Potentiality Of Stressed Alfalfa Plants To Modify Soil Traits

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Abstract: Leguminous plants play a key role in the sustainability of agricultural systems. Nevertheless, it is to somewhat scarce to find investigations about the interaction of such plants with the soil in which they grow under stress. A pot experiment was conducted to investigate the influence of graduated water regimes on the chemical features of different soil types, varying in sand proportion, before and after their cultivation with alfalfa (*Medicago sativa*) plants. The results indicated that soil cultivation with the studied plants, whether water-satisfied or not, generally increased the amount of soil ammonia, nitrate, peptide, total soluble and total nitrogen. On contrary, the amount of soil amino, amide, nitrite and protein nitrogen generally decreased after cultivation. Concerning soil ionic composition, the post-planting values of soil sodium, calcium, magnesium and sodium potassium ratio were significantly lower than their pre-cultivation synonyms. Furthermore, the percentages of decline caused by cultivating well-watered plants were higher than those caused by their moderately-droughted relatives which were in turn higher than those caused by severely-droughted ones. For soil chloride, potassium, sodium adsorption ratio and potassium adsorption ratio, culturing water-unstressed plants reduced these ionic fractions while droughted plants markedly favored such values. As a general feature, the maximum titers of all soil nitrogenous and ionic constituents were recorded for soil with the least sand proportion. The other chemical soil characteristics (pH, electric conductivity, organic carbon, organic matter, calcium carbonate, bicarbonates, sulphates and total soluble salts) were all fluctuated in a random fashion among the various soil types before and after planting water-stressed or control alfalfa plants. Thus, the results obtained herein recommend alfalfa as a pioneer plant that can be introduced to infertile and/ or dry lands with a paramount efficacy to enhance soil chemical properties.

Keywords: Alfalfa, Drought, Sand Proportion, Soil Properties

1. Introduction

In a cropping system, biological nitrogen fixation is the distinguishing characteristic of leguminous plants. Most legumes have the capability of forming symbiotic correlation with alpha or beta proteobacteria collectively known as *Rhizobia*. These bacteria utilize solar energy gathered by the plant to break down the bond in inert atmospheric nitrogen forming various reactive nitrogen species. By the means of such symbiotic relation, the need of a legume crop to nitrogen fertilizers will be minimized with little demand for soil nitrogen reserves [1]. *Medicago* is a herbal genus belonging to the family Fabaceae with about 83 different species [2]. Among these species, *Medicago sativa* or alfalfa is the most highly economic perennial [3]. Alfalfa is characterized by its profound potentiality to survive in arid and semi-arid regions with an extensive tolerance to long-term drought without any disturbance in its regrowth process [4]. With respect to soil requirement, alfalfa prefers loose deep soils where it adapts by a deeply-penetrating root system [5].

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Small [5] documented alfalfa as the world's most environment-friendly crop with remarkable potency to make agriculture more compatible with today's goal of minimizing negative ecological impacts. He supported his judgment by the facts that; (i) The symbiotic bacteria in alfalfa root nodules can fix up to 560 kg of atmospheric nitrogen per hectare per year; so alfalfa can gradually improve soil fertility and is therefore widely used in crop rotation. (ii) Alfalfa is an energy-efficient crop with minimal or no nitrogen fertilizers demand. (iii) Alfalfa has an extensive root system that reduces erosion by holding soil together and improving water infiltration. (iv) Alfalfa can be grown for several years without reseeding, an energy-conserving feature that reduces tillage and soil erosion by maintaining continuous green cover. (v) Alfalfa grows vigorously providing harmonious weed control with little need for herbicides. As an illation, the persistence, drought tolerance as well as the ability to colonize eroded areas and to fix atmospheric nitrogen all makes alfalfa intrinsic pioneer plant. *Via* the present study, the authors hope to attract the attention on the ability of alfalfa plants to fix nitrogen and thus gradually improve soil characteristics and reduce fertilizers input. Moreover, the ability of alfalfa plants to alter soil properties under various environmental circumstances would be explored by growing the studied plants in different types of soil with applying graduated levels of drought. Thereafter, the chemical features of the soil in which the plants are cultivated would be compared before and after planting.

2. Materials and Methods

2.1. Plant Material, Soil Samples & Growth Conditions

Seeds of alfalfa (*Medicago sativa* L., cultivar Nubaria 1) were procured from El-Nubaria, El-Behira Governorate, Egypt. For cultivation, clay and sand were mixed to obtain three types of soil with 33, 67 and 100% sand proportion. Plastic pots were packed with the soil samples then twenty seeds were sown in each pot. Thirty days after emergence, the seedlings were thinned to five per pot. When the seedlings were 45-day old, each of the three groups of pots was subdivided into three sets so that the plants were subjected to three levels of water

regimes; (1) control, where soil was never allowed to dry out and plants were irrigated when required, (2) moderate drought by withholding 33% of irrigation water and (3) severe drought by withholding 67% of irrigation water. Therefore, the resulting twelve soil samples collected before and after planting for 75 days could be marked as the percent of sand only before planting (33, 67 or 100s) or + the percent of watering after planting (33, 67 or 100w). Air-dried soil samples were collected, sieved to remove coarse gravel and packed in plastic bags to be ready for chemical analyses. The chemical variables estimated included soil nitrogenous constituents (ammonia, amino, amide, nitrite, nitrate, peptide, protein, total soluble and total nitrogen), soil ionic contents (Cl^-, Na^+, K^+, Ca^{++} and Mg^{++}), ionic ratios (sodium potassium ratio "SPR", sodium adsorption ratio "SAR" and potassium adsorption ratio "PAR"), as well as other chemical characteristics (pH, electric conductivity, organic carbon, organic matter, calcium carbonate, carbonates, bicarbonates, sulphates and total soluble salts). The estimation of organic carbon, organic matter, calcium carbonate and total nitrogen was carried out directly using the soil samples, while the other variables were determined using soil-water extract (1: 5, w/ v).

2.2. Determination of Nitrogenous Constituents

As adopted by Yemm and Willis [6], the difference between total soluble nitrogen and the sum of ammonia, amino, amide, nitrite plus nitrate fractions gave the value of peptide nitrogen. In addition, subtracting total soluble nitrogen from total nitrogen gave the value of protein nitrogen.

2.2.1. Determination of ammonia nitrogen

Ammonia nitrogen was estimated by the method adopted by Delory [7] as modified by Naguib [8]. An aliquot of the soil-water extract was mixed with 1 ml of 1 N NaOH and 0.5 ml of 0.5% $ZnSO_4$. The mixture was made up to 14 ml with distilled water before 1 ml of Nessler's reagent was added, shaken well and allowed to stand for 5 minutes then the optical density was measured at 450 nm.

2.2.2. Determination of amide nitrogen

As designed by Naguib [8], 10 ml of the soil extract was mixed with 2.5 ml of 10 N H_2SO_4. The mixture was refluxed for 4 hours at 100°C, neutralized with NaOH and made up to 50 ml. Ammonia nitrogen was estimated using Nessler's reagent as described before. The difference between this value and that blamed for free ammonia is equivalent to amide nitrogen.

2.2.3. Determination of amino nitrogen

Amino nitrogen was estimated according to Muting and Kaiser [9] where 0.1 ml of the soil extract was deproteinized with 1.5 ml of ethanol/ acetone mixture with the addition of 0.1 ml of phosphate buffer (pH 6.5) plus 2 ml of ninhydrin reagent and boiling in water bath for 20 minutes. After cooling, methanol was added up to 10 ml and the optical density was read at 580 nm.

2.2.4. Determination of nitrite nitrogen

An aliquot of the soil extract was mixed with 40 ml of distilled water and 2 ml of solution I (3.3 g of sulphanilic acid dissolved in 750 ml of distilled water and 250 ml of glacial acetic acid) and II (0.5 g of α-naphthylamine boiled in 100 ml of distilled water for 5 minutes, with the addition of 250 ml of glacial acetic acid and raising up to 1 L with distilled water). The contents were made up to 50 ml, allowed to stand for 30 minutes and measured at 525 nm [10].

2.2.5. Determination of nitrate nitrogen

As referenced from Snell and Snell [11], the soil extract was evaporated to dryness then 2 ml of phenol-disulphonic acid was added followed by mixing with 15 ml of cold distilled water after 10 minutes. Drops of NaOH solution were then added until slightly alkaline. The solution was made up to 50 ml and measured at 430 nm.

2.2.6. Determination of total soluble nitrogen

Following the conventional semimicro-modification of Kjeldahl method [12], an aliquot of the soil extract was heated for at least 8 hours with 0.5 g of catalyst (80 g K_2SO_4, 20 g $CuSO_4.5H_2O$ and 0.3 g SeO_2), 2 ml of concentrated H_2SO_4 and 1 ml of distilled water. The solution was treated with 12 ml of 40% NaOH and steam distilled in conventional manner into 5 ml of 0.05 N H_2SO_4. The distillate was then made up to volume and ammonia was estimated as formerly described.

2.2.7. Determination of total nitrogen

Total nitrogen was determined following Chinbal *et al*. [13] as described for total soluble nitrogen except that dry soil was used instead of the soil extract.

2.3. Determination of Ionic Contents & Ratios

According to Hansen and Munns [14], Cl^- levels were determined by volumetric titration against $AgNO_3$ using potassium chromate as indicator. Following the method described by Chapman and Pratt [15], flame photometer (model PFP7/C) was used for determining Na^+ and K^+, while Ca^{++} and Mg^{++} were measured by titration using EDTA as a complexmetric reagent as well as murexide and eriochrome black T as indicators. SAR and PAR were calculated according to McKell and Goodin [16] as follows:

$$SAR = Na^+ \{(Ca^{++} + Mg^{++})/2\}^{1/2}$$

$$PAR = K^+ \{(Ca^{++} + Mg^{++})/2\}^{1/2}$$

2.4. Determination of Soil Other Characteristics

These include soil reaction, electric conductivity as well as the percentage of organic carbon, organic matter, calcium carbonate, carbonates, bicarbonates sulphates and total soluble sugars.

2.4.1. Determination of soil reaction & conductivity

Electrical pH meter (model Adwa, AD- 1000 Romani) and conductivity meter (model CD- 4301) were used to determine soil reaction (pH) and electric conductivity (EC) of soil-water extracts; respectively.

2.4.2. Determination of organic carbon & matter

Using Walkely and Black rapid titration method as mentioned by Piper [17] and Jackson [18], 10 ml of 1 N potassium dichromate was added to 2 g of the soil sample followed by 20 ml of concentrated H_2SO_4, shaken well and allowed to stand for 30 minutes. Two hundred ml of distilled water, 10 ml of 85% phosphoric acid and 1 ml of diphenylamine were added to be titrated against 1 N ferrous sulphate. A blank titration was carried out to calculate the percentage of organic carbon and organic matter from the relations:

Organic Carbon = [(Blank titration – Sample titration) / Soil weight] × 0.003 × 100

Organic Matter = Soil organic carbon × 1.724

2.4.3. Determination of calcium carbonate

According to Jackson [18], 100 ml of 1 N HCl was added to 5 g of soil then stirred vigorously for an hour. Settling was allowed and 20 ml of the suspended liquid was transferred into a conical flask followed by the addition of 6-8 drops of phenolphthalein indicator to be titrated against 1 N NaOH with carrying out a blank titration. The percentage of calcium carbonate could be calculated as:

Calcium Carbonate = (Blank titration – Sample titration) × 5

2.4.4. Determination of carbonates & bicarbonates

Carbonates and bicarbonates were determined by titration method using 0.1 N HCl. Phenolphthalein and methyl orange were used as indicators for carbonates and bicarbonates; respectively [19]. Phenolphthalein was added to 50 ml of soil extract to be titrated against 0.1 N HCl till the color was just discharged (V_1). To the same solution, methyl orange was added and the titration was continued till the color changed from yellow to orange (V_2). The percentage of carbonates and bicarbonates could be calculated from the relations:

Carbonates = V_1 × (Total volume of extract / Volume of extract used) × (100 / Soil weight) × 0.0060

Bicarbonates = ($V_2 – 2V_1$) × (Total volume of extract / Volume of extract used) × (100 / Soil weight) × 0.0061

2.4.5. Determination of sulphates

According to Jackson [18], the amount of sulphates was computed as the difference between cations and anions determined in the soil extract as follows:

Sulphates = ($Na^+ + K^+ + Ca^+ + Mg^{++}$) – ($Cl^- + CO_3^{--} + HCO_3^-$)

2.4.6. Determination of total soluble salts

As described by Jackson [18], 100 ml of the soil extract was put in a weighed crucible to be evaporated till the volume was reduced to about 5 ml. Two ml of H_2O_2 was added to oxidize the soluble organic matter and evaporation was completed. The crucible was then dried at 105°C, cooled and weighed. The difference between the crucible weights (wt) is thus the weight of total soluble salts. The percentage of total soluble salts could be calculated as:

Total Soluble Salts = wt × (Total volume of extract / Volume of extract used) × (100 / Soil weight)

2.5. Statistical Data Analysis

For each the aforementioned investigation, double replicates were taken and only the mean values were represented with standard deviation. A statistical test for significant differences between means at P ≤ 0.05 was performed using CoHort/ CoStat software. The treatments were applied to analysis of variance (ANOVA) procedure to determine the least significant difference (LSD) and mean standard error (MSE) so that small letters were denoted, where different letters refer to significant variation with higher degree of variance as the letters are far from each other.

3. Results And Discussion

The effect of plant invasions on soil properties, especially when the plants are stressed, has rarely been investigated. Data in table 1 manifested that the amounts of soil ammonia nitrogen before cultivation were significantly lower than those after cultivation with well-watered or moderately-droughted alfalfa plants. However, the amounts of soil ammonia nitrogen before cultivation were significantly higher than those after cultivation with severely-droughted plants. *Medicago sativa* as well as its close relatives are usually taken as leading models when studying plant-bacteria symbiotic relations particularly the process of nitrogen fixation and legume genomics [20]. In the current study, the recorded increment in soil ammonia because of its cultivation with unstressed or moderately-droughted alfalfa plants is a logic result of nitrogen fixation carried out by the nitrogen-fixing bacteria that symbiotically aggregate with alfalfa roots. Through the process of nitrogen fixation, atmospheric nitrogen is converted into ammonia. Nevertheless, environmental stresses could simultaneously and adversely affect the efficacy of this process.

Table 1

Effect of different water regimes on nitrogenous constituents of different soil types before and after their cultivation with alfalfa plants. Data listed represent the mean values ± standard deviation with different letters referring to significant variation with least significant difference (LSD) and mean standard error (MSE) at P ≤ 0.05.

Treatment	Nitrogenous Constituents (mg g^{-1} dry weight)				
	Ammonia N	Amino N	Amide N	Nitrite N	Nitrate N
33s	0.114 ^{c} ± 0.002	0.123 ^{a} ± 0.01	0.116 ^{a} ± 0.005	0.145 ^{a} ± 0.001	0.116 ^{f} ± 0.001
67s	0.099 ^{e} ± 0.002	0.099 ^{cd} ± 0.004	0.097 ^{b} ± 0.01	0.120 ^{c} ± 0.001	0.106 ^{gh} ± 0.003
100s	0.088 ^{f} ± 0.001	0.087 ^{ef} ± 0	0.067 ^{ef} ± 0	0.108 ^{d} ± 0.001	0.092 ^{i} ± 0.005
33s + 100w	0.142 ^{a} ± 0.001	0.096 ^{de} ± 0	0.079 ^{cd} ± 0.005	0.110 ^{d} ± 0.001	0.145 ^{b} ± 0.001
67s + 100w	0.125 ^{b} ± 0.003	0.061 ^{gh} ± 0.004	0.060 ^{f} ± 0	0.070 ^{g} ± 0.001	0.140 ^{bc} ± 0
100s + 100w	0.091 ^{f} ± 0.001	0.051 ^{h} ± 0.004	0.037 ^{g} ± 0	0.065 ^{g} ± 0.001	0.120 ^{ef} ± 0.001
33s + 67w	0.122 ^{b} ± 0.004	0.109 ^{bc} ± 0.004	0.097 ^{b} ± 0	0.133 ^{b} ± 0.005	0.136 ^{cd} ± 0.002
67s + 67w	0.114 ^{c} ± 0.002	0.077 ^{f} ± 0	0.071 ^{de} ± 0.006	0.098 ^{e} ± 0.006	0.122 ^{e} ± 0
100s + 67w	0.100 ^{e} ± 0.001	0.063 ^{g} ± 0.006	0.060 ^{f} ± 0	0.083 ^{f} ± 0.003	0.107 ^{g} ± 0.003
33s + 33w	0.106 ^{d} ± 0.002	0.118 ^{ab} ± 0.003	0.101 ^{b} ± 0.005	0.141 ^{a} ± 0.002	0.132 ^{d} ± 0.001
67s + 33w	0.092 ^{f} ± 0.001	0.090 ^{de} ± 0.004	0.082 ^{c} ± 0	0.106 ^{d} ± 0.001	0.178 ^{a} ± 0.004
100s + 33w	0.083 ^{g} ± 0.001	0.077 ^{f} ± 0.007	0.067 ^{ef} ± 0	0.097 ^{e} ± 0.004	0.101 ^{h} ± 0.005
LSD	0.005	0.010	0.009	0.006	0.006
MSE	0.002	0.005	0.004	0.003	0.003

Treatment	Nitrogenous Constituents (mg g^{-1} dry weight)			
	Peptide N	Protein N	Total soluble N	Total N
33s	0.61 ^{de} ± 0.14	0.87 ^{a} ± 0.13	1.22 ^{d} ± 0.13	2.09 ^{cd} ± 0
67s	0.35 ^{gh} ± 0.05	0.83 ^{ab} ± 0.04	0.87 ^{gh} ± 0.04	1.69 ^{f} ± 0
100s	0.28 ^{h} ± 0.09	0.48 ^{c} ± 0.06	0.72 ^{i} ± 0.08	1.19 ^{h} ± 0.14
33s + 100w	1.40 ^{a} ± 0.01	0.67 ^{b} ± 0.07	1.97 ^{a} ± 0	2.64 ^{a} ± 0.07
67s + 100w	0.71 ^{cd} ± 0.05	0.78 ^{ab} ± 0.03	1.16 ^{de} ± 0.04	1.94 ^{de} ± 0.07
100s + 100w	0.71 ^{cd} ± 0.002	0.32 ^{c} ± 0.14	1.07 ^{ef} ± 0	1.39 ^{g} ± 0.14
33s + 67w	1.11 ^{b} ± 0.05	0.74 ^{ab} ± 0.11	1.70 ^{b} ± 0.04	2.44 ^{b} ± 0.07
67s + 67w	0.59 ^{de} ± 0.001	0.77 ^{ab} ± 0.07	1.07 ^{ef} ± 0	1.84 ^{ef} ± 0.07
100s + 67w	0.51 ^{ef} ± 0.04	0.37 ^{c} ± 0.04	0.93 ^{gh} ± 0.04	1.29 ^{gh} ± 0
33s + 33w	0.81 ^{c} ± 0.05	0.84 ^{ab} ± 0.11	1.40 ^{c} ± 0.04	2.24 ^{c} ± 0.07
67s + 33w	0.41 ^{fg} ± 0.003	0.84 ^{ab} ± 0	0.96 ^{fg} ± 0	1.79 ^{ef} ± 0
100s + 33w	0.41 ^{fg} ± 0.01	0.41 ^{c} ± 0.07	0.84 ^{h} ± 0	1.24 ^{gh} ± 0.07
LSD	0.128	0.183	0.113	0.166
MSE	0.059	0.084	0.052	0.076

Among the environmental constraints, drought is a major factor limiting nitrogen fixation. However, the mechanism involved in such limitation and the origin of inhibition are still argumentative and rarely investigated in forage legumes [21]. In this context, Gil-Quintana *et al*. [21] elucidated that water deficit inhibited nitrogen fixation in the nodules of *Medicago truncatula* plants. Thus, it is not surprising that the exposure of alfalfa plants herein to severe drought by withholding more than half the amount of irrigation water (33% watering) resulted in remarkable inhibition of their symbiotic nitrogen fixation as indicated by lower soil ammonia content after its cultivation with severely-droughted plants. In a similar trend, the results in table 1 cleared that the amounts of soil nitrate, peptide, total soluble and total nitrogen before cultivation were significantly lower than those after soil cultivation with water-stressed or unstressed plants. Also, the degree of increment resulted from cultivation without water stress was higher than that resulted from cultivation with moderate drought which was also higher than that accompanying severe drought. The recorded increase in soil nitrate nitrogen after cultivating it with the studied plants may be ascribed to the process of nitrification which involves the conversion of ammonia into nitrate by the nitrifying bacteria. With the estimated increase in soil peptide nitrogen, it is normal that both soil total soluble and total nitrogen also increased in response to soil cultivation. In a reversed manner, data in table 1 revealed that the amount of soil amino, amide, nitrite and protein nitrogen generally decreased after soil cultivation with the water-stressed or unstressed plants. This may indicate the depletion of soil organic nitrogen forms (amino, amide and protein nitrogen) probably due to their consumption by the growing plants. Also, the amount of soil nitrite was declined after cultivation as a consequence of the increased nitrate amount. Regarding the types of soil used in cultivation, the values of all soil nitrogenous constituents were reported in the present study to generally decrease with raising sand proportion (Table 1). In this regard, it is well known that soil in which sand predominates, the soil pores or interspaces are large and continuous so that water along with both organic and inorganic compounds may move freely. In this case, porosity is high favoring the leaching of various nitrogen compounds to the deep layers away from the associated growing plants. The results represented in table 2 indicated that the post-cultivation values of soil Na^+, Ca^{++}, Mg^{++} and SPR were lower than the pre-cultivation values. Moreover, the percentages of decrease caused by cultivating well-watered plants were higher than those caused by moderately-droughted plants which were in turn higher than those caused by severely-droughted ones. For soil Cl^-, K^+, SAR and PAR, soil cultivation with water-unstressed plants reduced these ionic contents and ratios. Meanwhile, cultivating droughted alfalfa plants resulted in marked increase in these values. As a general feature, the highest values of soil ionic contents were reported for soil with the least sand proportion. While evaluating the agro-physiological status of alfalfa plants grown in the various types of the studied soil with the three irrigation systems of the present research, the growth vigor of water-satisfied alfalfa plants was better than that of their droughted comparative plants [22]. Consequently, the well-watered plants could utilize the estimated elements (Na^+, K^+, Ca^{++}, Mg^{++} and Cl^-) more efficiently resulting in marked reduction in the amount of these elements in soil as a result of their withdrawal by normally-growing plants.

TABLE 2

EFFECT OF DIFFERENT WATER REGIMES ON IONIC CONTENTS AND RATIOS OF DIFFERENT SOIL TYPES BEFORE AND AFTER THEIR CULTIVATION WITH ALFALFA PLANTS. DATA LISTED REPRESENT THE MEAN VALUES ± STANDARD DEVIATION WITH DIFFERENT LETTERS REFERRING TO SIGNIFICANT VARIATION WITH LEAST SIGNIFICANT DIFFERENCE (LSD) AND MEAN STANDARD ERROR (MSE) AT P ≤ 0.05.

Treatment	Ionic Content (µmole g^{-1} dry weight)				
	Cl^-	Na^+	K^+	Ca^{++}	Mg^{++}
33s	14.8^{d} ± 1	36.3^{a} ± 0.29	21.6^{a} ± 0.07	8.5^{a} ± 0.7	7.0^{a} ± 0
67s	11.3^{e} ± 0	34.6^{c} ± 0.08	21.1^{f} ± 0.06	6.0^{b} ± 0	5.0^{b} ± 0
100s	8.5^{f} ± 0	28.7^{g} ± 0.25	18.8^{k} ± 0.08	4.0^{de} ± 0	4.5^{b} ± 0.7
33s + 100w	5.6^{g} ± 0	29.5^{f} ± 0.46	20.0^{i} ± 0.04	4.0^{de} ± 0	2.5^{de} ± 0.7
67s + 100w	5.1^{g} ± 0	28.6^{g} ± 0.04	19.6^{j} ± 0.16	2.9^{g} ± 0.1	2.1^{de} ± 0.1
100s + 100w	4.7^{g} ± 0.2	26.3^{i} ± 0.06	18.6^{l} ± 0.03	2.6^{g} ± 0	1.4^{e} ± 0
33s + 67w	28.2^{b} ± 0	35.2^{b} ± 0.30	24.1^{b} ± 0.14	4.5^{cd} ± 0.7	5.0^{b} ± 1.4
67s + 67w	14.8^{d} ± 1	32.5^{e} ± 0.06	23.5^{c} ± 0.06	3.6^{ef} ± 0.3	2.9^{d} ± 1
100s + 67w	9.9^{ef} ± 0	27.2^{h} ± 0.09	20.9^{g} ± 0.04	2.9^{g} ± 0.1	2.1^{de} ± 0.1
33s + 33w	32.4^{a} ± 2	36.1^{a} ± 0.04	24.9^{a} ± 0.08	5.0^{c} ± 0	6.5^{a} ± 0.7
67s + 33w	16.9^{c} ± 0	33.9^{d} ± 0.18	22.4^{d} ± 0.15	3.7^{ef} ± 0.1	4.3^{bc} ± 0.1
100s + 33w	11.3^{e} ± 0	27.5^{h} ± 0.04	20.4^{h} ± 0.07	3.0^{fg} ± 0	3.0^{cd} ± 0
LSD	1.54	0.45	0.20	0.67	1.34
MSE	0.71	0.21	0.09	0.31	0.62

Treatment	Ionic Ratio		
	SPR	SAR	PAR
33s	1.69^{a} ± 0.02	65.4^{d} ± 3.0	38.8^{cd} ± 2.24
67s	1.64^{b} ± 0.01	54.7^{e} ± 0.13	33.4^{g} ± 0.10
100s	1.53^{c} ± 0.02	40.5^{hi} ± 0.35	26.6^{i} ± 0.11
33s + 100w	1.48^{d} ± 0.02	53.1^{ef} ± 3.72	36.0^{f} ± 2.04
67s + 100w	1.46^{d} ± 0.01	45.2^{g} ± 0.05	31.0^{h} ± 0.25
100s + 100w	1.42^{e} ± 0	37.3^{i} ± 0.08	26.3^{i} ± 0.05
33s + 67w	1.46^{d} ± 0	87.9^{b} ± 1.73	60.2^{b} ± 1.35
67s + 67w	1.39^{f} ± 0.01	72.6^{c} ± 0.13	52.5^{c} ± 0.13
100s + 67w	1.30^{h} ± 0	51.0^{f} ± 0.17	39.2^{e} ± 0.07
33s + 33w	1.45^{d} ± 0	97.2^{a} ± 2.25	67.1^{a} ± 1.42
67s + 33w	1.51^{c} ± 0	71.8^{c} ± 0.39	47.5^{d} ±0.32
100s + 33w	1.35^{g} ± 0	43.6^{gh} ± 0.04	32.2^{gh} ± 0.11
LSD	0.03	3.53	2.29
MSE	0.01	1.62	1.05

With respect to soil Cl^- and K^+, while soil cultivation with unstressed alfalfa plants decreased the amount of these two elements; the cultivation with droughted plants markedly increased these values. The recorded boost in soil Cl^- and K^+ amounts when cultivated with stressed plants may be a consequence of stress-induced injury of plant biomembranes that could lead to the leakage of some cellular constituents, such as certain minerals, from plant root to the surrounding soil. In this context, it is well documented that water deficit could mediate the overproduction of reactive oxygen species that drastically harm plants by lipid peroxidation, protein deterioration, DNA fragmentation and eventually cell death [23]. Peroxidation of lipids disrupts membrane integrity of the plant cell causing essential solutes to leak out from the cells. As the finding reported for soil nitrogenous fractions, the highest values of all the estimated soil ionic contents and

ratios were recorded for soil with 33% sand. This could be similarly ascribed to the capability of clay particles to retain its chemical constituents because of the lower occurrence of soil pores as compared with the highly porous sandy soil. The results of the present investigation also elucidated that other chemical soil characteristics (pH, electric conductivity, organic carbon and matter, calcium carbonate, bicarbonates, sulphates and total soluble salts) were all fluctuated in a random fashion between the different soil types after and before their cultivation with the stressed or unstressed alfalfa plants (Table 3).

TABLE 3

EFFECT OF DIFFERENT WATER REGIMES ON CHEMICAL CHARACTERISTICS OF DIFFERENT SOIL TYPES BEFORE AND AFTER THEIR CULTIVATION WITH ALFALFA PLANTS. DATA LISTED REPRESENT THE MEAN VALUES ± STANDARD DEVIATION WITH DIFFERENT LETTERS REFERRING TO SIGNIFICANT VARIATION WITH LEAST SIGNIFICANT DIFFERENCE (LSD) AND MEAN STANDARD ERROR (MSE) AT P ≤ 0.05.

Treatment	Chemical Characteristics			
	pH	EC (mS)	Organic carbon (%)	Organic matter (%)
33s	8.47 ^{d} ± 0.007	0.73 ^{e} ± 0	0.39 ^{b} ± 0	0.67 ^{b} ± 0
67s	8.61 ^{c} ± 0.007	0.51 ^{f} ± 0.014	0.27 ^{e} ± 0	0.47 ^{e} ± 0
100s	8.91 ^{a} ± 0.007	0.20 ^{i} ± 0	0.12 ^{i} ± 0	0.21 ^{i} ± 0
33s + 100w	8.61 ^{c} ± 0.014	0.31 ^{g} ± 0	0.42 ^{a} ± 0	0.72 ^{a} ± 0
67s + 100w	8.71 ^{b} ± 0.014	0.21 ^{i} ± 0.007	0.30 ^{d} ± 0	0.72 ^{a} ± 0
100s + 100w	8.94 ^{a} ± 0.071	0.15 ^{j} ± 0	0.15 ^{h} ± 0	0.26 ^{h} ± 0
33s + 67w	8.06 ^{i} ± 0.014	1.21 ^{a} ± 0.014	0.37 ^{c} ± 0.014	0.64 ^{c} ± 0.021
67s + 67w	8.27 ^{f} ± 0	0.96 ^{c} ± 0.021	0.25 ^{f} ± 0.014	0.43 ^{f} ± 0.021
100s + 67w	8.36 ^{e} ± 0.007	0.27 ^{h} ± 0.007	0.10 ^{j} ± 0.014	0.17 ^{j} ± 0.021
33s + 33w	8.12 ^{h} ± 0.014	1.12 ^{b} ± 0.007	0.30 ^{d} ± 0	0.52 ^{d} ± 0
67s + 33w	8.20 ^{g} ± 0	0.80 ^{d} ± 0	0.23 ^{g} ± 0	0.39 ^{g} ± 0
100s + 33w	8.28 ^{f} ± 0	0.25 ^{h} ± 0	0.09 ^{j} ± 0	0.16 ^{j} ± 0
LSD	0.049	0.020	0.015	0.021
MSE	0.022	0.009	0.007	0.010

Treatment	Chemical Characteristics (%)				
	$CaCO_3$	CO_3^{--}	HCO_3^-	SO_4^{--}	Total soluble salts
33s	3.4 ^{a} ± 0.6	0.00 ± 0	0.073 ^{a} ± 0	0.095 ^{ab} ± 0.007	1.85 ^{e} ± 0.07
67s	2.2 ^{b} ± 0.2	0.00 ± 0	0.067 ^{b} ± 0	0.093 ^{bc} ± 0	1.10 ^{g} ± 0.14
100s	1.9 ^{bcd} ± 0.1	0.00 ± 0	0.061 ^{c} ± 0	0.077 ^{e} ± 0.001	0.85 ^{h} ± 0.07
33s + 100w	2.0 ^{bc} ± 0	0.00 ± 0	0.049 ^{ef} ± 0	0.101 ^{a} ± 0.002	0.70 ^{hi} ± 0.14
67s + 100w	1.4 ^{de} ± 0.1	0.00 ± 0	0.046 ^{f} ± 0.004	0.097 ^{ab} ± 0.004	0.55 ^{i} ± 0.07
100s + 100w	1.0 ^{efg} ± 0	0.00 ± 0	0.037 ^{g} ± 0	0.096 ^{ab} ±0.001	0.20 ^{j} ± 0.14
33s + 67w	1.5 ^{cde} ± 0	0.00 ± 0	0.061 ^{c} ± 0	0.047 ^{g} ± 0.008	4.00 ^{a} ± 0.14
67s + 67w	1.0 ^{efg} ± 0	0.00 ± 0	0.052 ^{de} ± 0.004	0.086 ^{cd} ± 0.001	3.70 ^{b} ± 0.14
100s + 67w	0.8 ^{fg} ± 0.4	0.00 ± 0	0.049 ^{ef} ± 0	0.080 ^{de} ± 0.001	1.35 ^{f} ± 0.07
33s + 33w	1.3 ^{ef} ± 0.4	0.00 ± 0	0.064 ^{bc} ± 0.004	0.040 ^{g} ± 0.004	2.80 ^{c} ± 0
67s + 33w	0.8 ^{fg} ± 0.4	0.00 ± 0	0.061 ^{c} ± 0	0.072 ^{ef} ± 0.001	2.50 ^{d} ± 0.14
100s + 33w	0.5 ^{g} ± 0	0.00 ± 0	0.055 ^{d} ± 0	0.069 ^{f} ± 0	0.90 ^{gh} ± 0
LSD	0.56	0.00	0.005	0.008	0.24
MSE	0.25	0.00	0.002	0.004	0.11

Chemical analysis of soil revealed that the different types of soil used in this study contained almost no or undetectable amounts of carbonates. On contrary, the amount of soil bicarbonates as well as calcium carbonate significantly decreased after soil cultivation with stressed or unstressed alfalfa plants. This change may reflect the tendency of the studied alfalfa plants under all growth conditions to consume as much bicarbonates and calcium carbonate as possible probably as available inorganic carbon sources essentially required for enhanced plant growth and development. With respect to soil electric conductivity and total soluble salts, the change in theses soil chemical parameters was almost the same; as it is well known that soil electric conductivity results mainly from the availability of total soluble salts. The pattern of data cleared that soil electric conductivity as well as the amount of total soluble salts were reduced after cultivating the soil with undroughted plants, probably due to the plant consumption of soil soluble salts. On the other hand, soil cultivation with droughted plants increased soil electric conductivity with marked increase in the amount of total soluble salts perhaps because of the stress-stimulated root membrane leakage as previously postulated for soil Cl^- and K^+ content. For soil pH, sulphates, organic carbon and organic matter, these increased by cultivating the studied plants without the application of drought. The ability of plants to increase soil organic carbon and matter is usually the main cause of increased soil fertility. On the other hand, these parameters were found to decrease when the soil was cultivated with the droughted plants.

4. CONCLUSION

Based on the results of the present investigation, *Medicago sativa* can be employed as a plant with an efficient role in land remediation even under stress conditions. To introduce new plants to the regions that are both dry (due to drought) and infertile (because of high soil sand proportion), the studied alfalfa plant can be useful.

ACKNOWLEDGMENT

The authors are grateful to the researchers in Ecology Lab, Botany Department, Faculty of Science, Mansoura University, for their valuable consultations while undertaking the experimental section.

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