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## Growth, relative chlorophyll content and concentration of inorganic solutes in sunflowers plants supplemented with marine macroalgae organic residue<sup>1</sup>

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### ABSTRACT

One effect of the tidal dynamics is the accumulation during of large volumes of macroalgae the low tide, named “arribadas algae”. The objective of this work was to analyze the effects of using arribada alga eorganic residues (AAOR) on the initial growth of sunflower plants, where the following were evaluated: plant growth, relative chlorophyll content and inorganic solids content (Na, K, Cl and NO<sub>3</sub>). The algae were collected on Praia do Pacheco in Caucaia, state of Ceará, Brazil, washed in running water and placed to dry in the sun for seven days, then dried in a forced circulation oven at 80 °C and crushed. The sunflower seeds were sown in 5 L plastic buckets, containing: 1) 100% (in volume) of sand; 2) sand + 100% of nitrogen recommendation - NR in commercial fertilizer (80 kg of N.ha<sup>-1</sup>); 3) sand + 50% of the NR in macroalgae; 4) sand + 100% of NR in macroalgae; and 5) sand + 150% of the NR in macroalgae. The use of AAOR improved the growth of the analyzed variables in relation to the plants grown in substrate with sand or sand + fertilizer. The best results were observed at 50% of the NR in AAOR. The addition of increasing concentrations of AAOR allowed a greater accumulation of beneficial ions such as K and NO<sub>3</sub><sup>-</sup> while no significant differences were observed for toxic ions such as Na and Cl in relation to plants supplemented with fertilizer or sand. The use of AAOR in substrates can be an alternative to fertilizers in plant cultivation since it reduces production costs besides being a sustainable destination.

**Keywords:** arribadas algae; *Helianthus annuus* L; organic residue.

### RESUMO

#### Crescimento, teores relativos de clorofila e concentrações de solutos inorgânicos em girassóis suplementados com resíduo orgânico de macroalgas marinhas

Um dos efeitos da dinâmica das marés é o acúmulo de grandes volumes de macroalgas durante a maré baixa, denominadas de “algas arribadas”. O trabalho objetivou analisar os efeitos da utilização de resíduos orgânicos de algas arribadas (ROAA) sobre o crescimento inicial de plantas de girassol avaliando-se: o crescimento das plantas, os teores relativos de clorofila e teores de solutos inorgânicos (Na, K, Cl e NO<sub>3</sub><sup>-</sup>). As algas foram coletadas na Praia do Pacheco em Caucaia-CE, Brasil, lavadas em água corrente e colocadas para secar a sol pleno durante sete dias, em seguida, secas em estufa de circulação forçada a 80 °C e trituradas. As sementes de girassol foram semeadas em baldes de plástico com capacidade para cinco litros, contendo: 1) 100% (em volume) de areia; 2) areia + 100% da recomendação de nitrogênio - RN em fertilizante comercial (80 kg de N.ha<sup>-1</sup>); 3) areia + 50% da RN em macroalgas; 4) areia + 100% da RN em macroalgas; e 5) areia + 150% da RN em macroalgas. A utilização de ROAA ocasionou melhoria nas variáveis de crescimento analisadas em relação às plantas crescendo em substrato com areia ou areia + fertilizante. Os melhores resultados foram

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observados a 50% da RN em ROAA. A adição de concentrações crescentes de ROAA possibilitou maior acúmulo de íons benéficos como K e  $\text{NO}_3^-$  enquanto que não foram observadas diferenças significativas para íons tóxicos como Na e Cl em relação às plantas suplementadas com fertilizante ou areia. A utilização de ROAA nos substratos pode ser uma alternativa aos fertilizantes no cultivo de plantas, reduzindo custos de produção, além de ser uma destinação sustentável.

**Palavras-chave:** algas arribadas; *Helianthus annuus* L; resíduo orgânico.

## INTRODUCTION

The production of seedlings is an important stage of the productive system; however, it is highly dependent on inputs. The use of renewable materials as a source of nutrients, besides being an interesting solution for waste disposal, is also an alternative for reducing the high input costs required for agricultural production (Trazzi *et al.*, 2013).

The use of waste is an environmental management practice foreseen in the Brazilian National Solid Waste Policy (PNRS), Law no. 12.305/2010. The environmental aspect is also added, since the use of organic residues also allows the reduction in the production costs.

Brazil is a country rich in marine macroalgae diversity. It has approximately 800 taxa of identified marine macroalgae. The use of this resource represents a great potential for farming (Machado *et al.*, 2011).

One effect of tidal dynamics and the action of sea currents and winds is the accumulation of large volumes of algae in the scorching regions during low tide. These algae are referred to as “arribadas algae”. The removal of those arribadas algae is common for a later disposal by urban cleaning companies (Nova *et al.*, 2014). However, it is generally known that this removal does not occur in any sand strip of the coast of Ceará state, Brazil. Thus, due to the economic, ecological importance and richness of nutrients, the objective of this work is to analyze the use of local macroalgae species for the fertilization of sunflower plants.

In order to verify the viability of the use of arribadas algae harvested for the production of substrates for plants, sunflower (*Helianthus annuus* L.) was chosen as the plant material. The crop shows desirable characteristics from an agronomic point of view, such as a short life cycle, high productivity, quality and oil yield, thus being a constituent of grain and biodiesel production systems (Araújo *et al.*, 2012).

Sunflower is widely used in human diet because it presents an excellent quality oil, high stability to oxidation and nutritional and functional characteristics of the human diet. Regarding animal feeding, it is used in the form of bran, as silage and in diets for birds with their achenes. It

is an important source of proteins for animal diets, besides the integrated participation in the production of honey, since it presents attractive potential of the flower for the bees (Freire, 2016). Among the various forms of use, the generation of energy is highlighted, as it is used as a raw material in the production of biodiesel.

The hybrid cultivar BRS 323 presents productivity and precocity, which makes it suitable for the different productive systems of the major agricultural regions in Brazil. The oil content is between 40% and 44%. It is recommended for the Brazilian states: Alagoas, Bahia, Ceará, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rondônia, Santa Catarina, São Paulo, Sergipe and the Distrito Federal (Embrapa, 2014).

## MATERIAL AND METHODS

This work was carried out in a greenhouse located at Instituto Federal de Educação Ciência e Tecnologia (IFCE), in Maracanaú *campus*, Ceará, Brazil, from June to August in 2017.

Algae were collected in Praia do Pacheco, Caucaia, Ceará, on April 15, 2017 from 13:00 h to 15:00 h at a 0.4-m tide, according to the tide table of Porto do Pecém-CE and waning gibbous moon. Samples of algae that were over the strip of sand completely uncovered by the sea during the low tide were manually collected. The samples were taken still fresh preserved at 4 °C in a thermal box and sent to the laboratory of Macroalgae located at the Instituto de Ciências do Mar – Labomar at the Universidade Federal do Ceará for identification.

Three species of red algae were found: *Glacilaria ornata*, *Glacilaria cervicornis*, *Glacilaria cearensis*, which represented the largest proportion of the sample (approximately 70%). The following were detected in smaller proportions: *Gelidiopsis gracilis*, *Botryocladia occidentalis*, *Caulerpa prolifera*, *Ulva fasciata*, *Hypnea pseudomusciformis*, *Gelidium coarctatum*.

After, masses of different species of algae were collected jointly to prepare the substrates for the cultivation of sunflower plants. Following removal, the algae were washed in running water and arranged to be

dried in the sun for about 7 days. Subsequently, the material was sent to Laboratório de Bioquímica e Fisiologia Vegetal (Laboratory of Biochemistry and Plant Physiology) at IFCE, Maracanaú *campus* for completion of the drying process in an oven with forced air circulation at 80 °C until reaching constant mass. After total drying, the material was crushed using mortar and pestle and called arribadasalgae organic residues (AAOR). Following this procedure, a sample of the material was sent for analysis in Laboratório de Solos/Água (Soil/Water Laboratory) at the Universidade Federal do Ceará (Table 1), where the total nitrogen concentration (N) was determined at 39.8 g.kg<sup>-1</sup> used in calculations for the AAOR amounts added to the substrates. For the mixed organic fertilizer treatment, data of the analyzes which were carried out were provided by the manufacturer. The fertilizer was a mixture of cattle manure, sugarcane bagasse, wood sawdust, chicken manure and agricultural gypsum.

The sunflower seeds (cultivar BRS 323 granted by Embrapa Produtos e Mercados) were sown in 5 L plastic buckets. The experiment was set up in a completely randomized design, with five treatments: 1) 100% (in volume) of sand; 2) Sand + 100% nitrogen recommendation (NR) in mixed commercial organic fertilizer (following recommendation of the 80 kg of N.ha<sup>-1</sup>); 3) Sand + 50% of NR in macroalgae; 4) Sand + 100% of NR in macroalgae; and 5) Sand + 150% of NR in macroalgae.

The sand used in all treatments was obtained in the river, with an average grain size (grains between 0.50 and 0.250 mm), and prior to the utilization for the preparation of substrates, washing with distilled water was performed to leach the likely presence of salts. Each treatment consisted of 12 replicates, each consisting of a pot with two plants. The treatments were irrigated at 80% field capacity. Data of each collection were submitted to analysis of variance (ANOVA) and the means compared by the test of Tukey ( $p < 0.05$ ) using Sigma Plot 11.0 software.

Two measurements of the relative contents of chlorophyll and number of leaves at 15 and 22 days after sowing (DAS) and two evaluations for height, stem diameters, leaf area and dry mass at 24 and 27 DAS were performed. The dry mass was determined after oven drying at 60 °C until reaching constant mass. The shoot

constituted by leaves + petioles + stems and the roots and total (shoot + root) were measured as well.

For determination of concentrations of inorganic solutes, dried leaf extracts obtained from the maceration in mortar of 1g of dry mass to 4.0 mL of deionized water were prepared. The macerate was then filtered on fine mesh nylon fabric and centrifuged at 12,000 x for 15 min. The supernatant was used as extract in the analyzes.

Chlorophyll relative contents were measured on the first fully expanded leaf counted from the apex using the Chlorophyll Meter SPAD-502 gear and data expressed as arbitrary units of the equipment (SPAD Index). The stem diameters were measured using a pachymeter, the number of leaves were manually counted, and the height of the aerial part was measured using a ruler, from the base of the stem to the insertion of the last fully expanded leaf.

## RESULTS AND DISCUSSION

At 15 and 22 days after sowing (DAS), chlorophyll relative contents (Figure 1A) presented the greatest values for the plants in the treatment supplemented with organic algae residue (AAOR) at 150% of the nitrogen recommendation (NR). Next, the treatments at 100 and 50% of the NR in AAOR stood out, not statistically differing from each other. The lowest values were observed in the commercial fertilizer treatment and sand. At 15 DAS, the 150% NR in AAOR was 18% greater than the groups at 50 and 100% of the NR in AAOR, and 80% greater than the commercial fertilizer and sand. At 22 DAS, the 150% of the NR in AAOR group was 23% greater than the groups of 50 and 100 of the NR in AAOR and 69% greater than the commercial fertilizer and sand groups.

For the height (Figure 1B) at 24 DAS, the 50% NR in AAOR treatment displayed an average value of 22.8cm per plant, while the groups of 100 and 150% of the NR in AAOR showed average values of 18.5 and 15.8 cm respectively. Thus, the 50% of the NR in AAOR treatment presented values 23% greater than the treatment of 100% of the NR in AAOR and 44% greater than that of 150% of the NR in AAOR. The lowest results were found in the commercial fertilizer and sand groups, 11.5 and 13 cm respectively, not statistically different from each other. At 27 DAS, treatment of 50% of the NR in AAOR reached an

**Table 1:** Chemical analysis of the arribada algae organic residue (AAOR) and the mixed organic fertilizer used in the substrate composition for sunflower plant cultivation

Treatments	Chemical characterization								
	g. kg <sup>-1</sup>					mg. kg <sup>-1</sup>			
	N total	P	K	Ca	Mg	Fe	Cu	Zn	Mn
AAOR	39.8	1.29	13.8	18.86	7.71	1,022.8	8.1	41.1	652.8
Commercial Fertilizer	2.2	8.43	8.10	1.52	1.85	3,445.5	1.80	89.3	380.1

average height of 26.7 cm, 16% greater than treatments 100 and 150%, which reached averages of 24.8 and 21.3 cm, and did not differ statistically from each other, and 90% greater than the commercial fertilizer and sand treatments, which reached averages of 13.8 and 14.2 cm.

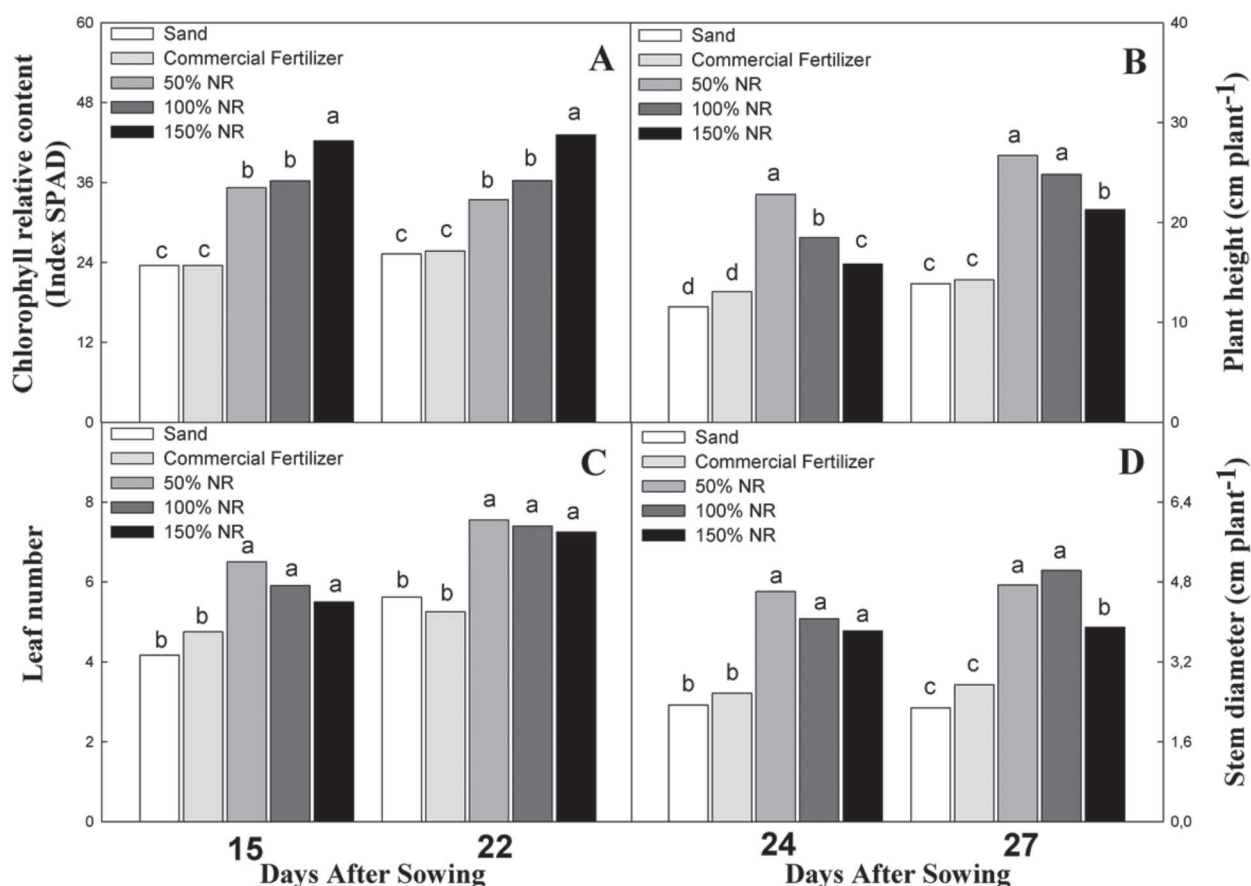
The number of leaves (Figure 1C) followed the same pattern for the two collections, where the three AAOR supplemented groups did not differ from each other and presented greater results than the commercial fertilizer and sand groups, which did not differ from each other. At 15 DAS, the AAOR supplemented treatments presented six leaves per plant, on average, 35% greater than the others, which presented 4.3 leaves per plant, on average. At 22 DAS, AAOR supplemented treatments reached an average of 7.5 leaves per plant, being 36% greater than the others, which presented an average of 5.4 leaves per plant.

In relation to stem diameter (Figure 1D), at 24 DAS, the AAOR supplemented treatments did not differ among themselves and presented an average of 4.2 cm per plant, resulting in values 70% greater than the commercial fertilizer and sand treatments, which did not differ between themselves and presented an average of 2.4 cm per plant.

At 27 DAS, the 50 and 100% NR groups, which did not statistically differ among each other and presented an average of 5 cm per plant, 25% greater than the treatment at 150% of the NR, which presented an average of 4 cm per plant. The treatment at 50% of NR was even 95% greater than commercial fertilizer and sand, which presented an average of 2.6 cm per plant.

Overall, the arribadas algae organic compound (AAOR) presents higher nutrient concentrations than the commercial fertilizer used in the experiment (Table 1). It is believed that this greater nutritional availability may have contributed to the increase of the biometric variables observed in Figure 1, where all AAOR supplemented groups presented greater results than the sand and commercial fertilizer groups. In addition, it is possible that AAOR presents greater availability of nutrients when compared to the mixed organic fertilizer, since it is crushed and easily absorbed.

It was observed that the accumulation of chlorophyll relative contents (Figure 1A) was proportional to the increase in AAOR concentrations. It is believed that this behavior is due to the greater concentration of Mg found



**Figure 1:** Chlorophyll relative contents (A), plant height (B), leaf number (C) and stem diameter (D) of sunflower plants in substrates containing: sand, commercial fertilizer or Arribada Algae Organic Residue at 50, 100 and 150% nitrogen recommendation -NR. Bars followed by different letters are the statistical differences among treatments in each evaluation period according to the test of Tukey ( $p < 0.05$ ).



in the organic compound in comparison to the commercial fertilizer (Table 1) because Mg is a forming element of the chlorophyll molecule, being essential for photosynthesis (Grangeiro *et al.*, 2011).

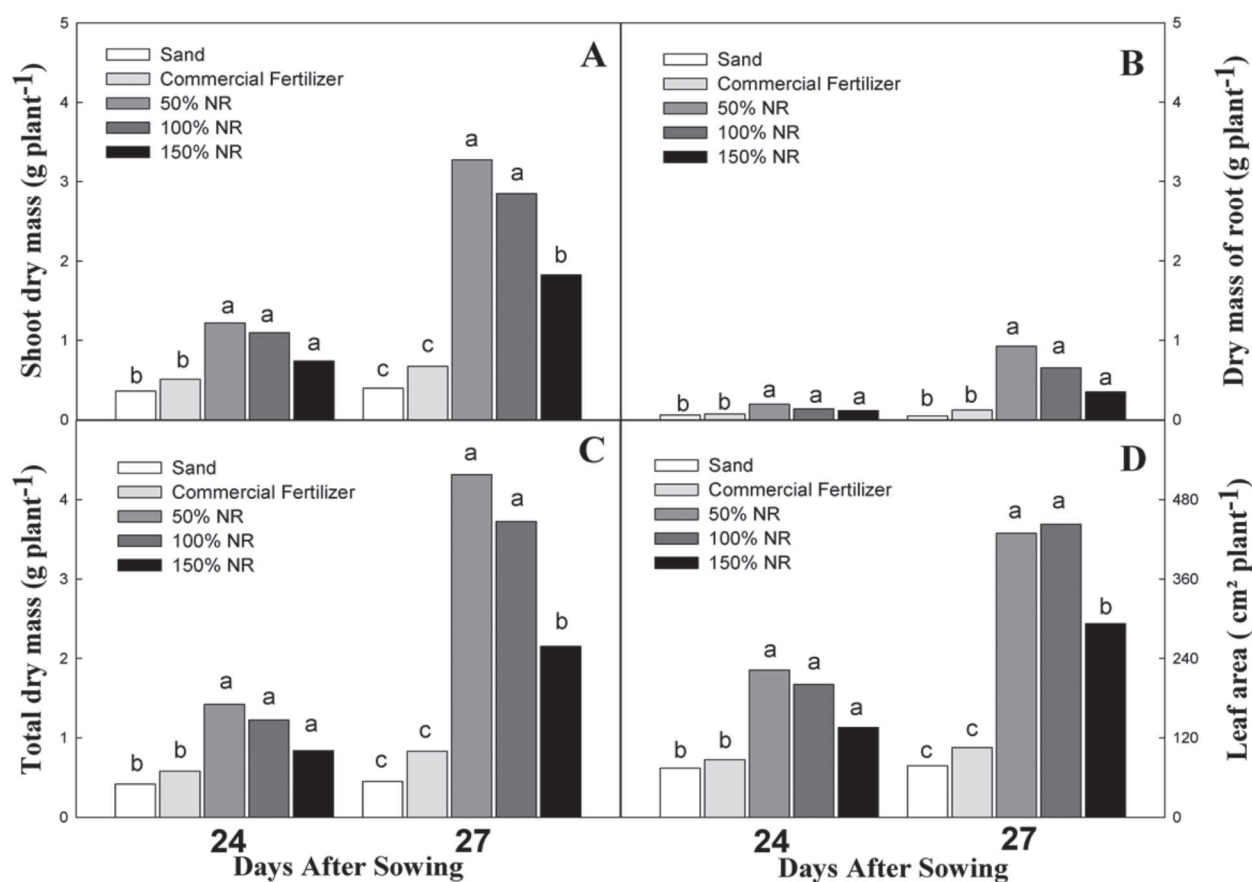
Regarding height (Figure 1B) and diameter (Figure 1D), the greatest results were found for the 50 and 100% NR in AAOR group, while, although it had received larger amounts of nutrients, the 150% NR in AAOR group presented slightly lower values. It is believed that this has occurred because of the high concentration of Fe present in AAOR. Although Fe is an essential micronutrient for the normal growth and development of plants, its excess may cause nutritional dysfunctions and affect plant physiology (Adamski *et al.*, 2011). Despite being a key micronutrient for the development of plant species, in some situations, Fe is able of being absorbed and accumulated in toxic concentrations (Sahrawat, 2005). Laurett *et al.* (2017) observed that Fe concentrations in nutrient solution over  $45 \mu\text{mol.L}^{-1}$  reduced the development of lettuce *Vitoria de Santo Antão* and *Rococó rucola*. Thus, its greater concentration in the 150% NR in AAOR group may have limited the development in height (Figure 1B) and diameter (Figure 1D) when compared to the 50% and 100% NR in AAOR groups.

At 24 DAS, AAOR supplemented treatments presented average values of  $1 \text{ g plant}^{-1}$  of shoot dry mass (Figure 2A), a values that is 134% greater than sand and commercial fertilizer treatment, which presented average values of  $0.43 \text{ g plant}^{-1}$ . At 27 DAS, treatments at 50 and 100% of the NR did not differ between each other and presented  $3 \text{ g plant}^{-1}$ , on average, 68% higher than the treatment at 150% and 472% in relation to the commercial fertilizer and sand treatments, which presented average values of 0.4 and  $0.7 \text{ g plant}^{-1}$ , respectively.

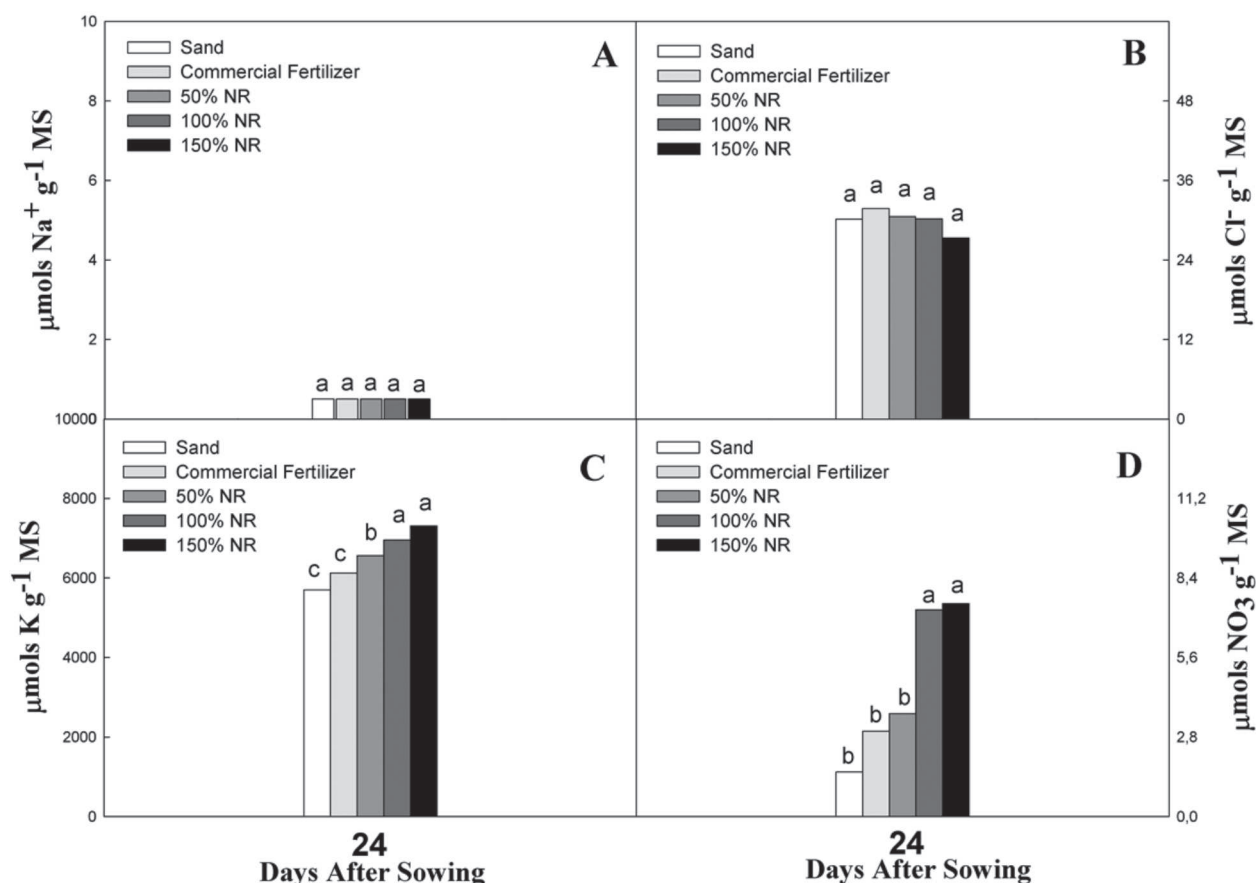
For root dry mass (Figure 2B), the greatest values were observed in the treatments containing AAOR that did not differ among each other. At 27 DAS, the average of these values was  $0.65 \text{ g plant}^{-1}$ . The superiority was 675% in relation to commercial fertilizer and sand treatments, which presented an average of  $0.08 \text{ g plant}^{-1}$ .

Total dry mass (Figure 2C) presented a behavior similar to that of the aerial part dry mass. It stood out at 27 DAS, the values for the 50% NR in AAOR that were 16, 100, 419 and 867% greater than the values for 100, 150% of the NR and commercial fertilizer and sand treatments, respectively.

Other authors observed similar results. Rayorath *et al.* (2008), in a study with *Arabidopsis thaliana*, verified dry mass of the highest aerial parts in plant species under the



**Figure 2:** Shoot dry mass (A), dry mass of root (B), total dry mass (C) leaf area (D) of sunflower plants in substrates containing: sand, commercial fertilizer or Arribada Algae Organic Residue at 50, 100 and 150% nitrogen recommendation -NR. Bars followed by different letters are the statistical differences among treatments in each evaluation period according to the test of Tukey ( $p < 0.05$ ).



**Figure 3:** Sodium content (A), chloride (B), potassium (C) and nitrate (D) in sunflower leaves of plants in substrates containing: sand, commercial fertilizer or Arribada Algae Organic Residue at 50, 100 and 150% nitrogen recommendation -NR. Bars followed by different letters are the statistical differences among treatments in each evaluation period according to the test of Tukey ( $p \leq 0.05$ ).

effects of marine algae compounds as well as Kumar & Sahoo (2011) in wheat plants and Matysiak *et al.* (2011) in maize.

Regarding leaf area (Figure 2D), the behavior was similar to that observed for dry mass. At 27 DAS, 50 and 100% of NR in AAOR treatments did not differ from each other and were superior to 150% of NR, commercial fertilizer and sand treatments by 49, 315 and 462%.

By the methodology used, the analysis of the Na contents did not find significant concentrations in any of the groups evaluated (Figure 3A). In relation to Cl (Figure 3B), no significant differences were found in the treatments used in the experiment.

In relation to K (Figure 3C), the 100 and 150% treatments stood out, not differing from each other, being 8% greater than the 50% NR and 20% higher than the commercial fertilizer and sand treatment.

This result is considered positive, taking into account that K is an element that acts directly in plant nutrition, and performs several functions in the plant, such as: control of cellular turgidity, activation of enzymes involved in the respiration process and photosynthesis, regulation of the opening and closing of stomata, and transport of carbohydrate (Kano *et al.*, 2010).

Yet, for nitrate ( $\text{NO}_3^-$ ) (Figure 3D), 100 and 150% NR in AAOR treatments were not statistically different among each other and were 172% greater than the average of the other treatments. This fact demonstrates a direct correspondence between the AAOR supplementation and leaf concentrations of  $\text{NO}_3^-$ .

This relationship may be regarded as a positive factor, considering that  $\text{NO}_3^-$  is an ion that benefits plant species. The  $\text{NO}_3^-$  is related to metabolic regulation, absorption and assimilation of N, which is essential in the production of proteins and nucleic acids, and to the mechanisms of regulation of nitrogen metabolism in the plant (Bredemeier & Mundstock, 2000).

## CONCLUSION

The use of arribadas algae organic residue (AAOR) resulted in an enhancement in the growth variables in relation to the plants growing in substrate with sand or sand + mixed organic fertilizer.

The use of AAOR in substrates can be an alternative to fertilizers in plant cultivation since it reduces production costs besides being a sustainable destination.

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