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Biomass Production and Antioxidative Enzyme Activities of Sunflower Plants Growing in Substrates Containing Sediment from a Tropical Reservoir

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Abstract

Many Brazilian reservoirs are intensely submitted to the silting process, particularly the small and medium size ones. The study aimed to examine the feasibility of using silt sediment to grow sunflower plants under conditions of water stress, by evaluating its effects on the relative chlorophyll contents, dry matter and antioxidative enzyme system. The study was conducted under greenhouse conditions at the Instituto Federal do Ceará Campus Maracanaú, Brazil. The sunflower seeds were sown in buckets containing 1) sand; 2) sand + manure/mixed organic fertilizer; 3) sand + 91.8 g of sediment, and 4) sand + 183.6 g of sediment. The sediment was collected from the Tijuquinha reservoir, Northeast of Brazil. The plants were watered daily to 70% field capacity. At 16 days after sowing, irrigation to half of each group of seedlings was suspended. The experimental design was completely randomized in a 2 × 4 factorial with five replicates. The data of each harvest time were analysed by analysis of variance and the means were compared by Tukey's test ($P \leq 0.05$). The addition of silt sediment improved the variables (relative chlorophyll content, and shoot and total dry matters) compared to plants grown in substrate containing sand and sand + compost/mixed organic fertilizer, respectively. In general, a greater increase in the variables was observed with the 200% nitrogen recommendation treatment than the other treatments studied. It is possible that the silt sediment from reservoirs can be an alternative to chemical fertilizers for plant cultivation, reducing production costs, providing improvements in the quality of potable water and restoring the storage capacity of surface reservoirs lost by siltation.

Keywords: *Helianthus annuus* L., silting, drought stress, antioxidative enzymes

1. Introduction

Dams are the main water storage forms in the Brazilian semiarid region. These reservoirs complement water abstraction for population supply in addition to meeting the needs of agricultural activities in an area dominated by intermittent rivers (Araújo & Medeiros, 2013). Over 90% of the water in the state of Ceará is provided by surface reservoirs (Araújo et al., 2006).

However, the water quality in these reservoirs is negatively affected by siltation, which compromises its use for human consumption and can lead to eutrophication. One of the main causes of siltation is the leaching of soil nutrients by rainfall that is then retained in the bottom of the reservoirs. These sediments are derived from erosion of exposed soil, due to the removal of vegetation or to inadequate soil management. Carvalho et al. (2000) found that the sediments serve as catalysts, carriers and pollutant fixing agents. According to Cabral et al. (2005), many Brazilian reservoirs are intensely submitted to the silting process, particularly the small and medium size ones.

Siltation can cause a change in the reservoir's geometry, making the water sources shallower and, thereby, more susceptible to water losses by evaporative. The water retention capacity of the reservoir is also reduced (Araújo et al., 2006). In the state of Ceará, siltation generates an average 2% reduction in the capacity of the reservoir every 10 years (Araújo & Medeiros, 2013). Thus, preventive and corrective actions are essential to maintaining the quality and supply of water in these water bodies. Irregular rainfall and inadequate water quality are among the main factors affecting agricultural production in the Northeastern region of Brazilbecause, among the resources used by plants, water is considered the most critical.

The semiarid region of Ceará contains soils with low organic matter and nutrients, there by, reducingcrop yields (Feitosa et al., 2013). Conversely, dam sediment contains a high concentration of nutrients, such as nitrogen, phosphorus and organic matter, which allow the use of this type of waste in Brazilian agriculture (Feitosa et al., 2013). The application of this sediment may improve the chemical and physical characteristics of the soil, thus, improving the quality and crop yields and reducing production costs (Abreu-Júnior et al., 2005).

The sunflower (*Helianthus annus* L.) is a plant of the Asteraceae family, which originates from North America and has been cultivated in large areas throughout the world. According to Guerra and Picksius (2005), sunflower oil is among the potential sources of biofuel production in Brazil.

The currentstudy aimed to assessthe viability of usingsilt sediment from the Tijuquinha dam in Ceará, Brazil, to grow sunflower plants under conditions of water restriction, by evaluating its effects on the relative chlorophyll contents, dry mass production, antioxidative enzyme system and lipid peroxidation in the plants.

2. Materials and Methods

The experiment was conducted from August to December 2015 under greenhouse conditions at the Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE) in Maracanaú, Ceará, Brazil (03º52′S; 38º37′W). The mean values of temperature and relative air humidity in the greenhouse were 33.3 ºC and 54%, respectively.

The selected sunflower seeds (*Helianthus annus* L.) were disinfected with sodium hypochlorite solution (0.7%). The sowing occurred in plastic buckets (5 L) containing: 1) fine-granulometry sand; 2) fine-granulometry sand + manure/mixed organic fertilizer (11.8% N); 3) fine-granulometry sand + 91.8 g of dam sediment; 4) fine-granulometry sand + 183.6 g of dam sediment.

The sediment was collected in October 2015 from the Tijuquinha dam, in the city of Baturité, Ceará. After collection, the sediment was placed in an oven at 60 °C to complete drying. The sediment was then macerated to obtenir homogeneous mixture and finally added to the treatments in the proportions described above.

The amount of mixed organic fertilizer obtained commercially, and sediment (91.8 g) were calculated according to the recommendation for planting, which is 80 kg nitrogen per hectare, in addition to the compounds nitrogen concentrations. However, to verify a possible increase in plant growth, this concentration was doubled in the plants treated with 183.6 g of dam sediment. Table 1 presents the results of the chemical analysis of the sediments.

Table 1. Chemical analysis of the Tijuquinha dam sediment used in the composition of the substrates for growing *Helianthus annuus* L. plants

N	P	C	OM	Ca^{2+}	Mg	Al^{3+}	Na^{+}	K^{+}	S	$H^{+} + Al^{3+}$
---------- g kg^{-1} ----------										
1,634	0,523	24,96	43,03	4,9	5,2	0,65	0,34	0,41	10,8	7,1

Note. N: Total Nitrogen; P: Phosphorus; C: carbon; OM: organic matter; Ca^{2+}: calcium; Mg: magnesium; Al^{3+}: aluminum; Na^{+}: sodium; K^{+}: potassium; S: sum of bases.

Source: Laboratório de Solo/Água da UFC/FUNCEME, 2013.

The plants were irrigated daily to 70% of field capacity. At 16 days after sowing (DAS), the irrigation to half of each group of seedlings described above, was suspended.

Two evaluations were performed, at 21 (5 days under water restriction) and 23 DAS (7 days under water restriction), respectively. The chlorophyll relative contents were measured in the first fully expanded leaf using an SPAD-502 chlorophyll meter. Later, a group of plants was harvested and the material was dried in a forced-air oven at 60 ºC to complete drying for the determination of shoot (leaves + petiole + stems) (SDM), root (RDM) and total (shoot + roots) (TDM) dry matter, respectively.

For biochemical analyses, fresh leaves and roots were frozen in liquid nitrogen and stored frozen (-25 °C). Later, extracts of plant materials were prepared for the determination of antioxidative enzyme activities. Leaf tissue (1 g) was homogenized in a mortar and pestle with 4 ml of ice-cold extraction buffer (100 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA). For ascorbate peroxidase (APX) estimation, 2 mM ascorbic acid was added to the extraction buffer.

The activities of catalase (CAT), guaiacol peroxidase (GPX), APX and superoxide dismutase (SOD) were determined. CAT activity was determined according to Haver and McHale (1987), by the decrease in absorbance at 240 nm due to the consumption of hydrogen peroxide (H_2O_2).The method described by Kar and Mishra method (1976) was used to measure the GPX activity. This assay is based on an increase in absorbance at 470 nm due to the formation of tetraguaiacol. The APX activity was evaluated by the method of Nakano and Asada (1981), which measures the oxidation of ascorbate by the decrease in absorbance at 290 nm. The SOD activity was determined by Beauchamp and Fridovich's method (1971), and the reaction measured by the increase in absorbance at 560 nm due to the photoreduction of p-nitrobluetetrazolium (NBT) toblue formazan.

The activities of CAT, APX and GPX, were expressed as mol H_2O_2/min/g fresh mass (FM), and SOD in activity units (U)/g FM, where U is defined as the amount of enzyme required to cause 50% inhibition of NBT photoreduction. Each extract was measured in duplicate.

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction, according to Buege and Aust (1978). The results were expressed as nmol MDA/FM.

The experimental design was completely randomized, arranged in a 2 (irrigated or non-irrigated) × 4 (sand, sand + fertilizer, sand + 91.8 g of dam sediment, sand + 183.6 g of dam sediment) factorial, with five replicates. Each replicate consisted of a bucket containing two plants. The data for each harvest time were analysed separately by analysis of variance (ANOVA) and the means were compared by Tukey's test ($P \leq 0.05$) using Sigma Plot 11.0 software.

3. Results

Figure 1 shows the leaf chlorophyll relative contents of the sunflower plants. It was found that water restriction did not reduce this variable in relation to their respective controls. However, at 23 DAS, higher means were observed for plants with sediment in their substrate. This increase was most pronounced for the treatment with 200% nitrogen recommendation (NR) in sediment, which was 15 and 23% superior to sand treatment under control conditions and water restriction, respectively.

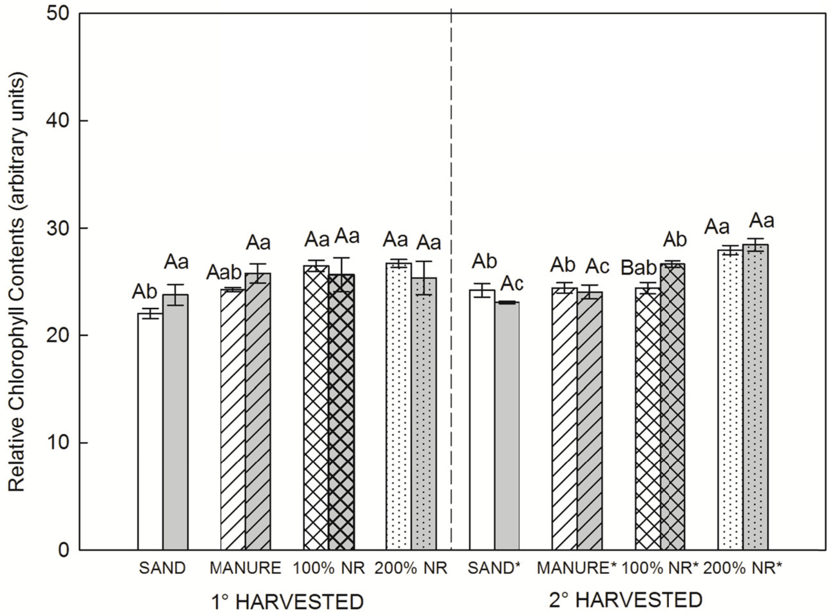

Figure 1. Relative chlorophyll content of sunflower plants at 21 days after sowing (DAS) (1st harvest - 5 days after suspension of irrigation) and 23 DAS (2nd harvest - 7 days after the suspension of irrigation) under control (white bars) or water stress (grey bars) conditions. Different capital letters indicate significant differences due to the type of irrigation (control and stress), while different lower case letters indicate significant differences due to the substrate (sand, sand + fertilizer, sand + 100% NR, sand + 200% NR), according to Tukey's test ($P \leq 0.05$)

Note. Statistical analyses were performed independently a teach collection. Bars represent the mean values of five repetitions ± standard error.

As observed for the leaf chlorophyll relative content, the suspension of irrigation did not cause a reduction in the production of SDM, RDM and TDM at the two harvest times (Figure 2). For the SDM, it was found that even under the same irrigation condition, the plants of the treatment with sediment on the substrate showed higher values than the other treatments. There were no differences between treatments with sediment in plants irrigated daily. However, the average of the two treatments was 60 and 113% higher than the sand treatment at the first and second harvests, respectively. The treatment with 200% NR in plants under water restriction was 79 and 97% higher than the sand treatment at 21 and 23 DAS, respectively (Figure 2A).

The RDM presented different behaviour to the SDM (Figure 2B). At the first harvest, there were generally no significant differences among all treatments under both irrigation conditions. At the second harvest, there was a 32% decrease in the RDM of the sand treated plants compared to the other treatments underwater restriction. In plants with irrigation suspension, there were no statistical differences between the treatments. This result may have been due to the large loss of material during the harvest.

It can be observed in Figure 2C that the TDM in plants with sediment treatment under control conditions and water restriction, respectively, were higher than other treatments. At 21 DAS, and under control conditions, 100% NR treatment excelled, being 61% higher than the sand treatment. In water restriction conditions, there were no significant differences between sediment treatments, which were 24% higher than the sand treatment. At 23 DAS, higher means were observed for the 200% NR treatment underwater restriction, with a superiority of 124% compared to the sand treatment.

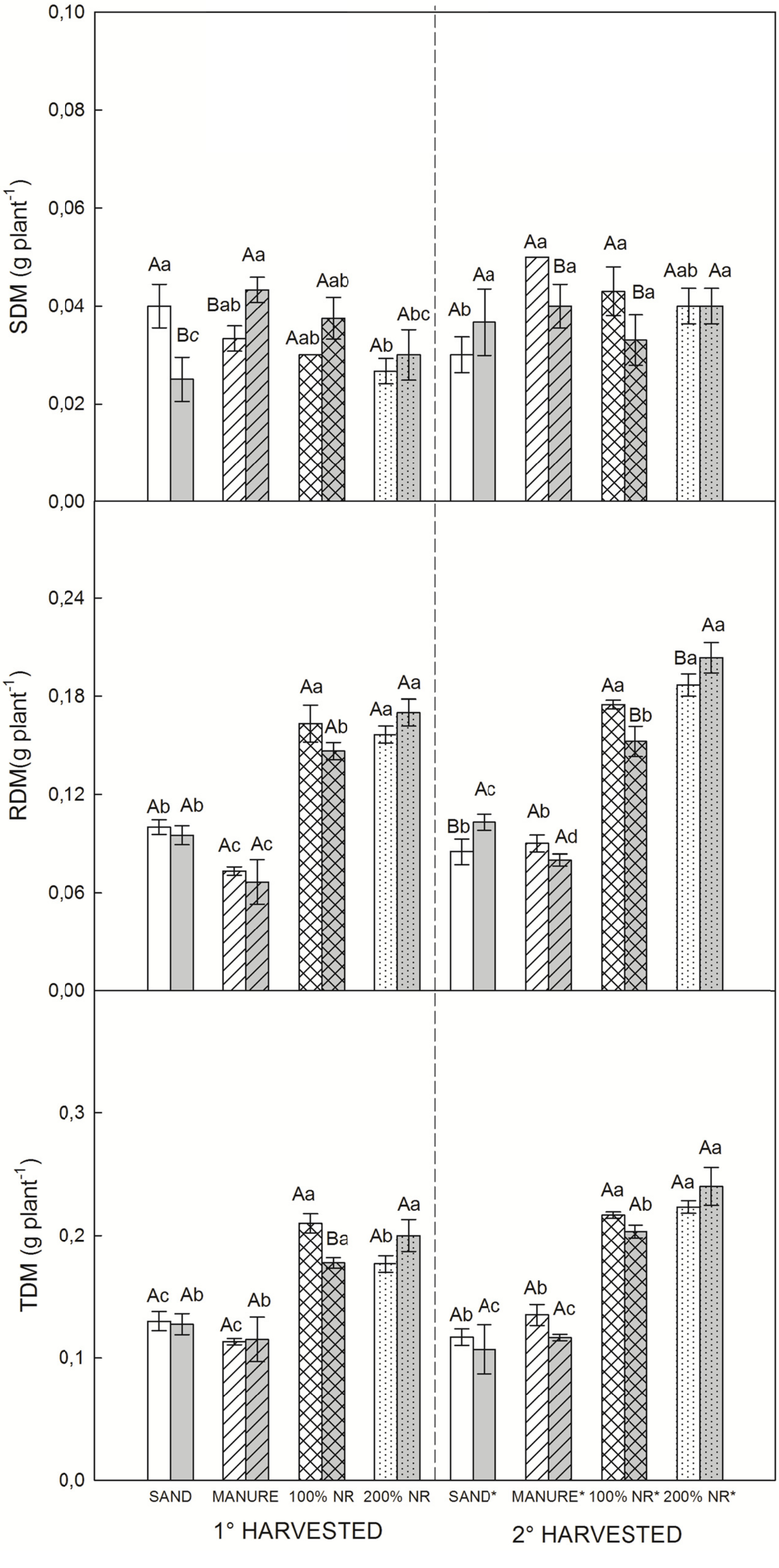

Figure 2. Shoot (A), roots (B) and total dry masses (C) of sunflower plants at 21 days after sowing (DAS) (1st harvest - 5 days after suspension of irrigation) and 23 DAS (2nd harvest - 7 days after the suspension of irrigation) under control (white bars) or water stress (grey bars) conditions

Note. Additional details in the legend of Figure 1.

At the first and second harvests, under both control conditions and water restriction, the leaf activity of SOD was higher in plants grown in substrates containing sediment (Figure 3A) than the other treatments. At 21 DAS, under water restriction conditions, treatments with sediment were statistically superior to fertilizer treatments, although the same did not occur when the results were compared with the sand treatment. However, at 23 DAS, the leaf activity of SOD in the plants treated with 200% NR was higher than the other treatments.

Similarly to SOD, the CAT leaf activity was also higher in plants grown in the substrate with than without dam sediment, under both control conditions and water restriction, respectively (Figure 3B).

Under control conditions, the CAT leaf activity of the plants treated with sediment was 50% higher at the first harvest and 45% higher at the second harvest, than the treatment with sand. The 100% NR treatment excelled the other treatments at the first harvest under water restriction. There were no significant differences between the sediment treatments at 23 DAS, which were 36% higher than the sand treatment.

The APX proved to be considerably higher at 23 DAS under water restriction conditions. The enzyme activity was higher in the treatments with than without sediment under these conditions, and on average 15% higher compared to the sand treatment (Figure 3C).

The guaiacol peroxidase activity (GPX) was higher in the 200% NR treatment in the first harvest and under control conditions, being 220% higher than the sand treatment (Figure 3D). There were no significant differences between treatments with sediment under water restriction conditions, but they were still higher than the others treatments. The same behavior was observed in plants irrigated daily, at 23 DAS. The 200% NR treatment and was 240% higher than the sand treatment under water restriction conditions.

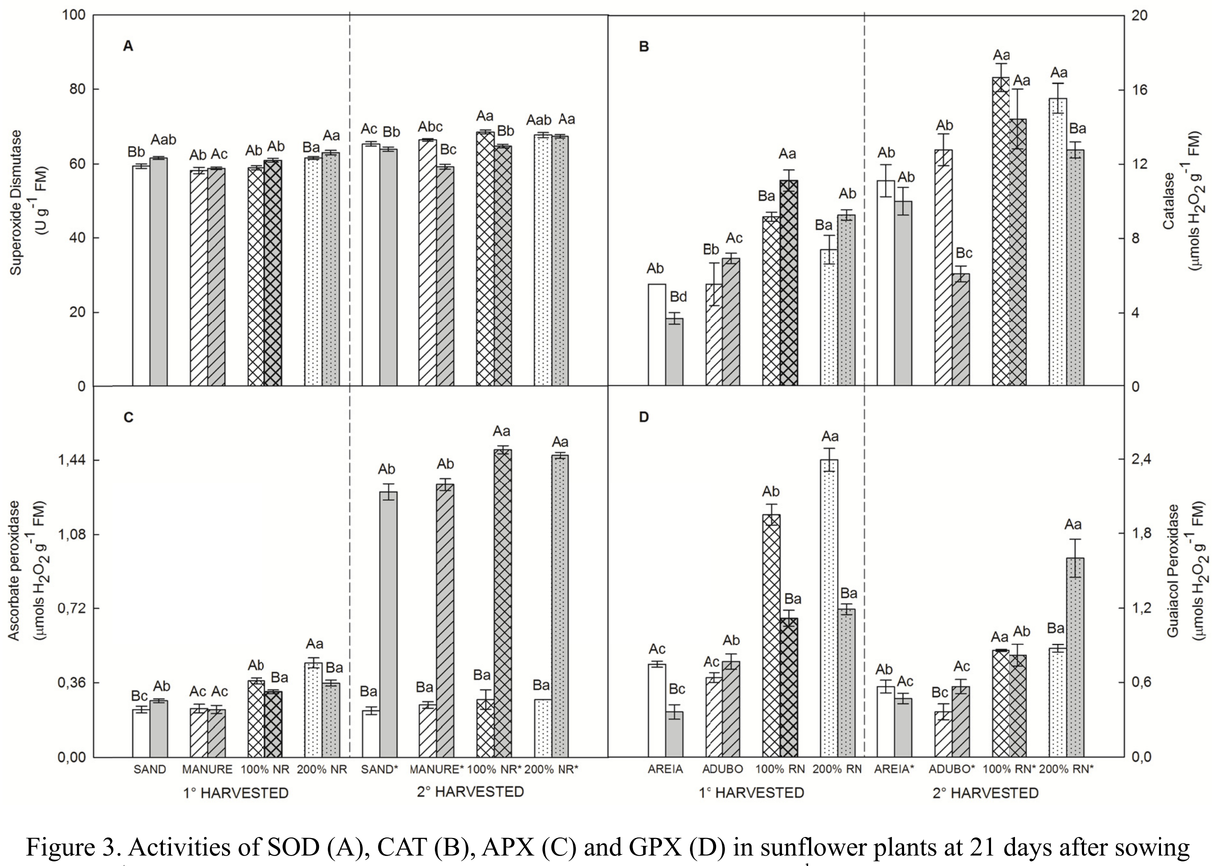

Figure 3. Activities of SOD (A), CAT (B), APX (C) and GPX (D) in sunflower plants at 21 days after sowing (DAS) (1st harvest - 5 days after suspension of irrigation) and 23 DAS (2nd harvest - 7 days after the suspension of irrigation) under control (white bars) or water stress (grey bars) conditions

Note. Additional details in the legend of Figure 1.

As observed for the leaves, the roots of sunflower plants grown in dam sediment containing substrate also showed higher enzymatic activities compared to the sand treatment. No CAT activity was identified in the roots. For SOD

(Figure 4A), it was found that at the first harvest, the 100% NR treatment was superior to the other treatment sunderboth irrigation conditions. At the second harvest, the 200% NR treatment showed excellent performance, with averages 38 and 58% higher than the sand treatment under control conditions and water restriction, respectively.

It was observed that at 21 DAS, the water restriction did not cause an increase in the APX activity in the roots. However, at 21 DAS the APX activity was higher in the 200% NR treatment. At 23 DAS, the 100% NR treatment differed significantly from the sand treatment under both conditions of irrigation, respectively, and the sand treatment average was 35 and 15% lower than the 100% NR treatment in plants irrigated and non-irrigated, respectively (Figure 4B).

In general, GPX activity in the roots was higher in plants grown under water restriction conditions. It was observed that the 200% NR treatment was superior to the other treatments at the two harvest times under both control and water restriction conditions, respectively. The 200% NR treatment was on average, 174 and 40% higher compared to the sand treatment at the first harvest and 312 and 311% higher than the sand treatment at the second harvest, under control and water restriction conditions, respectively (Figure 4C).

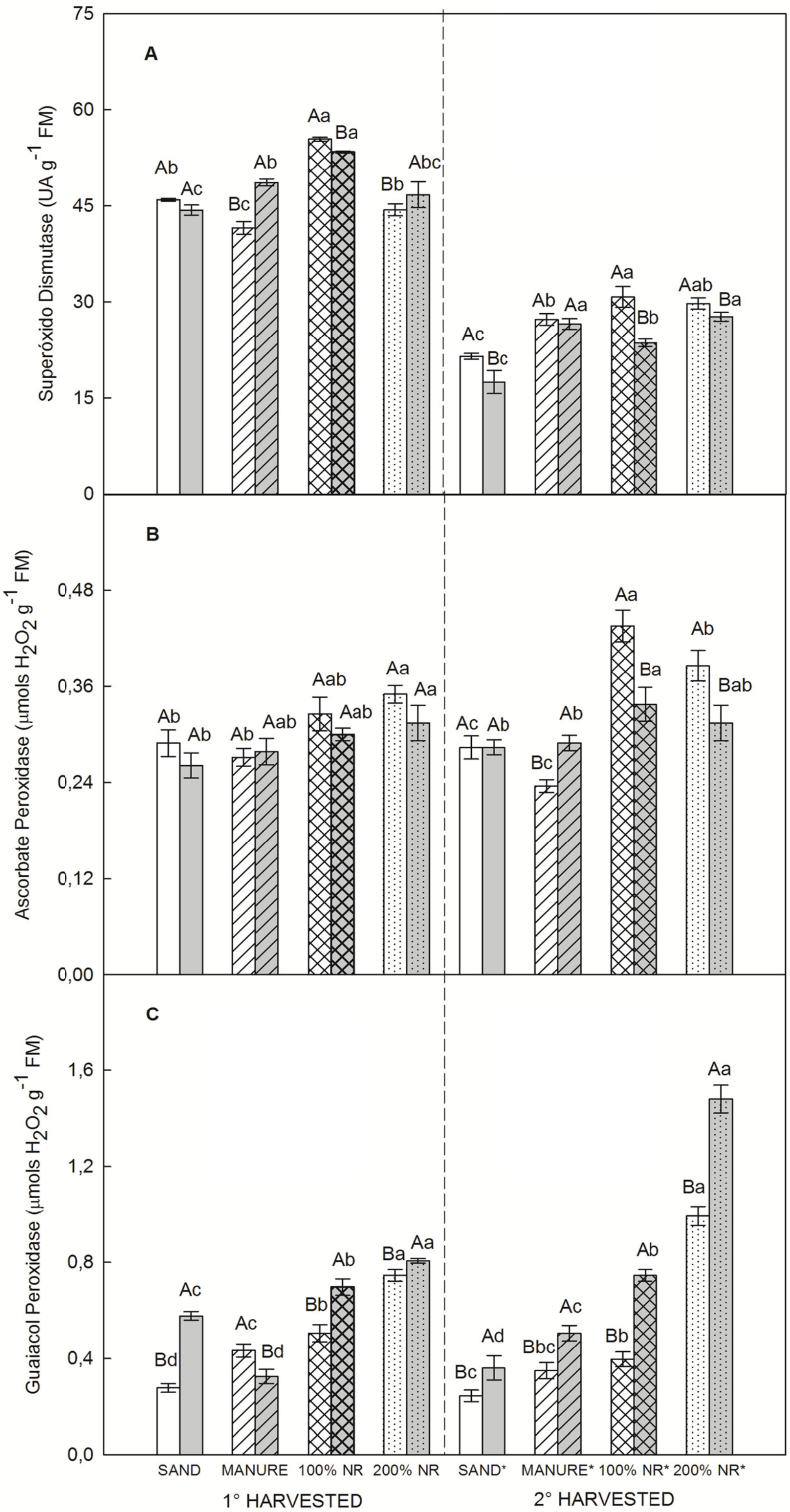

Figure 4. Activities of SOD (A) APX (B) and GPX (C) in sunflower plants at 21 days after sowing (DAS) (1st harvest - 5 days after suspension of irrigation) and 23 DAS (2nd harvest - 7 days after the suspension of irrigation) under control (white bars) or water stress (grey bars) conditions

Note. Additional details in the legend of Figure 1.

According to Figure 5, in general, at the two harvests, the MDA contents of the sunflower plants increased due to irrigation suspension. However, the plants with sediment in the substrate showed lower values compared to those without sediment. At 21 DAS, the 100% NR treatment was 68 and 92% lower compared to the sand treatment, under control and water restriction conditions, respectively. At 23 DAS, the 200% NR treatment was 80 and 74% lower than the sand treatment under control and water restriction conditions, respectively.

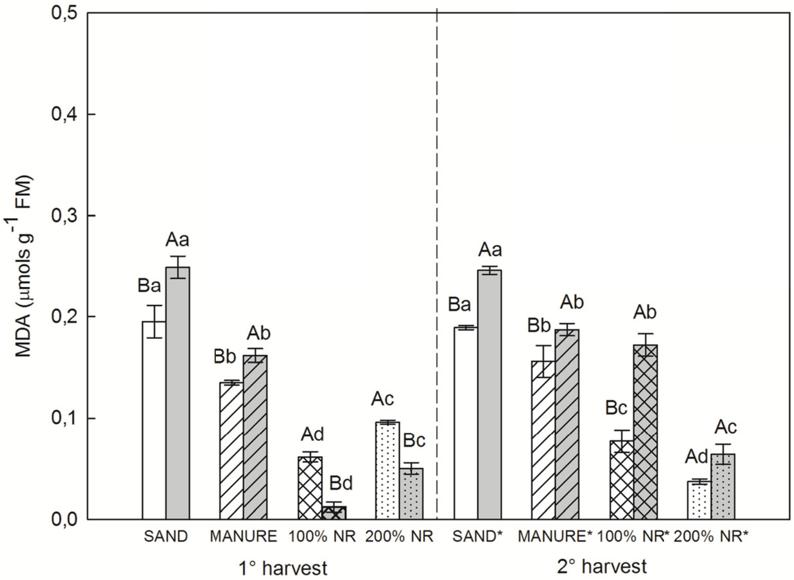

Figure 5. MDA contents in sunflower plants at 21 days after sowing (DAS) (1st harvest - 5 days after suspension of irrigation) and 23 DAS (2nd harvest - 7 days after the suspension of irrigation) under control (white bars) or water stress (grey bars) conditions

Note. Additional details in the legend of Figure 1.

4. Discussion

The determination of relative chlorophyll content is highly relevant because the photosynthetic activity of the plant depends on the leaf's ability to absorb light (Emrich et al., 2011). Water stress can cause hormonal dysfunction in plants linked to the increase in ethylene concentration in the leaves, which induces senescence (Taiz & Zeiger, 2009) and reduced absorption of nutrients, such as nitrogen, which is the constituent of the chlorophyll molecule (Lenhard, 2008). It is believed that such behaviour was not observed in the current study because the plants were not under severe water stress conditions (Figure 1).

As nitrogen is a constituent of the chlorophyll molecule, there is generally a high correlation between the relative chlorophyll content and the concentration of nitrogen in the soil (Soratto et al., 2004). It is suggested that the increase in relative chlorophyll content in the leaves of plants grown in substrates containing dam sediment can reflect a better nutritional status, or availability, particularly for nitrogen. Mizobata et al. (2016) also observed increases in relative chlorophyll content in *Hymenaea stilbocarpa* plants with the addition of organic matter in the soil.

In general, alack or excess of water is harmful to plant growth and development. An important step for irrigation management is the determination of water requirements for the various stages of plant development (Amorim Neto et al., 1996). Awater deficit in the soil can lead to plant water stress, causing physiological and morphological changes, such as a reduction in cell growth, reduced leaf area and increase in leaf abscission. It can also reduce the association between the biomass of the root to shoot, close stomata and decrease photosynthesis, severely affecting the production of plant biomass (Taiz & Zeiger, 2009).

The water restriction period to which the plants were exposed was not long enough to cause severe damage and changes in plant growth (Figure 2). Studies on various water depth levels for sunflower irrigation indicate that increasing the amount of available water leads to an increase in parameters, such as TDM and the dry mass of the inflorescence. Silva et al. (2007) studied the effects of four irrigation water depths (117.20, 350.84, 428.70 and 522.14 mm) over108 d and found a higher production, oil content and plant height for the 522.14 mm depth. Nobre et al. (2010) observed a linear increase in the SDM of sunflower, with increasing water depth. Castro et al. (2006) found that water stress decreased the dry mass yield of plants.

The beneficial effects of adding organic waste to the soil have been previously demonstrated in the literature. For instance, Mizobata et al. (2016) observed an increase in the SDM in *H. stilbocarpa* plants when organic residue was added to the substrate. Amaral et al. (2016) demonstrated that a substrate containing 50% plant compound provided higher RDM, SDM and TDM in plants of *Leucaena leucocephala* (Lam.) de Wit. However, there are limited studies published that evaluate the use of eutrophic dam sediments, such as that from the Tijuquinha dam, in the composition of substrates for plants. Thus, the current study proves pivotal to demonstrate the benefits of adding sediment to the sunflower plants soil, both under control and water restriction conditions, respectively. It is believed that the increase in plant growth associated with the sediment treatments may be due to the presence and availability of nutrients in their composition, such as nitrogen, phosphorus, calcium and magnesium, which increases soil fertility and enhances its chemical properties.

Plants use several integrated events to adjust to water stress conditions, which involves morphological, anatomical, cellular and biochemical changes. Among these events is the reduction of the stomatal aperture, which aims to reduce the loss of water by evapotranspiration (Gill & Tuteja, 2010). However, this process can lead to increased production of reactive oxygen species (ROS) including the radical superoxide ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), H_2O_2 and singlet oxygen ($^1O_2$) (Jaleel et al., 2007). ROS production is a normal event during plant growth and development. These ROS species act as signalling molecules in plants. However, under biotic and abiotic stresses, the ROS production can be increased and cause damage to biomolecules (Apel & Hirt, 2004).

In response to ROS production, plants have an antioxidative enzyme system that is an important defence against free radicals generated under stress conditions. Some of the enzymes that scavenge ROS are SOD, which catalyses the dismutation of superoxide in H_2O_2 and O_2, as well as CAT, APX and GPX, which can split the H_2O_2 molecule. The balance of this enzymatic system depends on several factors, such as the type, duration and intensity of the stress that the plant experiences (Larcher, 2000).

In the present study, there were increases in the activities of antioxidative enzymes in plants grown in substrates containing sediment. In leaves, CAT was the main enzyme responsible for H_2O_2 scavenging, and this enzyme was much higher than the other enzymes evaluated. CAT activity was not detected in the roots, where GPX was the main enzyme responsible for H_2O_2 scavenging. It is possible that the higher antioxidative enzymes activities in treatments with dam sediments may contribute to an increase in sunflower growth (dry mass) compared to plants grown in substrates containing sand or fertilizer.

Under water restriction, the production of free radicals is significantly increased, which can lead to a sequence of events that starts with lipid peroxidation, followed by degradation of the cell membranes and apoptosis (Greggains et al., 2000). However, the ROS scavenging by the antioxidative enzymes acts to minimize lipid peroxidation that can be considered the main symptom of oxidative damage to cell membranes (Hernandez et al., 2000). An increase in CAT activity could decrease the intracellular H_2O_2 concentration, reducing lipid peroxidation and the damage to plant membranes under water restriction conditions. Therefore, it is possible that the increased enzymes activities in plants grown in substrates containing dam sediment caused the decrease in MDA contents observed in the sunflower leaves (Figure 5).

In the experimental conditions used in the current study, the addition of silt sediment from the Tijuquinha dam provided improvements in the relative chlorophyll content, SDM and TDM compared to plants grown in substrate containing sand and sand + compost/mixed organic fertilizer, respectively. Also, the 7-d water restriction was not capable of causing a drastic reduction in plant growth, independent of the substrate used. The application of double NR in the sediment (200% RN treatment) caused a greater increase in the variables analysed than the other treatments. Therefore, dam sediments can be an alternative to chemical fertilizers for plant cultivation. Furthermore, withdrawal of such material from dams can provide improvements in the quality of water supply and can recover the superficial reservoir storage, partially lost by the sedimentation process.

5. Conclusions

In the experimental conditions used in this experiment, the addition of silted dam sediment of the Tijuquinha dam provided improvements in the variables: relative chlorophyll content, shoot and total dry masses when compared to plants growing in substrate containing sand or sand + compost/mixed organic fertilizer.

It was found that the water restriction of 7 days was not capable of causing drastic reduction in plant growth, independent of the substrate used.

The application of double nitrogen recommendation in sediment (200% RN treatment) caused greater increase in the variables analyzed in comparison to the other treatments.

The dam sediments could be an alternative to chemical fertilizers for plant cultivation. Furthermore, withdrawal of such dams background material can provide improvements in the quality of water supply and can recover the superficial reservoir storage, partially lost by sedimentation process.

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