower plants. Plant Cell Environ. 23, 1005–1024, http://doi.org/10.1046/j.1365-3040.2000.00595.x.

Vance, C. P., Uhde-Stone, C., Allan, D. L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157, 423–447, http://doi.org/10.1046/j.1469-8137.2003.00695.x.

Yamada, M., Kuroda, C., Fujiyama, H., 2016a. Growth promotion by sodium in amaranthaceous plants. J. Plant Nutr. 39, 1186–1193, http://doi.org/10.1080/01904167.2015.1069341.

Yamada, M., Kuroda, C., Fujiyama, H., 2016b. Function of sodium and potassium in growth of sodium-loving Amaranthaceae species. Soil Sci. Plant Nutr. 62, 20–26, http://doi.org/10.1080/00380768.2015.1075365.