



EFFECT OF WASTEWATER AND PHOSPHATE FERTILIZATION ON PHOTOSYNTHETIC PIGMENTS AND MACRONUTRIENTS OF *Moringa oleifera* Lam.

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ABSTRACT

The use of unconventional resources, such as treated domestic wastewater, has proven to be a promising alternative for agricultural irrigation in semi-arid regions, contributing to water sustainability. This study evaluated the effects of fertigation with wastewater and phosphate fertilization on photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids) and the concentration of macronutrients (N, P and K) in leaves of *Moringa oleifera* Lam. The experiment was conducted in a greenhouse at the Professor Ignácio Salcedo Experimental Station of the National Institute of Semiarid (INSA), in Campina Grande-PB, in a haplic PLANOSOL soil. The experimental design was in randomized blocks, with a 5 x 2 factorial scheme (five concentrations of wastewater and two levels of phosphate fertilization), totaling 10 treatments. Irrigation was performed manually, and the drained volume was collected to calculate evapotranspiration. At 90 days, the levels of chlorophyll A, B and carotenoids did not differ significantly between the treatments with wastewater, but phosphate fertilization increased the levels of N and P in the plant. Wastewater, applied at up to 75%, increased the N level and, at 100%, increased P and K. It is concluded that wastewater, with adequate management, is viable for *Moringa* cultivation, but caution is required due to possible chemical interactions and content proportions in the soil.

Keywords: Irrigation efficiency; Water reuse; Fertigation; Chlorophylls; Sustainability

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EFEITO DA ÁGUA RESIDUÁRIA E ADUBAÇÃO FOSFATADA EM PIGMENTOS FOTOSSINTÉTICOS E MACRONUTRIENTES DE *Moringa oleifera* Lam.

RESUMO – O uso de recursos não convencionais, como a água residuária doméstica tratada, tem se mostrado uma alternativa promissora para a irrigação agrícola em regiões semiáridas, contribuindo para a sustentabilidade hídrica. Este estudo avaliou os efeitos da fertirrigação com água residuária e da adubação fosfatada sobre os pigmentos fotossintéticos (clorofila A, clorofila B e carotenoides) e a concentração de macronutrientes (N, P e K) em folhas de *Moringa oleifera* Lam. O experimento foi conduzido em casa de vegetação na Estação Experimental Professor Ignácio Salcedo, do Instituto Nacional do Semiárido (INSA), em Campina Grande-PB, em solo do tipo PLANOSSOLO háplico. O delineamento experimental foi em blocos ao acaso, com esquema fatorial 5 x 2 (cinco concentrações de água residuária e dois níveis de adubação fosfatada), totalizando 10 tratamentos. As irrigações foram manuais e o volume drenado foi coletado para cálculo da evapotranspiração. Aos 90 dias, os teores de clorofila A, B e carotenoides não diferiram significativamente entre os tratamentos com água residuária, mas a adubação fosfatada aumentou os teores de N e P na planta. A água residuária, aplicada em até 75%, elevou o teor de N e, em 100%, incrementou P e K. Conclui-se que a água residuária, com manejo adequado, é viável para a cultura da *Moringa*, mas exige cautela devido a possíveis interações químicas e proporções de conteúdo no solo.

Palavras-Chave: Eficiência na irrigação; Reutilização hídrica; Fertirrigação; Clorofilas; Sustentabilidade

1. INTRODUCTION

The controlled use of treated domestic wastewater in agriculture has been an important alternative to meet water demand and provide essential nutrients for plants, such as nitrogen, phosphorus, and potassium, thereby contributing to agricultural

performance and the sustainable use of natural resources (Maimone & Harder, 2014; Medeiros et al., 2017). Oliveira et al. (2014) emphasized that this practice can reduce dependence on chemical fertilizers, increase crop productivity, and improve soil properties through the addition of organic matter. This benefit is particularly relevant in semi-arid regions, such as Northeast Brazil, where irregular rainfall and drought periods directly affect agriculture (Santos et al., 2016).

In semi-arid regions, the use of treated domestic wastewater emerges as an effective water resource alternative, while simultaneously providing essential nutrients that support agricultural production and the sustainability of rural communities (Fonteles et al., 2015). Among the nutrients present in treated domestic wastewater, phosphorus plays a critical role, particularly given the deficiency of this nutrient in Brazilian soils, where its application is essential for crop growth and development. Additionally, potassium is vital to plant physiology, influencing enzymatic activity as well as water and nutrient uptake (Silva et al., 2017; Lima et al., 2019).

Fertigation with phosphorus-enriched wastewater represents a promising technique, as it not only maximizes nutrient utilization but also reduces the reliance on conventional fertilizers, contributing to agricultural sustainability (Pereira et al., 2016). However, the use of this practice remains underexplored for tree species such as *Moringa oleifera* Lam., a plant of high nutritional value with the potential to enhance animal feed, particularly in regions where access to alternative nutritional sources is limited (Carvalho et al., 2017).

Nevertheless, irrigation with wastewater requires careful management, as, despite its benefits, it can pose risks of soil contamination and plant toxicity if not managed properly (Santos et al., 2016). The reuse of wastewater, by supplying NPK, is a sustainable strategy that supports plant development and conserves water resources, while minimizing environmental impacts (Carvalho et al., 2017). Simultaneously, phosphorus fertilization, which is essential for root development, photosynthesis, and energy production in plants, is indispensable for crops grown in tropical soils that are often phosphorus-deficient (Cimó et al., 2018; Silva et al., 2018).

In this context, this study evaluated the

effects of fertigation with wastewater and phosphorus fertilization on the composition of photosynthetic pigments (chlorophyll A, chlorophyll B, and carotenoids) and the concentration of macronutrients (N, P, and K) in *Moringa oleifera* leaves. Additionally, it aimed to understand the benefits and limitations of the combined use of wastewater and phosphorus fertilization, contributing to more sustainable and efficient agricultural practices under the conditions of the Brazilian semi-arid region.

2. MATERIAL AND METHODS

2.1 Location and experimental area

The experiment was conducted in a greenhouse at the Professor Ignácio Salcedo Experimental Station, part of the National Institute for the Semi-Arid Region (INSA), a Research Unit of the Ministry of Science, Technology, and Innovation (MCTI), located in Campina Grande-PB, Brazil (7°16'41" S and 35°57'59" W), at an average altitude of

470 meters, situated within the Brazilian semi-arid region, a climatic transition zone of the municipality. The local climate is classified as Aw'i, according to Köppen's climate classification, which is hot and humid, with an annual maximum temperature of 28.7 °C and a minimum of 19.8 °C, showing slight variation throughout the year.

2.2 Characterization of the soil and water used in the experiment

The soil used in the experiment was classified as a HAPLIC PLANOSOL, according to EMBRAPA (2018). This type of soil forms in plain or depression areas and has a light texture, ranging from sand to loamy sand. The soil was analyzed at the Soil Chemistry and Fertility Laboratory, part of the Department of Soil and Rural Engineering at the Center for Agricultural Sciences of the Federal University of Paraíba (UFPB), to assess its physical, chemical, and fertility properties (Tables 1 and 2).

Table 1. Soil physical parameters before experiment installation

Tabela 1. Parâmetros físicos do solo antes da instalação do experimento

Sand (g.kg ⁻¹)	Silt (g.kg ⁻¹)	Clay (g.kg ⁻¹)	Dispersed clay (g.kg ⁻¹)	Degree of flocculation (kg.dm ⁻³)
821	108	72	38	472
Soil density (g.cm ⁻³)	Particle density (g.cm ⁻³)	Total porosity (m ³ .m ⁻³)	Humidity 0,01 – 0,03 – 1,50 Mpa (g.kg ⁻¹)	
1,55	2,61	0,41	83 – 56 – 533	

Table 2. Chemical parameters and soil fertility before the experiment was set up

Tabela 2. Parâmetros químicos e fertilidade do solo antes da instalação do experimento

Parameter	Value	Parameter	Value
pH H ₂ O (1:2,5)	5,4	AL ⁺³ (cmolc.dm ⁻³)	0
P (mg.dm ⁻³)	14,16	Ca ⁺² (cmolc. dm ⁻³)	2,48
S-SO ₄ ⁻² (mg.dm ⁻³)	-	Mg ⁺² (cmolc.dm ⁻³)	1,33
K ⁺ (mg .dm ⁻³)	139,9	SB (cmolc. dm ⁻³)	4,22
Na ⁺ (cmolc. dm ⁻³)	0,06	CTC (cmolc.dm ⁻³)	7,01
H ⁺ + Al ⁺³ (cmolc. dm ⁻³)	2,79	M.O (g.kg-1)	13,45

The chemical analysis of the soil, conducted using the Mehlich Method, indicated an initial phosphorus (P) content of 14.16 mg.dm³. To raise this level to 35.16 mg.dm³ of P, 7.9 g.kg⁻¹ of phosphate amendment was applied to each pot, using purified monoammonium phosphate (MAP) (Dripsol®) purchased locally. This amendment has a minimum guaranteed content of 61% P₂O₅, as regulated by Brazilian fertilizer legislation (Alvarez et al., 1999).

The wastewater initially used (for 30 days) for seedling irrigation was collected at the EXTRABES Experimental Station of UFCG, in partnership with UEPB and CAGEPA. The effluent, originating from a residential building, was treated in an anaerobic dynamic membrane bioreactor with a hydraulic retention time of 18 hours and a capacity of 54 liters, processing 72 liters per day. From the 31st day onward, water from another source was used, collected from the INSA/MCTI Treatment Station, where UASB reactors treated effluent from the administrative headquarters.

Potable water (public supply) was used to prepare the doses to be applied, serving as a control to assess the quality of the water used for irrigation during the experiment. All samples were analyzed for pH, electrical conductivity, major cations, bicarbonates, chlorides, sulfates, and other water quality parameters in the laboratories of UFCG, following the methodology described by Melo et al. (2023).

2.3 Experimental design, treatments, and planting system

The statistical design adopted was a randomized block design with four replicates, arranged in a 5 x 2 factorial scheme, totaling 10 treatments. Factor 1 consisted of five concentration levels (100%, 75%, 50%, 25%, and 0%) of wastewater diluted in potable water for irrigating Moringa. Factor 2 comprised two fertilization methods: with phosphate fertilization (PF) and without fertilization (WF).

The 10 treatments were arranged in 40 experimental plots, distributed across 4 blocks, with each plot containing 3 pots (each pot holding 2 plants, where direct seeding was carried out using 4 seeds, retaining only the two most vigorous plants). This totaled 120

pots and 240 plants. The cylindrical pots (34 cm in height and 29 cm in diameter) were equipped with a drainage system consisting of a 20 mm “90°” elbow fitting connected to a 2L PET bottle for collecting drained water. The pots were lined with fiberglass mesh to prevent soil loss. Each pot was filled with 3.4 kg of gravel for drainage and 20 kg of dry, sieved soil, classified as HAPLIC PLANOSOL, sourced from the Borborema Depression region in Paraíba.

2.4 Irrigation characteristics

Irrigation was conducted manually three times a week using a graduated container to measure the amount of water applied to each pot, always in the late afternoon, with the application directed to the soil. The volume of drained water was collected the following morning, and evapotranspiration was calculated as the difference between the volume applied and the volume drained, determining the amount of water to be applied the next day. To achieve leaching fractions of 0.15 and 0.20 during each phase of the crop cycle, Equation 1 (Rhoades, 1974) was used:

$$VI = ((VW - VD)) / ((1 - LF)) \quad (\text{Eq. 1})$$

Where: VI = volume of water to be applied in the irrigation (mL), VW = volume of water applied in the previous irrigation or period (mL), VD = volume of water drained in the previous irrigation or period (mL), and LF = leaching fraction (0.15 and 0.20).

The volume of water applied in the irrigation was calculated based on these fractions and the concentrations of wastewater for each treatment (Table 3), detailing the water input during the intervals of 30, 60, 90, and 110 days after the initiation of treatments (DAIT).

2.5 Phosphorus, Potassium, Nitrite, and Nitrate input in irrigation water

At the beginning of germination, with an average germination rate of 90%, when the seedlings showed development of the hypocotyl and epicotyl, with pairs of leaves and cotyledon opening, each pot received 7.9 g.kg⁻¹ of phosphorus corrective through

Table 3. Percentages of wastewater and potable water in irrigation in each treatment

Tabela 3. Percentagens de águas residuária e potável na irrigação em cada tratamento

Water input by treatment	At 30 DAIT	At 60 DAIT
T1 = 100% (WW)	3,2 L - WW + 0 L - PW	12 L - WW + 0 L - PW
T2 = 75% (WW) + 25% (PW)	2,4 L - WW + 0,8 L - PW	9 L - WW + 3 L - PW
T3 = 50% (WW) + 50% (PW)	1,6 L - WW + 1,6 L - PW	6 L - WW + 6 L - PW
T4 = 25% (WW) + 75% (PW)	0,8 L - WW + 2,4 L - PW	3 L - WW + 9 L - PW
T5 = 100% (PW)	0 L - WW + 3,2 L - PW	0 L - WW + 12 L - PW
Water input by treatment	At 90 DAIT	At 110 DAIT
T1 = 100% (WW)	29,8 L - WW + 0 L - PW	45,8 L - WW + 0 L - PW
T2 = 75% (WW) + 25% (PW)	22,4 L - WW + 7,4 L - PW	34,4 L - WW + 11,4 L - PW
T3 = 50% (WW) + 50% (PW)	14,9 L - WW + 14,9 L - PW	22,9 L - WW + 22,9 L - PW
T4 = 25% (WW) + 75% (PW)	7,4 L - WW + 22,4 L - PW	11,4 L - WW + 34,4 L - PW
T5 = 100% (PW)	0 L - WW + 29,8 L - PW	0 L - WW + 45,8 L - PW

fertilization with purified monoammonium phosphate (MAP) (Dripsol®) in the treatments with phosphate fertilization to promote root growth. The irrigation volume varied throughout the experiment, resulting from the plants' increased water requirements, but the volume of water applied was kept constant in each treatment. The nutrient quantities in the irrigation water were calculated according to equations (2 to 6) for the different treatments, using the concentrations of phosphorus, nitrite, nitrate in mg.L⁻¹, and potassium in meq.L⁻¹, obtained from the laboratory analysis.

$$T1 = \text{Nutrient input} = Vwa * QNWI \quad (\text{Eq. 2})$$

$$T2 = \text{Nutrient input} = Vwa * ((0,75 * QNWI) + (0,25 * QNPW)) \quad (\text{Eq. 3})$$

$$T3 = \text{Nutrient input} = Vwa * ((0,50 * QNWI) + (0,50 * QNPW)) \quad (\text{Eq. 4})$$

$$T4 = \text{Nutrient input} = Vwa * ((0,25 * QNWI) + (0,75 * QNPW)) \quad (\text{Eq. 5})$$

$$T5 = \text{Nutrient input} = Vwa * QNPW \quad (\text{Eq. 6})$$

Where: T = Treatment; Vwa = Volume of water applied; QNWI = Quantity of nutrients in wastewater irrigation; QNPW = Quantity of

nutrients in potable water.

2.6 Analyzed variables

During the periods of 30, 60, 90, and 110 days after the initiation of treatments (DAIT), leaf samples were collected, dried in an oven, ground, stored in labeled plastic bags, and sent to the chemical analysis laboratory at UFPB for determination of nitrogen, total phosphorus, and potassium levels, following the methods outlined by Tedesco et al. (1995) and Miyazawa et al. (1999). Nitrogen was determined by the Kjeldahl method, phosphorus by visible spectrophotometry, and potassium by flame photometry. At 90 days, photosynthetic pigments were assessed in samples from healthy leaves, collected from the third leaf below the apex of the plants between 07:00 and 07:40. These samples were sent to the Plant Analysis Laboratory at UFCG for chlorophyll A, chlorophyll B, and carotenoid analysis.

2.7 Statistical Analysis

The variables were initially subjected to residual normality analysis using the Shapiro-Wilk test and to verification of variance homogeneity, ensuring the validity of the assumptions for applying the statistical models. After this step, the F-test was performed, and when the interaction was significant, it

was analyzed using polynomial regression (linear and quadratic) for the proportion of wastewater. For the phosphate fertilization factor, the Tukey test was applied at a 5% probability level, supported by the Sisvar software (Ferreira, 2014).

3. RESULTS

The phosphorus, potassium, nitrate, and nitrite input accumulated through irrigation with potable water and treated domestic wastewater, presented in Tables 4 to 7, corresponds to the intervals of 30, 60, 90, and 110 days after the initiation of treatments (DAIT), along with the accumulated dosage of each nutrient per treatment.

The highest accumulation of phosphorus, potassium, nitrate, and nitrite occurred in Treatment 1 (100% wastewater) and was observed at the end of the 110 days after the initiation of treatments, with maximum accumulations of 787.55 mg L⁻¹, 27.36 meq

L⁻¹, 99.05 mg L⁻¹, and 3.585 mg L⁻¹ of P, K, nitrite, and nitrate, respectively (Tables 4 to 7).

The analysis of variance for the quantities of Nitrogen (N), Phosphorus (P), and Potassium (K) in the leaves of *Moringa oleifera* plants shows significant effects of wastewater (WW) based on the F-test. At the 1% probability level, significant effects were observed for nitrogen and potassium. For phosphorus, the significant effect occurred at the 5% level. Phosphate fertilization (PF) influenced nitrogen at the 5% level and phosphorus and potassium at the 1% level. Additionally, significant interaction effects between WW and PF were observed for nitrogen and phosphorus at the 5% and 1% levels, respectively, while differences for potassium were not significant (Table 8).

The analysis of the interaction between WW (wastewater) and WF (without fertilization) in relation to the nitrogen content in the leaves of *Moringa* (Figure 1) showed a quadratic effect, with the proportion of wastewater that resulted in the highest nitrogen content in the leaves

Table 4. Phosphorus (mg L⁻¹) accumulated in irrigation at 30, 60, 90 and 110 DAIT in each treatment without phosphate fertilization

Tabela 4. Fósforo (mg L⁻¹) acumulado na irrigação aos 30, 60, 90 e 110 DAIT em cada tratamento sem adubação fosfatada

At 30 DAIT	At 60 DAIT	At 90 DAIT	At 110 DAIT
T1 – WF = 41,18	T1 – WF = 195,18	T1 – WF = 507,55	T1 – WF = 787,55
T2 – WF = 31,52	T2 – WF = 148,78	T2 – WF = 386,63	T2 – WF = 599,83
T3 – WF = 21,87	T3 – WF = 102,39	T3 – WF = 265,71	T3 – WF = 412,11
T4 – WF = 12,21	T4 – WF = 55,99	T4 – WF = 144,79	T4 – WF = 224,39
T5 – WF = 2,56	T5 – WF = 9,60	T5 – WF = 23,88	T5 – WF = 36,68

Table 5. Potassium (meq L⁻¹) accumulated in irrigation at 30, 60, 90 and 110 DAIT in each treatment without phosphate fertilization

Tabela 5. Potássio (meq L⁻¹) acumulado na irrigação aos 30, 60, 90 e 110 DAIT em cada tratamento sem adubação fosfatada

At 30 DAIT	At 60 DAIT	At 90 DAIT	At 110 DAIT
T1 – WF = 2,20	T1 – WF = 7,39	T1 – WF = 17,92	T1 – WF = 27,36
T2 – WF = 1,73	T2 – WF = 5,87	T2 – WF = 14,26	T2 – WF = 21,78
T3 – WF = 1,27	T3 – WF = 4,35	T3 – WF = 10,60	T3 – WF = 16,2
T4 – WF = 0,81	T4 – WF = 2,83	T4 – WF = 6,94	T4 – WF = 10,62
T5 – WF = 0,35	T5 – WF = 1,32	T5 – WF = 3,28	T5 – WF = 5,04

Table 6. Nitrate (mg L^{-1}) accumulated in irrigation at 30, 60, 90 and 110 DAIT in each treatment without phosphate fertilization

Tabela 6. Nitrato (mg L^{-1}) acumulado na irrigação aos 30, 60, 90 e 110 DAIT em cada tratamento sem adubação fosfatada

At 30 DAIT	At 60 DAIT	At 90 DAIT	At 110 DAIT
T1 – WF = 3,52	T1 – WF = 23,23	T1 – WF = 63,21	T1 – WF = 99,05
T2 – WF = 2,92	T2 – WF = 18,47	T2 – WF = 50,01	T2 – WF = 78,29
T3 – WF = 2,32	T3 – WF = 13,71	T3 – WF = 36,82	T3 – WF = 57,54
T4 – WF = 1,72	T4 – WF = 8,95	T4 – WF = 23,63	T4 – WF = 36,79
T5 – WF = 1,12	T5 – WF = 4,2	T5 – WF = 10,44	T5 – WF = 16,04

Table 7. Nitrite (mg L^{-1}) accumulated in irrigation at 30, 60, 90 and 110 DAIT in each treatment without phosphate fertilization

Tabela 7. Nitrito (mg L^{-1}) acumulado de nas irrigações aos 30, 60, 90 e 110 DAIT em cada tratamento sem adubação fosfatada

At 30 DAIT	At 60 DAIT	At 90 DAIT	At 110 DAIT
T1 – WF = 0,179	T1 – WF = 0,844	T1 – WF = 2,289	T1 – WF = 3,585
T2 – WF = 0,146	T2 – WF = 0,678	T2 – WF = 1,828	T2 – WF = 2,860
T3 – WF = 0,113	T3 – WF = 0,512	T3 – WF = 1,368	T3 – WF = 2,136
T4 – WF = 0,080	T4 – WF = 0,346	T4 – WF = 0,907	T4 – WF = 1,411
T5 – WF = 0,048	T5 – WF = 0,18	T5 – WF = 0,447	T5 – WF = 0,687

Table 8. Mean square of the amounts of nitrogen, phosphorus, and potassium in *Moringa oleifera* seedlings in each treatment

Tabela 8. Quadrado médio das quantidades de nitrogênio, fósforo e potássio nas mudas de *Moringa oleifera* em cada tratamento

Source of variation	DF	Mean square		
		N (mg)	P (mg)	K (mg)
Wastewater (WW)	4	93,40**	47,82*	35,89**
Linear Reg.	1	343,46**	48,92 ^{ns}	110,45**
Quadratic Reg.	1	20,82 ^{ns}	136,88**	26,01 ^{ns}
Phosphatic Fertilization (PF)	1	70,25*	3476,17**	329,24**
WW *PF	4	37,41*	200,87**	7,07 ^{ns}
Block	3	24,9	105,51	6,09
Residue	27	6,03	16,21	3,5
CV%	-	7,3	10,34	8,96
General mean	-	33,64	38,94	20,88

^{ns} Not significant at the 0.05 probability level, by the F test; *, ** Significant at the 0.05 and 0.01 probability levels, respectively, by the F test.

being 96.3%, corresponding to 37.15 g.kg⁻¹).

Figure 2 shows the phosphorus content in the leaf tissue of Moringa plants at 90 days after the application of treatments, based on the interaction between the proportion of wastewater and phosphate fertilization. The data can be found in Figure 2

In the unfolding of the interaction between WW (wastewater) and PF (phosphate fertilization), it was observed that as the proportion of wastewater increased, the phosphorus content in the leaves of the plants decreased (Figure 2). The lowest phosphorus content occurred with 100% WW, corresponding to 40.6 g.kg⁻¹. This may be associated with the phosphorus content in the

soil from the contribution of the wastewater (787.55 mg L⁻¹) and the additional input through phosphate fertilization (7.9 g.kg⁻¹).

In the interaction between WW (wastewater) and WF (without phosphate fertilization), it was observed that as the proportion of wastewater increased, the phosphorus content in the leaf tissue also increased. This effect may be linked to the phosphorus input in the soil from the wastewater, with the maximum being at 100% WW, corresponding to 34.12 mg L⁻¹.

The potassium content (g.kg⁻¹) in the leaves of Moringa gradually declined until 90 days after the application of treatments with wastewater (Figure 3).

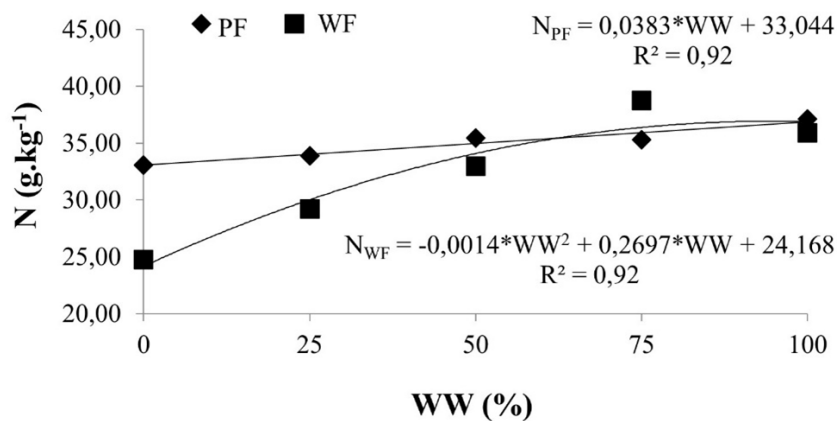


Figure 1. Nitrogen content in Moringa leaves 90 days after the start of treatments

Figura 1. Teor de nitrogênio nas folhas de Moringa aos 90 dias após o início dos tratamentos

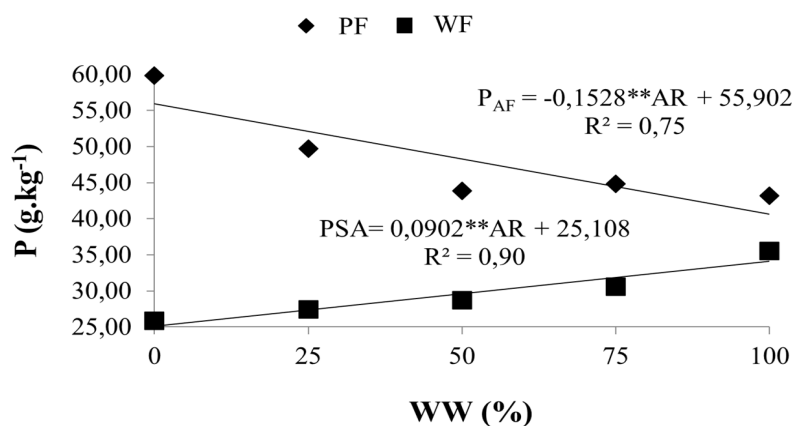


Figure 2. Phosphorus content in Moringa leaves 90 days after the start of treatments

Figura 2. Teor de fósforo nas folhas de Moringa aos 90 dias após o início dos tratamentos

As the proportion of wastewater increased, the potassium content in the leaves decreased linearly, which may be related to the amount of potassium supplied by the water through irrigation (Figure 3). When the phosphorus level increased, it inhibited the absorption of potassium by the plants. In fact, the phosphorus increment was 41.18 mg L⁻¹, 195.18 mg L⁻¹, 507.55 mg L⁻¹, and 787.55 mg L⁻¹ at 30, 60, 90, and 110 DAIT, respectively (Table 6).

The potassium content (g.kg⁻¹) in the leaves, in relation to phosphate fertilization in *Moringa* at 90 days after the application of treatments, showed a significant effect

of phosphate fertilization on the potassium content (Figure 4).

In the analysis of variance, the quantities of chlorophyll a, b, and carotenoids at 90 days after the application of treatments on *Moringa oleifera* seedlings did not show significant effects on the contents of chlorophyll a, b, carotenoids, or total chlorophyll in any of the factors studied.

4. DISCUSSION

The minerals N, P, and K are essential for plants as they promote proper development

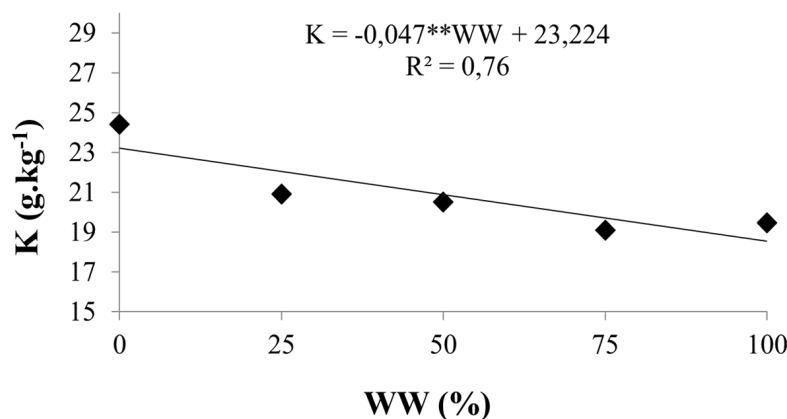


Figure 3 Potassium content in *Moringa* leaves 90 days after the start of treatments

Figura 3. Teor de potássio nas folhas de *Moringa* aos 90 dias após o início dos tratamentos

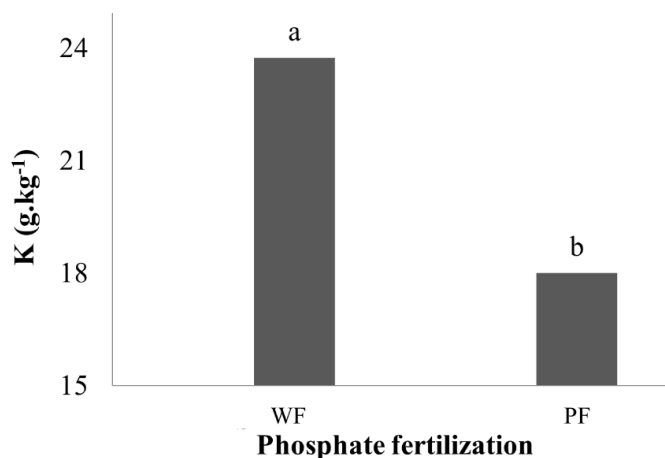


Figure 4. Potassium content in *Moringa* leaves 90 days after the start of treatments as a function of phosphate fertilization

Figura 4. Teor de potássio nas folhas de *Moringa* aos 90 dias após o início dos tratamentos em função da adubação fosfatada

Table 9. Analysis of variance of the amounts of Chlorophyll a, b, carotenoids, 90 days after application of treatments in *Moringa oleifera* seedlings

Tabela 9. Análise de variância das quantidades de Clorofila a, b, carotenoides, aos 90 dias após aplicação dos tratamentos nas mudas de *Moringa oleifera*

Source of variation	GL	Mean square	
		A	B
		(mg.gMF ⁻¹)	(mg.gMF ⁻¹)
Wastewater (WW)	4	0,0012 ^{ns}	0,0002 ^{ns}
Wastewater (WW)	1	0,0000 ^{ns}	0,0000 ^{ns}
Linear Reg.	1	0,0000 ^{ns}	0,0006 ^{ns}
Quadratic Reg.	1	0,0013 ^{ns}	0,0000 ^{ns}
Phosphatic Fertilization (PF)	4	0,0058 ^{ns}	0,0011 ^{ns}
WW *PF	3	0,0019	0
Block	27	0,0015	0,0004
CV%	-	16,13	29,53
General mean	-	0,24	0,07

Source of variation	GL	Mean square	
		CAR	TOTAL
		(mg.gMF ⁻¹)	(mg.gMF ⁻¹)
Wastewater (WW)	4	0,0003 ^{ns}	0,0020 ^{ns}
Wastewater (WW)	1	0,0000 ^{ns}	0,0000 ^{ns}
Linear Reg.	1	0,0009 ^{ns}	0,0006 ^{ns}
Quadratic Reg.	1	0,0003 ^{ns}	0,0021 ^{ns}
Phosphatic Fertilization (PF)	4	0,0004 ^{ns}	0,0104 ^{ns}
WW *PF	3	0,0005	0,0021
Block	27	0,0001	0,0025
CV%	-	21,34	15,92
General mean	-	0,06	0,3149

^{ns} Not significant at the 0.05 probability level, by the F test; *, ** Significant at the 0.05 and 0.01 probability levels, respectively, by the F test

of the crops. Phosphorus (P) plays a key role in plant metabolism as it is a macronutrient involved in energy synthesis and transfer and is also a component of nucleic acids and other coenzymes (Grant et al., 2001). Phosphorus is one of the elements that most influences plant development, and its deficiency is related to its absorption form in soil particles, which increases its demand to meet the plants' needs (Melo et al., 2014).

Potassium (K) is a macronutrient that plays vital roles in the growth and development of plants, including stomatal regulation, the transport of photosynthates, and enzymatic

activity (Figueiredo et al., 2008). It also contributes to various physiological processes and the balance in the cation-anion relationship, in addition to interacting positively with nitrogen (Ferreira et al., 2019).

Nitrogen (N) is a macronutrient provided to plants in large quantities to meet the needs of crops, as it plays a crucial role in the formation of amino acids, proteins, structural functions, and energy transfer. It is also involved in processes such as respiration, photosynthesis, and cell differentiation and multiplication (Malavolta et al., 1997). However, this element has a very complex dynamic that complicates

its management, as the responses depend on a variety of edaphoclimatic conditions, including soil quality, the source of its origin, and the management techniques used (Lins et al., 2017).

The accumulation of N, P, and K (Tables 4 to 7) highlights the importance of wastewater for irrigation in Moringa cultivation. Studies by Vieira et al. (2008) showed that Moringa accumulated nutrients, particularly N, K, and P, in various parts of the plant, with the highest proportions found in the leaves. These concentrations were higher when compared to the average found in the leaf area of many other plant species. Souza et al. (2010) found that wastewater used in the irrigation of sunflowers provided a greater nutrient input to the plants, especially nitrogen (N) from the wastewater, which had a significant effect on the seedlings' diameter and leaf count at 14 and 28 days after transplanting (DAIT).

The growth of plants is directly related to the higher availability of assimilable phosphorus, as this mineral plays a role in cell division and cell enlargement, in addition to influencing the development of meristematic tissues (Cardoso et al., 2015).

Phosphorus, primarily in the form of phosphate ions (H_2PO_4), after being absorbed by plants, is incorporated into the plant's organic compounds, and transformed into phosphorylated sugars, nucleotides, and phospholipids. It is converted into energy in plant cells as it is assimilated during ATP formation. According to Malavolta et al. (1997), when incorporated into ATP, the phosphate group is formed and can be transferred through various reactions, forming several phosphorylated compounds (Taiz & Zeiger, 2013).

It is important to emphasize that nutrients such as Nitrogen (N) and Phosphorus (P) are essential for the proper development of plants and are readily available in domestic wastewater, making them accessible for irrigation in agriculture (Medeiros et al., 2015). The use of sewage water in the irrigation of sunflower crops, for example, resulted in higher values for the number of leaves per plant, as reported by Freitas et al. (2012).

When studying the effects of domestic wastewater on the irrigation of eucalyptus seedlings and comparing them with conventional fertigation treatments using

ammonium nitrate, potassium nitrate, and monoammonium phosphate (MAP), Augusto et al. (2007) found increases in the levels of N, P, and K in the plant leaves, which were higher than those in the conventional fertigation treatment. They justified that the higher the nutrient content supplied to the plants, the better their performance would be.

In this regard, Silveira & Higashi (1998) stated that in conventional fertigation treatments, the levels of nitrogen and potassium are appropriate for the plants, whereas, conversely, wastewater would have insufficient levels of these nutrients. However, according to these authors, the phosphorus content may be excessively high in the conventional treatment, whereas in wastewater, the phosphorus content is more appropriate.

The interaction between the factors WW x PF showed a linear effect, where, as the proportion of wastewater combined with phosphorus fertilization increased, the nitrogen content in the leaves also increased, reaching a maximum value of 36.87 g.kg^{-1} at 100% WW. This value was higher than those found by Moyo et al. (2011) and Teixeira (2012), which were 30.3 and 28.65 g.kg^{-1} , respectively, in dried and ground Moringa leaves.

Nitrogen (N) can be absorbed by plants in the form of NH_4^+ ions or as NO_3^- , which is predominantly available in the soil. Upon absorption, the plant incorporates this nutrient into amino acids, which in turn promotes protein synthesis, resulting in increased leaf growth and a subsequent increase in the photosynthetic area (Dechen & Nachtigall, 2007).

Almeida (2018) highlights the nutritional benefits of *Moringa oleifera*, concluding that the leaves of this plant are rich in nutrients, with high protein content and a significant concentration of vitamins and minerals. In plants, total nitrogen (TN) exists in three forms: true protein, which makes up 60% to 80% of TN, followed by non-protein nitrogen and lignified nitrogen in smaller amounts (Souza, Nogueira, & Batista, 2006). Regarding the effects of nutrient omission in Moringa plants during the early growth stages, Vieira et al. (2008) found that the omission of N, P, and K in nutrient composition affected plant development, reducing the growth of the aerial part and increasing root growth, with nitrogen omission having the most significant

impact on the root-to-shoot ratio.

Santos et al. (2008) reported increases in dry matter production in the aerial part of forest species when the phosphorus level was increased, demonstrating that phosphorus typically boosts dry matter production in the aerial part of plants, thereby raising the relative growth rate. These findings align with the results obtained in this study. Similar results were found by Freitas et al. (2017) when evaluating the growth of *Cassia grandis* L. f. seedlings with phosphorus fertilization and liming, highlighting the positive influence of phosphorus fertilization on the growth and quality of plants in the studied conditions across all evaluated variables. According to Teixeira et al. (2018), increasing phosphorus fertilization contributes to an increase in plant dry matter production, emphasizing the essential role of phosphorus in promoting greater plant development, especially since this mineral is often deficient in most soils in Brazil.

Phosphorus is an essential supplement and one of the most important nutrients for plant growth, especially in the early stages (Bezerra et al., 2014). In the studies conducted by Almeida (2018) to evaluate the nutritional value of *Moringa*, a high mineral content was found, including phosphorus with 204 mg per 100 g of dried leaf powder from this plant. His results also suggest a potential salt stress reaction in the plants due to the excess salts caused by the wastewater, where the higher the proportion of wastewater in the irrigation, the higher the concentration of salts in the soil solution.

According to Garcia et al. (2007), the significant effects of high soil salinity on the potassium levels in the above-ground parts of corn plants, 120 days after planting, linearly reduced potassium values as salinity levels in the soil solution increased. Cruz et al. (2006), when evaluating the concentration of macronutrients in yellow passion fruit plants, concluded that potassium was the most suppressed nutrient when the plant was subjected to salt stress.

Potassium (K) can be found in large quantities in the Earth's crust, being the seventh most abundant element, mainly in soils with primary minerals and in weathered soils in exchangeable, non-exchangeable, and water-soluble forms. To supply plants with this nutrient, the exchangeable K solution

in the soil (K^+ ion) is the form in which plants absorb it most quickly (Dechen & Nachtigall, 2007). Among all macronutrients, K is the second most required by most crops (Nachtigall & Raij, 2005). However, according to Albuquerque et al. (2011), excessive irrigation and high concentrations of K in the soil solution can impair plant development due to nutrient leaching and resulting nutrient imbalances.

When phosphate fertilization was used (Figure 4), the potassium content was lower (18.01 g.kg^{-1}) compared to the treatment without phosphate fertilization (23.75 g.kg^{-1}). This fact may be associated with the interaction between fertilization and potassium absorption by the plants. Torquato et al. (2011), studying different phosphorus doses in cowpea culture, found a significant reduction in K levels in the roots and leaves of this crop due to varying phosphorus doses. When other fertilizers are added to the soil, potassium leaching increases because it is displaced from negative charges by the cations added with the fertilizers (Novais et al., 2007). Tisdale et al. (1985) also states that the absorption of a specific mineral by the plant is directly influenced by the presence of other cations in the soil solution, meaning that one absorbed ion tends to affect the release of another ion and is also related to other cations on the exchange sites.

An important factor to consider in the pigment levels is that the nutrient supply in this study and the absorption of these nutrients by the plants, especially nitrogen, phosphorus, and potassium (Figures 1 to 4), may have contributed to maintaining the balance of chlorophyll a, b, carotenoids, and total chlorophyll in *Moringa* leaves at 90 DAIT. Figure 1 shows how much nitrogen the plants absorbed. According to Correia et al. (2005), nitrogen directly influences pigment levels in plants. A plant deficient in nitrogen has impaired growth, and its leaves become yellow due to the loss of chlorophyll, reflecting deficiencies in growth and productivity (Santos et al., 2004).

A well-fertilized soil effectively contributes to the growth and development of most agricultural crops (Freiberger et al., 2013). On the other hand, the deficiency of nutrients such as phosphorus causes starch to accumulate in the chloroplasts, reducing the translocation of carbohydrates and impairing enzyme activity that is dependent on phosphorylation,

especially those directly involved in nutrient absorption (Marschner, 1995).

Potassium is another essential nutrient for the balance of chlorophyll components, contributing to the maintenance of leaf turgidity, the translocation of sugars within plants, and the chemical and electrolytic balance of plants (Freiberger et al., 2013). Therefore, a plant deficient in potassium experiences reduced growth, which in turn lowers its photosynthetic capacity (Ferreira et al., 2004).

5. CONCLUSION

Chlorophyll A, Chlorophyll B, and Carotenoids in *Moringa oleifera* leaves, 90 days after the start of the experiment, did not show significant effects in the treatments with wastewater (WW) irrigation, regardless of the proportion used.

However, the phosphatic fertilization (PF) influenced the increase in N and P levels in the aerial part of *Moringa*. Moreover, wastewater (WW) influenced the increase in N, with the maximum concentration at 75% WW, and also contributed to the increase in P and K in the 100% wastewater treatment.

This research found that treated domestic wastewater (WW) used in agricultural irrigation is feasible and, to some extent, advantageous for *Moringa* cultivation. However, its use requires caution due to potential interactions with other components and proportions in the soil solution.

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AUTHOR CONTRIBUTIONS

MELO, A. R.: Conceptualization; Data curation; Data analysis; Writing of the original manuscript; Writing – review and editing; SILVA, P. F.: Supervision; Methodology; Data and experiment validation; Data analysis; Writing – review and editing; DANTAS NETO, J.: Project administration; Methodology;

Supervision; Data and experiment validation; Writing – review and editing; SILVA, F. A.: Conceptualization; Data analysis; Writing – review and editing; COSTA JUNIOR, D. D.: Conceptualization; Writing – review and editing; PÉREZ-MARIN, A. M.: Project administration; Methodology; Supervision; Data and experiment validation; Writing – review and editing.

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