

# Fish farming effluent and recirculation times on gas exchange and nutrition of hydroponic watercress

## Efluente da piscicultura e tempos de recirculação nas trocas gasosas e nutrição do agrião hidropônico

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**ABSTRACT** - The present study evaluated the potential use of fish farming effluent incorporated into the standard nutrient solution and recirculation time on gas exchange and macro and micronutrient contents of watercress in a hydroponic system. The study was conducted in randomized blocks, in a split-plot scheme with 4 replicates, in two experiments carried out in spring and summer seasons. Plots were composed of different proportions of nutrient solution (NS) and fish farming effluent (FFE): S1 (0% NS and 100% FFE), S2 (25% NS and 75% FFE), S3 (50% NS and 50% FFE), S4 (75% NS and 25% FFE), and S5 (100% NS and 0% FFE). Subplots consisted of two solution recirculation times (Time 1 – T1: 15 for 15 min and Time 2 – T2: 15 for 30 min), totaling 40 experimental plots. Net photosynthesis, stomatal conductance, transpiration, substomatal CO<sub>2</sub> concentration, SPAD index, and macro and micronutrient contents were evaluated. Net photosynthesis showed a quadratic fit in both cultivation cycles, with maximum values observed with use of 86.0% NS (spring) and 64.9% NS (summer). Leaf N and P contents indicate that the use of approximately 70% NS can be considered satisfactory, as it promoted the highest values of these macronutrients. Using fish farming effluent can enable the production of watercress in hydroponic systems, influencing the nutritional status of the crop.

**RESUMO** - O presente estudo avaliou o potencial uso do efluente da piscicultura, combinado a solução nutritiva padrão e tempo de recirculação, sob as trocas gasosas e teores de macros e micronutrientes do agrião d'água em um sistema hidropônico. O estudo foi conduzido em blocos casualizados, no esquema de parcelas subdivididas com 4 repetições, em dois experimentos realizados nas estações de primavera e verão. As parcelas foram compostas por diferentes proporções de solução nutritiva (SN) e efluente da piscicultura (EP): S1 (0% de SN e 100% de EP), S2 (25% de SN e 75% de EP), S3 (50% de SN e 50% de EP), S4 (75% de SN e 25% de EP), e S5 (100% de SN e 0% de EP). As subparcelas foram constituídas por dois tempos de recirculação da solução (Tempo 1 – T1: 15 por 15 min e Tempo 2 – T2: 15 por 30 min), totalizando 40 parcelas experimentais. Avaliou-se a fotossíntese líquida, condutância estomática, transpiração, concentração substomática de CO<sub>2</sub>, índice SPAD e teores de macro e micronutrientes. A fotossíntese líquida apresentou ajuste quadrático, em ambos os ciclos de cultivo, com máximos valores observados com uso de 86,0 (primavera) e 64,9% (verão) da SN. Os níveis foliares de N e P indicam que o uso de aproximadamente 70% da SN pode ser considerado satisfatório, já que proporcionou os maiores valores destes macronutrientes. A utilização de efluente da piscicultura pode viabilizar a produção de agrião em sistemas hidropônicos, influenciando o estado nutricional da cultura.

**Keywords:** *Nasturtium officinale*. Mineral nutrition. Photosynthesis.

**Palavras-chave:** *Nasturtium officinale*. Nutrição mineral. Fotossíntese.

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### INTRODUCTION

The reduction in water availability drives research aimed at reducing environmental impacts and optimizing the use of water in agriculture, in the face of climate change. On the other hand, with the growing demand for food and food security of the population, food production will need to increase water consumption (FAO, 2017), as well as the use of fertilizers (PENUÉLAS; COELLO; SARDANS, 2023).

Among the agricultural activities, fish farming can impact the environment, as the water from fish production contains feed residues and large amounts of nutrients, with a significant potential for pollution of watercourses from the discharge of effluents (ERONDU; ANYANWU, 2005). This effluent can be used in hydroponic cultivation since the nutrients from these waters can be reused for plant fertilization and nutrition (OLIVEIRA et al., 2023).

Plant mineral nutrition is associated with proper fertilizer management and fertilization programs. In hydroponic systems, the mineral elements essential for plant growth and development are supplied through nutrient solutions. Nutrient solutions are composed of nutrients and oxygen, dispersed in appropriate proportions and amounts, in ionic (cations and anions) or molecular form (PRADO, 2021), in order to meet the nutritional demand of the crop.

Among these vegetables produced in a hydroponic system, watercress



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(*Nasturtium officinale*) stands out as a crop that naturally develops in environments similar to the hydroponic medium (HASSANDOKHT; JAFARI; EBRAHIMI, 2021). In addition, watercress is a crop of high nutritional value, and it also contains many bioactive compounds associated with antioxidant, anti-inflammatory and chemotherapeutic properties (HASSANDOKHT; JAFARI; EBRAHIMI, 2021). Thus, this study aimed to evaluate the potential use of fish farming effluent arising from the production of Nile tilapia (*Oreochromis niloticus*) and solution recirculation times on the photosynthetic and nutritional parameters of watercress in a hydroponic system.

## MATERIAL AND METHODS

### Study location

The study was carried out in a greenhouse on the premises of the Department of Agricultural Engineering (DENA) of the Federal University of Ceará (UFC), Pici Campus, Fortaleza, CE, Brazil (3° 44" S, 38° 33" W and

altitude of 19.5 m). According to Köppen's classification, the local climate is Aw', tropical rainy, with two well-defined seasons (a drier period during the winter and a period with maximum rainfall during the autumn) (KÖPPEN, 1918). The average annual relative humidity is 77.5%, with a maximum temperature of 30.8 °C and a minimum temperature of 23.9 °C, according to climatic data collected in the period between 1981 and 2010 (INMET, 2022).

Two experiments were conducted in a greenhouse under hydroponic conditions, the first from October to November 2021 (spring) and the second from January to February 2022 (summer). The greenhouse had the following dimensions: 6.5 m wide by 12.0 m long, ceiling height of 3.5 m and total height with metal arch of 4.5 m. The roof was made with transparent agricultural plastic film (low-density polyethylene), 150 microns thick and treated against ultraviolet (UV) rays, and the sides were protected by anti-aphid screens. A thermo-hygrometer (Hygrometer Htc-2A) was installed inside the greenhouse (1.80 m high) to collect data on temperature and relative humidity during the experiments, which are presented in Table 1.

**Table 1.** Air temperature and relative humidity of the protected environment during the experiments.

Experiments	Air temperature (°C)			Relative humidity (%)		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Spring	37.0	32.0	34.7	64.0	43.0	53.6
Summer	37.3	23.7	32.7	95.0	45.0	66.0

### Experimental design and growing conditions

The experimental design was randomized blocks in a split-plot scheme, with 4 replicates, totaling 40 experimental plots. Plots were composed of five nutrient solutions, with different proportions of standard nutrient solution (NS) and fish farming effluent (FFE): S1 (100% FFE), S2 (25% NS and 75% FFE), S3 (50% NS and 50% FFE), S4 (75% NS and 25% FFE), and S5 (100% NS). Subplots consisted of two solution recirculation times (Time 1 – T1: 15x15min and Time 2 – T2: 15x30 min). The recirculation times were controlled by means of a timer (analog timer – Gc), which supplied the solution at an interval of 15 min operating for 15 min off (T1) and 15 min operating for 30 min off (T2).

Watercress was grown in the hydroponic system composed of 40 independent benches in NFT system (laminar flow of nutrient technique). Each plot consisted of a cultivation channel (PVC pipe of 100 mm in diameter and 2.70 m in length), a reservoir (50 L capacity) to store the nutrient solution, and an electric pump (0.25 CV) to pump the solution into the hydroponic channel at the highest part of the cultivation bench (injection of the solution into the channel with a flow rate of 1.5 L min<sup>-1</sup>), as described by Santos et al. (2022). Each hydroponic channel had ten holes of 5 cm in diameter for cultivating the plants, spaced 0.25 m apart. The channels were installed (spaced 0.25 m apart) on the cultivation bench at a 3.0% slope. The schedules for actuating the electric pumps according to the recirculation times of the solutions were carried out using analog timers, according to the treatments.

### Crop management and nutrient solutions

The experiments were carried out with watercress (*Nasturtium officinale*), cultivar 'Folha Larga', with seeds acquired from the company ISLA Sementes. The seedlings were produced in 96-cell trays containing coconut fiber substrate and irrigated with a nutrient solution recommended by Furlani (1998) diluted by 50%. Subsequently, at 30 days after sowing, the seedlings are transplanted to the hydroponic system, and treatments began to be applied. For the control treatment – S5 (without fish farming effluent), the nutrient solution (NS) was composed at a concentration of 100% of nutrient salts according to Furlani (1998).

Fish farming effluent was obtained from the rearing of Nile tilapia (*Oreochromis niloticus*), produced in an excavated tank (2.0 x 4.0 x 0.9 m) lined with tarpaulin, with a volume of 4.8 m<sup>3</sup> at its maximum capacity. The tank was located next to the greenhouse. The effluent used came from the growth phase of the fish (60 to 300 g), which were fed according to their weight and the recommendations of the feed manufacturer (Polinutri Alimentos S.A., São Paulo, Brazil). Aeration with a cascade in a recirculation system for the tank was performed with an electric pump (0.5 CV). The effluent was filtered in a decanter filter, containing an acrylic geotextile to remove suspended solids and a biological filter filled with expanded clay with nitrifying bacteria. Fish farming effluent samples were sent to the Laboratory for the Analysis of Soils, Waters, Plant Tissues and Fertilizers (FUNCEME/UFC Agreement) for chemical characterization (Table 2).

**Table 2.** Chemical characterization of the fish farming effluent used in the experiments.

Experiment	Cations (mmol <sub>e</sub> L <sup>-1</sup> )				Anions (mmol <sub>e</sub> L <sup>-1</sup> )			dS m <sup>-1</sup>	SAR	pH	(mg L <sup>-1</sup> )
	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>3-</sup>	CO <sub>3</sub> <sup>2-</sup>	EC			Dissolved solids
Spring	1.3	3.5	4.7	0.7	9.5	0.5	0.5	1.04	2.15	8.1	1040
Summer	1.2	2.0	4.5	0.7	7.9	0.1	0.3	0.85	2.52	9.2	850

SAR: Sodium Adsorption Ratio.

After filtering the effluents, they were stored in tanks with capacity of 1000 L. Treatment S1 was composed entirely of the fish farming effluent (without the addition of nutrient salts). The other mixtures of nutrient solution and fish farming effluent were obtained from the following combinations: S2: 25% nutrient solution and 75% fish farming effluent; S3: 50% nutrient solution and 50% fish farming effluent; S4: 75% nutrient solution and 25% fish farming effluent; S5: 100% nutrient solution and 0% fish farming effluent. Each solution

was stored in 240 L containers. Weekly monitoring was performed with measurements of the electrical conductivity (EC) and pH of each solution, using a portable conductivity meter (Tds & EC) and a pH meter (Akso - AK90), respectively (Table 3). However, no pH corrections to the ideal range (5.5 to 6.5) were made, simulating field conditions. The volume of the nutrient solution lost by evapotranspiration was replaced weekly and manually, using the corresponding mixture instead of water.

**Table 3.** Mean values of electrical conductivity (EC) and pH of nutrient solutions at the end of the experiments.

Nutrient Solutions (%)	Spring				Summer			
	Time I		Time II		Time I		Time II	
	EC (dS m <sup>-1</sup> )	pH						
S1 – 0% NS + 100%FFE	0.91	8.60	0.90	8.70	0.85	8.90	0.93	8.90
S2 – 25% NS + 75%FFE	1.26	8.20	1.25	8.30	1.34	8.50	1.37	8.50
S3 – 50% NS + 50%FFE	1.59	7.80	1.56	7.80	1.93	7.70	1.89	7.90
S4 – 75% NS + 25%FFE	2.05	6.80	2.00	6.80	2.16	6.70	2.20	7.10
S5 – 0% NS + 100%FFE	2.27	6.30	2.26	6.40	2.57	6.00	2.60	6.10

### Variables analyzed

At 26 days after transplanting (DAT), gas exchange and SPAD index were determined in the middle third region of the plants and in fully expanded leaves between 8 and 10 am, after selecting plants with uniform development of each hydroponic profile. Gas exchange was measured using a Portable Infrared Gas Analyzer (IRGA, model LCpro-SD). The variables measured were: net photosynthesis ( $P_n$ ) in  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ; transpiration ( $E$ ) in  $\text{mmol} \text{ m}^{-2} \text{ s}^{-1}$ ; stomatal conductance to water vapor ( $g_s$ ) in  $\text{mmol}(\text{H}_2\text{O}) \text{ m}^{-2} \text{ s}^{-1}$ ; internal  $\text{CO}_2$  concentration ( $C_i$ ) in  $\mu\text{mol} \text{ mol}^{-1}$ . Readings were performed under saturating light ( $1200 \mu\text{mol} \text{ m}^{-2} \text{ s}^{-1}$ ), with constant  $\text{CO}_2$  concentration (400 ppm) and ambient temperature. The SPAD index was determined with a portable chlorophyll meter (Chlorophyll Meter SPAD-502), with three readings taken (to obtain an average value) on fully expanded leaves exposed to sunlight.

At 26 DAT, the plants (leaves + roots) were collected, placed in previously identified paper bags, and dried in a forced air circulation oven (65 °C) until reaching constant mass. After drying, the samples were ground in a Wiley mill with a 20-mesh (0.841 mm) sieve. The samples were then sent to *Laboratório Athenas Consultoria Agrícola e Laboratório LTDA* for determination of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron

(B), according to the methodology described by Malavolta, Vitti and Oliveira (1989).

### Statistical analysis

The data were subjected to the F test of the analysis of variance, and the variables that showed significant effects were subjected to polynomial regression analysis, aiming to fit behavior models (linear and quadratic). Qualitative data were subjected to Tukey test ( $p < 0.01$  and  $p < 0.05$ ). The analyses were performed using the statistical program SISVAR (FERREIRA, 2011).

## RESULTS AND DISCUSSION

### Gas exchange and SPAD index

In the spring experiment, the nutrient solution significantly affected stomatal conductance ( $g_s$ ), transpiration ( $E$ ), substomatal  $\text{CO}_2$  concentration ( $C_i$ ) and SPAD index (Table 4). However, for  $g_s$  and  $E$  there was no adequate fit of the regression models used. For the summer experiment, the nutrient solution exerted a significant effect only on  $C_i$  and SPAD index. In addition, only the nutrient solution factor significantly influenced the net  $\text{CO}_2$  assimilation rate ( $P_n$ ) in both crop cycles.

**Table 4.** Summary of analysis of variance for net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ), substomatal  $CO_2$  concentration ( $C_i$ ), SPAD index in watercress cultivated with nutrient solutions prepared from fish farming effluent and recirculated for different times under hydroponic conditions.

Sources of variation	DF	Mean Square				
		Spring experiment				
		$P_n$	$g_s$	$E$	$C_i$	SPAD
Block	3	79.847 <sup>ns</sup>	0.0065 <sup>ns</sup>	0.7411 <sup>ns</sup>	1752.3979 <sup>ns</sup>	14.423 <sup>ns</sup>
Time (T)	1	65.142 <sup>ns</sup>	0.0373 <sup>ns</sup>	8.7456 <sup>ns</sup>	82.4375 <sup>ns</sup>	10.609 <sup>ns</sup>
Residual (a)	3	134.999	0.0429	5.8616	3480.1218	20.152
Solution (S)	4	139.489 <sup>**</sup>	0.0427 <sup>*</sup>	7.2862 <sup>*</sup>	3642.8781 <sup>**</sup>	1015.645 <sup>**</sup>
Interaction (T x S)	4	14.614 <sup>ns</sup>	0.0223 <sup>ns</sup>	3.4683 <sup>ns</sup>	122.2609 <sup>ns</sup>	6.769 <sup>ns</sup>
Residual (b)	24	10.866	0.0150	2.4896	204.0123	37.051
Total	39	-	-	-	-	-
CV - T (%)	-	28.45	56.94	31.71	18.56	13.31
CV - S (%)	-	25.09	33.72	20.67	4.49	18.05
Summer experiment						
Block	3	5.989 <sup>ns</sup>	0.002 <sup>ns</sup>	0.183 <sup>ns</sup>	390.449 <sup>ns</sup>	10.174 <sup>ns</sup>
Time (T)	1	0.207 <sup>ns</sup>	0.001 <sup>ns</sup>	0.089 <sup>ns</sup>	19.792 <sup>ns</sup>	128.881 <sup>*</sup>
Residual (a)	3	11.763	0.017	1.685	472.096	7.034
Solution (S)	4	116.118 <sup>**</sup>	0.030 <sup>ns</sup>	5.018 <sup>ns</sup>	6572.169 <sup>**</sup>	936.438 <sup>**</sup>
Interaction (T x S)	4	11.981 <sup>ns</sup>	0.007 <sup>ns</sup>	1.072 <sup>ns</sup>	18.519 <sup>ns</sup>	14.356 <sup>ns</sup>
Residual (b)	24	16.563	0.013	1.923	333.845	16.114
Total	39	-	-	-	-	-
CV - T (%)	-	22.48	37.01	22.15	7.24	8.43
CV - S (%)	-	26.67	32.94	23.66	6.09	12.76

\*\* and \* = significant at 1 and 5% probability levels by the F test, respectively, ns - not significant, DF - degrees of freedom, CV - coefficient of variation.

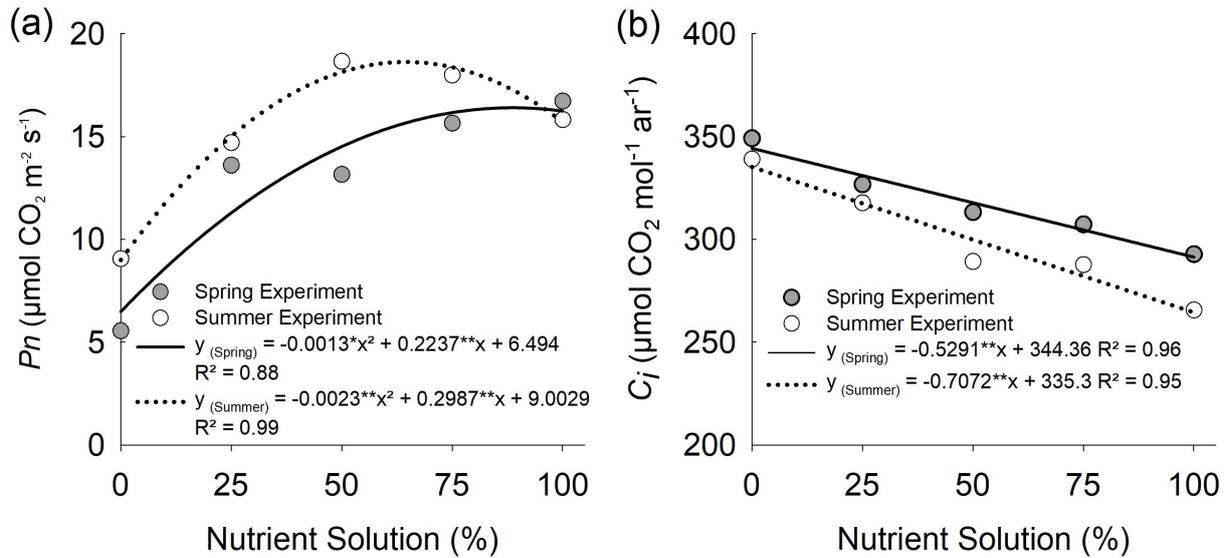
A quadratic model fitted to net photosynthesis ( $P_n$ ) data in both experiments, with maximum values observed at 26 DAT, using 86.0 and 64.9% NS for the spring and summer experiments, respectively (Figure 1a). From these values, the percentage increase in NS caused a reduction in  $A$ . This behavior is probably due to the reduction in the substomatal  $CO_2$  concentration ( $C_i$ ), caused by osmotic stress and high temperature. Several factors, both external and internal, can affect photosynthesis:  $CO_2$  and  $O_2$  concentrations, availability of water and nutrients, temperature and light, as well as the size, shape, architectural arrangement and pigment content of the leaves (MENDES; LUCENA; MEDEIROS, 2015).

The lower  $A$  values, observed for treatments with 0 and 25% NS, may be related to low nutrient availability due to high pH, which may affect water and ion absorption. This reduction was possibly due to the impairment in the formation and maintenance of chlorophylls, since nitrate and magnesium ions are essential for the photosynthetic process. In addition, high temperatures may have caused damage to the photosynthetic apparatus, compromising the integrity of the membranes, or even to the enzymatic activity of RuBisCO, responsible for  $CO_2$  fixation in the photosynthesis process (TAIZ; ZEIGER, 2013).

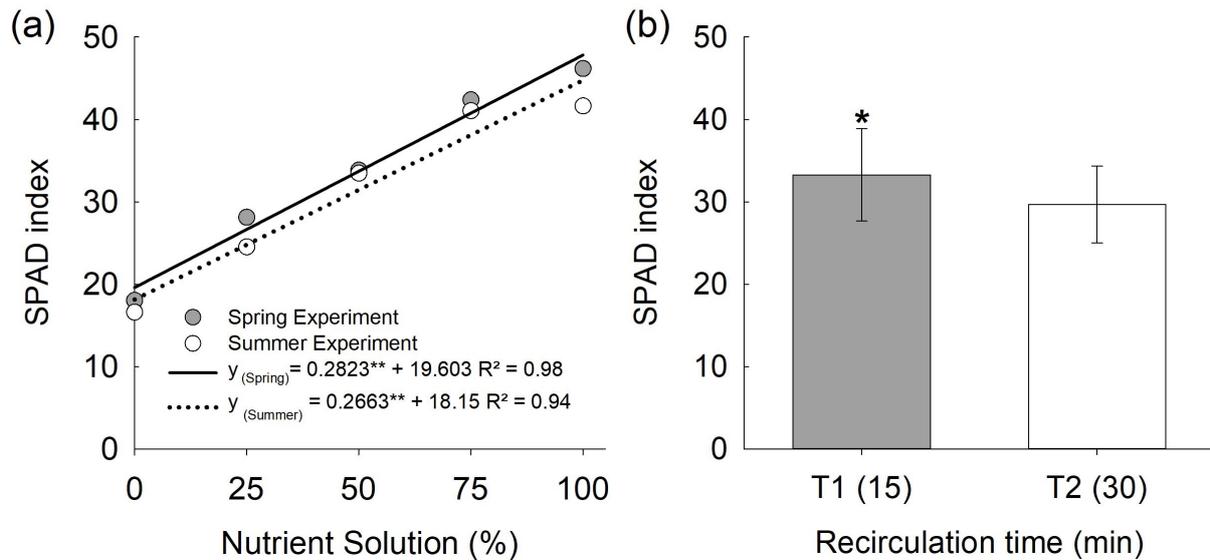
For  $C_i$  (Figure 1b), the pattern was opposite to that found for the SPAD index. The percentage increase of NS in the mixture caused a decrease in  $C_i$ . This reduction is possibly due to variations in the mechanism of opening and closing the stomata and, consequently, in the dynamics of gas exchange (SOUZA et al., 2020). Watercress crop shows better development under conditions of mild temperatures (15 and 25 °C), since its leaves are very sensitive to climate change

(PACOTTE, 2020). However, in this study, the plants were exposed to higher temperatures throughout both experiments, showing loss of turgor for most of the day. This condition, associated with the osmotic stress generated by the increased concentration of salts in the NS (DING et al., 2018), induces stomatal closure to reduce water loss via transpiration. In turn, with the closure of the stomata, there is a decrease in  $CO_2$  concentration in the intercellular spaces of the leaves ( $C_i$ ), due to its use in photosynthesis (SOUZA et al., 2020). However, as the concentration of the fish farming effluent decreased, there were reductions in the substomatal  $CO_2$  concentration ( $C_i$ ) of 0.5291 and 0.7072  $\mu\text{mol mol}^{-1}$  for the spring and summer experiments, respectively.

In addition, in the summer experiment, the SPAD index was also influenced by the interaction between nutrient solution and recirculation time (Table 4). The SPAD index showed increments in both experiments, equal to 0.282 and 0.266 for the spring and summer experiments, respectively (Figure 2a). In addition, in the summer experiment, the T1 recirculation time promoted a mean value of 33.2, higher than the value of 29.6 obtained with T2 (Figure 2b). This response of T1 as compared to T2 may be due to a greater absorption of NS and, consequently, of nitrogen, since the SPAD index is directly related to leaf N contents (BENATI; NAVA; MAYER, 2021). In the present study, plants subjected to T1 recirculation time were twice as much exposed to NS flow as plants under T2, but longer recirculation intervals may generate water deficit in hydroponic cultivation, due to the absence of substrate, and limit plant development (SOUZA et al., 2020).



**Figure 1.** Regression analyses for net photosynthesis (a) and stomatal conductance (b) in watercress cultivated with nutrient solutions prepared from fish farming effluent and recirculated for different times under hydroponic conditions.



**Figure 2.** Mean values of SPAD index for watercress as a function of nutrient solutions prepared from fish farming effluent (a) and as a function of the recirculation time of nutrient solutions (b).

Although several factors can interfere with the SPAD index, the supply of nutrients can be considered one of the most relevant, since the plant needs such nutrients to perform its vital functions, with the production of chlorophyll and sugars that are used to promote growth ((MENDES; LUCENA; MEDEIROS, 2015). Thus, in plant nutrition studies, this index is correlated with foliar diagnosis and the nutritional status of plants (BENATI; NAVA; MAYER, 2021). In this study, the low SPAD index, observed in plants subjected to solutions with 0 and 25% NS, are associated with symptoms of chlorosis and reduced growth. These symptoms are evidence of a more severe deficiency of nutrients, especially nitrogen (MENDES; LUCENA; MEDEIROS, 2015). In addition, in this study, nutrient deficiency possibly

occurred due to the high pH, which, throughout the experiment, ranged from 7.9 to 9.2 for the solutions with 0 and 25% NS, respectively (Table 3). On the other hand, the pH of the solutions with 75 and 100% NS ranged from 5.5 to 7.7 (Table 3). This, in turn, may have had an influence on the solubility of the nutrients and, indirectly, on the higher SPAD indices for the treatments with higher percentage of NS. In this context, at pH close to or equal to neutrality, all macronutrients are available. However, under alkaline conditions, the solubility of micronutrients (Cu, Zn, Fe, and Mn) decreases, and precipitation of phosphates and carbonates of these elements may occur (LIRA et al., 2019; MENDES; LUCENA; MEDEIROS, 2015).

### Macro and micronutrient contents

For both experiments, only nutrient solution (NS) had a significant effect on total nitrogen (N) contents. In the spring experiment, only the nutrient solution (NS) had a

significant effect on phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) contents. Regarding the summer experiment, there was only an effect of the NS factor on P and Mg accumulation (Table 5).

**Table 5.** Summary of the analysis of variance for the contents of macronutrients nitrogen, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in watercress cultivated with nutrient solutions prepared from fish farming effluent and recirculated for different times under hydroponic conditions.

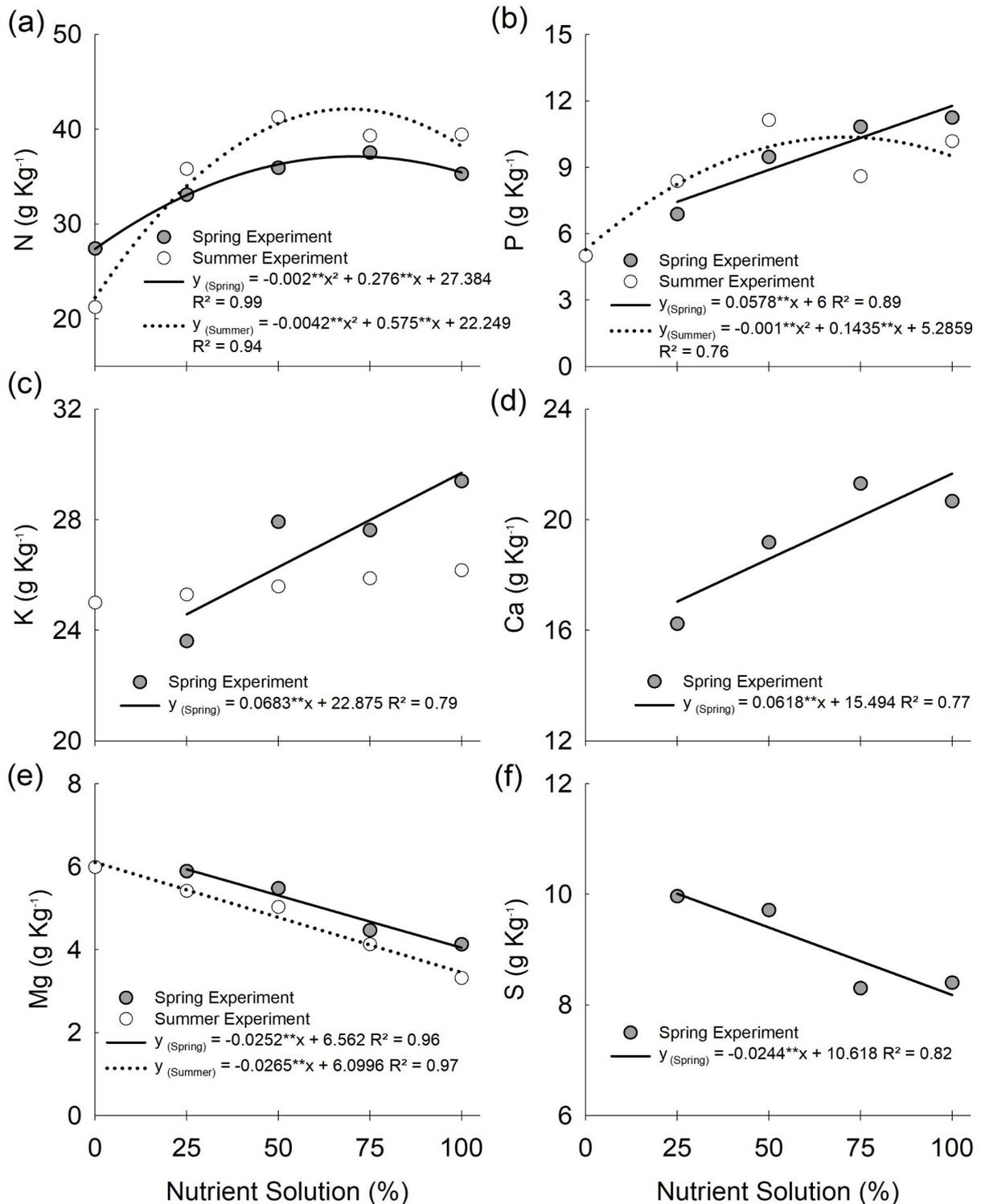
Sources of variation	DF	Mean Square					
		N	P	K	Ca	Mg	S
Spring Experiment							
Block	3	45.423 <sup>ns</sup>	8.658 <sup>ns</sup>	47.715 <sup>ns</sup>	6.798 <sup>ns</sup>	1.128 <sup>ns</sup>	3.794 <sup>ns</sup>
Time (T)	1	16.189 <sup>ns</sup>	0.245 <sup>ns</sup>	10.013 <sup>ns</sup>	0.750 <sup>ns</sup>	0.245 <sup>ns</sup>	1.280 <sup>ns</sup>
Residual (a)	3	86.179	2.119	80.808	5.051	0.162	8.401
Solution (S)	3	76.948 <sup>**</sup>	31.004 <sup>**</sup>	49.073 <sup>*</sup>	40.856 <sup>**</sup>	5.513 <sup>**</sup>	5.997 <sup>**</sup>
Interaction (T x S)	3	5.571 <sup>ns</sup>	1.178 <sup>ns</sup>	13.785 <sup>ns</sup>	4.836 <sup>ns</sup>	0.113 <sup>ns</sup>	1.029 <sup>ns</sup>
Residual (b)	18	15.057	2.712	12.305	5.831	0.228	0.689
Total	31	-	-	-	-	-	-
CV - T (%)	-	26.85	15.14	33.12	11.61	8.06	31.87
CV - S (%)	-	11.22	17.13	12.92	12.48	9.57	9.13
Summer Experiment							
Block	3	46.139 <sup>ns</sup>	2.959 <sup>ns</sup>	131.158 <sup>ns</sup>	6.163 <sup>ns</sup>	0.013 <sup>ns</sup>	2.334 <sup>ns</sup>
Time (T)	1	0.116 <sup>ns</sup>	8.100 <sup>ns</sup>	102.080 <sup>ns</sup>	3.721 <sup>ns</sup>	0.342 <sup>ns</sup>	1.936 <sup>ns</sup>
Residual (a)	3	42.769	3.499	146.077	15.599	0.402	4.619
Solution (S)	4	370.770 <sup>**</sup>	43.706 <sup>**</sup>	28.612 <sup>ns</sup>	35.339 <sup>ns</sup>	9.001 <sup>**</sup>	5.231 <sup>ns</sup>
Interaction (T x S)	4	8.065 <sup>ns</sup>	8.581 <sup>ns</sup>	65.849 <sup>ns</sup>	17.238 <sup>ns</sup>	0.453 <sup>ns</sup>	2.557 <sup>ns</sup>
Residual (b)	24	68.788	5.063	53.279	13.760	0.736	5.130
Total	39	-	-	-	-	-	-
CV - T (%)	-	17.88	21.59	38.32	21.46	13.29	20.30
CV - S (%)	-	22.68	25.97	23.14	20.15	17.98	21.39

\*\* and \* = significant at 1 and 5% probability level by the F test, respectively, <sup>ns</sup> - not significant, DF - degrees of freedom, CV - coefficient of variation.

Total nitrogen contents were described by the quadratic model in both experiments, with higher accumulations of 36.91 g kg<sup>-1</sup> (spring) and 41.93 g kg<sup>-1</sup> (summer) using 69 and 68.45% NS, respectively (Figure 3a). On the other hand, P contents in the spring experiment showed an increment of 0.0578 g kg<sup>-1</sup> for each unit increment of NS (Figure 3b). However, P contents in the summer experiment were described by the quadratic model, with the highest value of 10.43 g kg<sup>-1</sup> under 71.75% NS (Figure 3b). K and Ca contents, only in the spring experiment, showed increments of 0.0683 g kg<sup>-1</sup> and 0.0618 g kg<sup>-1</sup> for each unit increment of NS, respectively (Figures 3c and 3d). Mg contents also showed reductions of 0.0252 g kg<sup>-1</sup> (spring) and 0.0265 g kg<sup>-1</sup> (summer) (Figure 3e). In addition, S contents in the summer experiment showed a reduction of 0.0244 g kg<sup>-1</sup> for each unit increment of NS (Figure 3f).

These results suggest that the addition of approximately 30% of fish farming effluent to the nutrient

solution is able to promote better plant nutrition in terms of nitrogen and phosphorus. However, for the other macronutrients, the absorption of these ions is compromised. According to Trani et al. (2014), the macronutrient contents in watercress leaves suitable for the crop are (g kg<sup>-1</sup>): 45-60 of N, 3-12 of P, 35-50 of K, 15-35 of Ca, 3-10 of Mg and 4-7 of S. These values are consistent with those of the present study for P, Ca and Mg, lower than those of S and higher than those of N and K (Figure 3). Despite the non-ideal values of N, the quadratic behavior observed for this nutrient is strongly associated with the behavior of the growth, yield and photosynthesis variables and SPAD index, previously analyzed, since this element participates in the structure of metabolites fundamental to the development of plant organisms, such as amino acids, proteins, enzymes, coenzymes, nucleic acids and pigments. It is required in greater quantities by most plants (BRANDÃO FILHO et al., 2018).



**Figure 3.** Mean values for the leaf macronutrient contents of watercress as a function of nutrient solutions prepared from fish farming effluent: nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e) and sulphur (f).

In studies with hydroponic watercress, under different nutrient solutions and EC levels, the P content in plants increased linearly (0.381 g kg<sup>-1</sup>) for each unit increment of EC in NS (LIRA et al., 2018). These results are similar to those of the summer experiment in the present study, but differ in part, since the quadratic model yielded an optimal value of

10.43 g kg<sup>-1</sup> with the use of 71.75% NS (Figure 3b).

Regarding K content, the results reported by Lira et al. (2018) also showed a linear but decreasing behavior (Figure 3c). This is possibly due to the accumulation of NaCl used in the preparation of the nutrient solution by these authors. Consequently, Na can be transported by the same proteins as

K, such that the K content in leaves can decrease when there are high Na contents (SHABALA, 2013). This behavior differs from the results found in the present study, in which the NS was not prepared with NaCl, and K content increased as a function of the increase in NS electrical conductivity (Figure 3c).

For Mg, a decreasing behavior is observed, opposite to that of K (Figure 3e). This is possibly due to the decrease of Mg absorption rate induced by cations, due to the lower pH values associated with the increase in NS, such as K and Ca,

which also expressed a linear increase similar to K. This deficiency of Mg generated by the competitiveness of other cations is a phenomenon that can be frequently observed (BRANDÃO FILHO et al., 2018).

For the micronutrients, the significant influence of the nutrient solution (NS) on copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and boron (B) contents was also observed, in both experiments. In addition, leaf Fe contents in the summer experiment were significantly influenced by the interaction between nutrient solution and recirculation time (Table 6).

**Table 6.** Summary of the analysis of variance for the contents of the micronutrients copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and boron (B) in watercress cultivated with nutrient solutions prepared from fish farming effluent and recirculated for different times under hydroponic conditions.

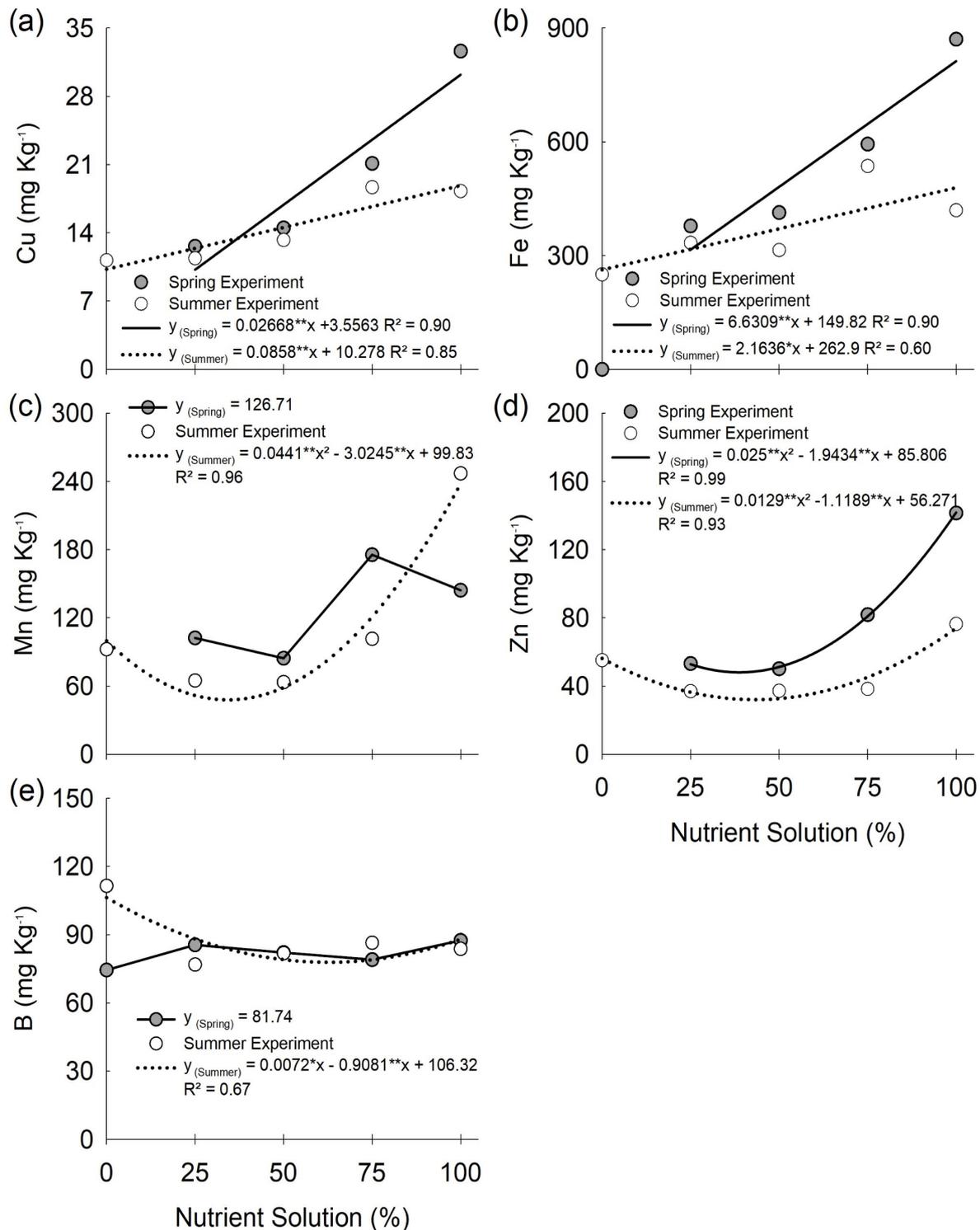
Sources of variation	DF	Mean Square				
		Cu	Fe	Mn	Zn	B
Spring experiment						
Block	3	104.335 <sup>ns</sup>	191737.209 <sup>ns</sup>	1458.100 <sup>ns</sup>	976.820 <sup>ns</sup>	326.174 <sup>ns</sup>
Time (T)	1	162.450 <sup>ns</sup>	19488.315 <sup>ns</sup>	1177.338 <sup>ns</sup>	0.101 <sup>ns</sup>	26.520 <sup>ns</sup>
Residual (a)	3	65.946	218894.744	6727.649	347.329	86.070
Solution (S)	3	654.889 <sup>**</sup>	405580.215 <sup>**</sup>	13516.029 <sup>**</sup>	14352.214 <sup>**</sup>	187.624 <sup>ns</sup>
Interaction (T x S)	3	112.894 <sup>ns</sup>	26794.788 <sup>ns</sup>	694.940 <sup>ns</sup>	17.872 <sup>ns</sup>	75.655 <sup>ns</sup>
Residual (b)	18	37.688	18547.842	1184.482	219.641	149.345
Total	31	-	-	-	-	-
CV - T (%)	-	40.15	82.92	64.73	22.81	7.00
CV - S (%)	-	30.35	24.14	27.16	18.14	9.22
Summer experiment						
Block	3	9.078 <sup>ns</sup>	28774.778 <sup>ns</sup>	2158.537 <sup>ns</sup>	8.070 <sup>ns</sup>	205.399 <sup>ns</sup>
Time (T)	1	4.489 <sup>ns</sup>	62845.256 <sup>ns</sup>	38.025 <sup>ns</sup>	13.689 <sup>ns</sup>	151.530 <sup>ns</sup>
Residual (a)	3	15.419	32052.223	2448.057	272.679	251.161
Solution (S)	4	107.713 <sup>**</sup>	97448.297 <sup>*</sup>	96.322 <sup>**</sup>	2371.878 <sup>**</sup>	222.828 <sup>ns</sup>
Interaction (T x S)	4	9.135 <sup>ns</sup>	24175.454 <sup>ns</sup>	96.322 <sup>ns</sup>	6.998 <sup>ns</sup>	439.434 <sup>*</sup>
Residual (b)	24	13.691	31637.584	1431.740	138.988	143.871
Total	39	-	-	-	-	-
CV - T (%)	-	26.95	48.25	43.43	33.78	18.66
CV - S (%)	-	25.4	47.93	33.21	24.12	14.12

\*\* and \* = significant at 1 and 5% probability levels by the F test, respectively, ns - not significant, DF - degrees of freedom, CV - coefficient of variation.

From the regression analysis of the effect of the nutrient solution on copper contents, increments of 0.2668 and 0.0858 mg kg<sup>-1</sup> were observed in the spring and summer experiments, respectively (Figure 4a). For leaf Fe contents, there was an increase in both experiments, equal to 6.6309 (spring) and 2.1636 mg kg<sup>-1</sup> (summer) per each unit increment of NS (Figure 4b). For leaf Mn contents, it was not possible to fit the equations for the spring experiment, and an average of 126.71 mg kg<sup>-1</sup> was obtained (Figure 4c). In the summer experiment, leaf Mn contents were described by the quadratic model, with a minimum value of 47.9 mg kg<sup>-1</sup> for 34.2% NS (Figure 4c). Leaf Zn contents, in both experiments, showed a quadratic fit, with minimum values of 32.0 and 38.8 mg kg<sup>-1</sup>, corresponding to 43.3 and 38.8% NS, for the spring and summer experiments, respectively (Figure 4d).

Micronutrients, unlike macronutrients, do not participate in plant structures and are therefore absorbed in small amounts on the order of mg kg<sup>-1</sup>. However, they are essential, as they act on the activation and constitution of enzymes and, therefore, the deficiency of any micronutrient can cause damage to plant development and growth (FERNANDES, 2006).

pH is one of the main factors that can affect nutrient availability and, consequently, micronutrient absorption by plants (FERNANDES, 2006). Under conditions of high pH, there is a reduction in the solubilization and absorption of Cu, Zn, Fe and Mn. This influence of pH was also observed in the present study. In general, Cu, Fe, Mn and Zn contents increased with the percentage increase of conventional NS in the mixture, which had lower pH values (Figure 4).



**Figure 4.** Mean values for the leaf micronutrient contents of watercress as a function of nutrient solutions prepared from fish farming effluent: copper (a), iron (b), manganese (c), zinc (d) and boron (e).

However, according to Trani et al. (2014), the leaf micronutrient contents (mg kg<sup>-1</sup>) suitable for watercress are: 7-20 of Cu, 50-300 of Fe, 50-300 of Mn and 25-100 of Zn. These values are lower than those of the present study, except for Mn, which is in accordance with the ideal range (Figure 4). Therefore, in general, there was no deficiency of the

micronutrients analyzed. However, the excess of these elements may have had an influence on the development of plants.

Leaf Cu contents above 20 mg kg<sup>-1</sup> may cause toxicity (MALAVOLTA; VITTI; OLIVEIRA, 1989). In the spring experiment, values above this limit were estimated with the

use of NS from a percentage of 61.63% (Figure 4a). Under the condition of Cu excess, in turn, toxicity can influence the loss of vigor of the roots (FERNANDES, 2006). Regarding Fe, values above the ideal limit of 300 mg kg<sup>-1</sup> were found from 22.65% NS (spring) and 17.15% NS (summer), which can generate restrictive effects on the vegetative growth of plants (JUCOSKI et al., 2016). For Zn, values above the ideal limit were also observed, with a mixture percentage between 75 and 100% NS (Figure 4d). Therefore, considering that the high pH reduced the availability of micronutrients in the treatments with 0 and 25% of conventional NS and that there was an excess of the elements Cu, Fe and Zn with the increase of NS in the mixture, it is verified that the partial replacement of NS with fish farming effluent promotes more adequate micronutrient values for the watercress crop.

For B contents, in the spring experiment, it was not possible to fit the equations for T1, which showed a mean value of 81.7 mg kg<sup>-1</sup> (Figure 4e). On the other hand, in the summer experiment, B contents showed a minimum value of 77.6 mg kg<sup>-1</sup> under 63.0% NS (Figure 4e). The values observed for this element, within the range of 75 to 115 mg kg<sup>-1</sup>, are well above the recommended range, corresponding to 25-60 mg kg<sup>-1</sup> (TRANI et al., 2014). However, among the treatments observed, the treatment with 0% NS and 100% fish farming effluent in T2, summer experiment, stood out with a B content of 111.5 mg kg<sup>-1</sup> (Figure 4e). This element is absorbed in greater quantities by plants under higher pH conditions, which justifies the highest value in the treatment with 0% NS compared to the values associated with the increment of NS.

According to Fernandes (2006), B performs several functions, including its action in the translocation of sugars and in the metabolism of carbohydrates, besides playing an important role in the metabolism of nitrogen, and its excess can generate toxicity, which in turn manifests itself in the form of yellowing of the leaves, extending to the margins. Therefore, since B contents exceed the adequate value in all treatments evaluated, the choice of the NS mixture should be made based on the lowest value, which in this case corresponds to 63.06% NS. Thus, replacing 36.94% NS with FFE can generate values closer to the adequate range for hydroponic watercress when compared to conventional NS.

In view of the above, it is considered that circulation times longer than 15 minutes are not beneficial for nutrient absorption and gas exchange, which confirms the results reported in previous studies (SOUZA et al., 2020). On the other hand, the combination of NS with FFE appears with promising results, as it is possible to replace part of the NS with the effluent, generating ideal combinations to increase nutrient absorption and positively influence the gas exchange of hydroponic watercress. In addition, it should be considered that the combination of NS and FFE can vary in terms of nutrient availability, being related to the tilapia development phase in the reservoirs (QUEIROZ et al., 2017), as the production of excrement can increase the nutritional load of the combination, and therefore greater attention is needed in the preparation of these solutions.

## CONCLUSION

N and P contents indicate that the use of approximately 70% of the nutrient solution can be considered satisfactory,

since the standard nutrient solution has concentrations of nutrients above the demand required for the watercress crop, which increases the production costs with fertilizers.

Partial replacement of the nutrient solution with fish farming effluent provides more suitable micronutrient values for the watercress crop.

Using fish farming effluent can enable the production of watercress in hydroponic systems, influencing the nutritional status of this crop.

Recirculation time at intervals of more than 15 minutes was not beneficial for nutrient absorption and gas exchange in the watercress crop, so further studies are needed.

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