Algerian Journal of Arid Regions
Journal Algérien des Régions Arides *JARA*

Article

Physiological responses of cork oak seedlings (Quercus suber L.) grown on solid substrates amended with sludge

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Abstract : Sludge are collectively used as amendment in various regions. They play an important role in the stability of grounds. Only, these Sludge contain (according to the technique of used purge) certain chemical elements which can be toxic for the organisms which would be in contact and vegetables are the most vulnerable in this toxicity. The chemical analyses of certain substances, namely, the proline, the soluble sugars and the chlorophyll allowed us to determine which stressful effect can lead Sludge on the oak cork and in which proportion. These results show that Sludge have a significant effect on the biochemical parameters and the stress provoked by these last ones is more marked on the substratum S4 in 80 % of Sludge.

Keywords : Bioindicator; chlorophyll; oak cork, prolines; residual sludge; stress; soluble sugars

Received : 06 June 2022
Accepted : 16 June 2022

Citation : Karoune, S.; Kechebar, M.S.;Deghiche-Diab, N.; Saad, S. Physiological responses of cork oak seedlings (*Quercussuber* L.) grown on solid substrates amended with sludge. *Journal Algérien des Régions Arides* 2022, 14 (2): 103–109.

1. Introduction

The physico-chemical composition of residual sludge, (fertilizers, heavy metals and salts) can cause disturbances in plants by inducing them in different types of stress (saline, water and osmotic) [1]. Their accumulation has been demonstrated in many species and in different stress, osmotic, water and thermal situations [2]; soluble sugars; abscisic acid (ABA) which is considered to be a stress hormone which would lead the plant to take protective measures against attacks, especially at the approach of low temperatures [3]. Photosynthetic activity can also be affected by environmental constraints. The recovery of residual sludge by spreading seems the most effective means, from an ecological and economic point of view, namely that this practice has a double objective: Take advantage of the natural biological capacities of soils to "digest" the sludge and reintroduce the elements into the natural cycles, Enhance the fertilizing properties of residual sludge for crops.

The use of residual sludge from urban wastewater treatment plants in forestry seems a priori to be less of a problem than in agriculture. In fact, the risks of toxicity to humans, for example by passage of heavy metals through the food chain, do not exist. As plant material, we chose the cork oak (*Quercussuber* L) which is a remarkable Algerian forest species, it presents a great economic value thanks to its physiological peculiarity which distinguishes it from other woods (production of cork), especially since this species is rare, since its distribution air is limited to the Mediterranean rim.

In Algeria the current state of the cork grove is worrying (stand at the end of its exploitation, deficiency of natural regeneration, low yield, gradual decrease in cork harvested annually), hence the absolute need to carry out actions of renovation and of forest rejuvenation. According to [4], examples of cork oak regeneration in Algeria are rare or even non-existent, in this context, recourse to planting can be recommended. Nurseries are currently unable to ensure a supply of quality cork oak plants, and this is due to the cultivation method practiced by nurserymen.

Indeed, in these so-called ground nurseries, the breeding of the plants is done in a traditional way, the containers used being plastic bags and the sowing is generally done in a substrate made up of a mixture of soil and sand 'oued, which causes root malformations which condemn, from the start, the success of reforestation. The use of other growing media is extremely expensive, however residual sludge, rich in organic matter and nutrients; nitrogen, potassium and phosphorus, can replace chemical fertilizers and serve as a basis for the preparation of a less expensive and more available growing medium. For this test, a physicochemical characterization of the sludge was made and its influences on the seedlings of the cork oak (*Quercus suber* L) from a biochemical point of view.

2. Materials and Methods

2.1. Used materiel

For this experiment, we used acorns from cork oak. They were collected from the ground at the end of November at IBN BADISS from the forest of the Wilaya de Constantine. They have a brown color, length varying between 2.5 and 4cm and a diameter between 1 and 1.5cm. These acorns were delivered to us by the nursery of DjebelOuahch – Constantine.

The sludge used in this experiment was produced in September 2006, it is dark black in color, unpleasant odor. It was taken at random from drying beds (both on the ends of the beds than in the center). These sludges are considered to be stable, dried out, in a solid state, the particles of which have an average size of about 6mm.

The soil was taken from a forest soil of the surface horizon "A", just at the below the humus layers about 10 to 15cm from the soil surface, at the level of the DjebelOuahche reserve.

2.2. Mixtures and substrates

The mixtures used consist of soil and residual sludge. By this test, we are trying to make a physicochemical characterization of the mud, and to see what will be the behavior of the cork oak plants in these substrates, with a view to substituting the mixture assumed ideal (50% blond peat + 50% composted pine bark) **[**5] and economically expensive, by locally available and less expensive materials. The substrates were mixed by hand and put into plastic pots. The composition and the name of the different substrates tested is giving in Table 1.

Table 1. Substrates and their composition.

Mixtures		
Substrates	**Soil**	**Sludge**
S 1	90%	10%
S 2	80%	20%
S3	60%	40%
S4	20%	80%
Control	100%	0%

2.3. *Experimental device*

The tests are carried out in complete random blocks with 10 repetitions. Each block is composed of 5 pots, each containing 3 acorns and each corresponding to a substrate with a total of 150 plants for the whole device.

2.4. Dosage of substances

The main substances are assayed at the level of the various organs of the seedlings (leaves stem and roots) at the apparent rest stage of the second wave of growth (the assay is carried out on the organs of the two waves (V1 and V2).

The proline contents were determined according to the method described by [6], soluble sugars by the method of [7] and chlorophyll by the technique of [8].

2.5. Statistical Analysis

All analyses were done in triplicate. Results were expressed as means ± standard deviations. The data were statistically analyzed using the Minitab 2000 statistical software. An independent t-test was used for comparison of means between groups. One-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference test were used to compare means among groups.

3. Results

3.1. Effect of sludge on proline accumulation

The comparison of the effect of different substrates (Figure 1) on the proline content of leaves, stems and roots in cork oak plants (*Quercussuber* L.), at rest stage apparent second wave of growth, shows that the maximum proline value is found in roots of substrate S4 (11.70µmol/mg MS) and the minimum value was recorded in the leaves of the first growth wave at substrate S1(0.34 µmol/mg MS).

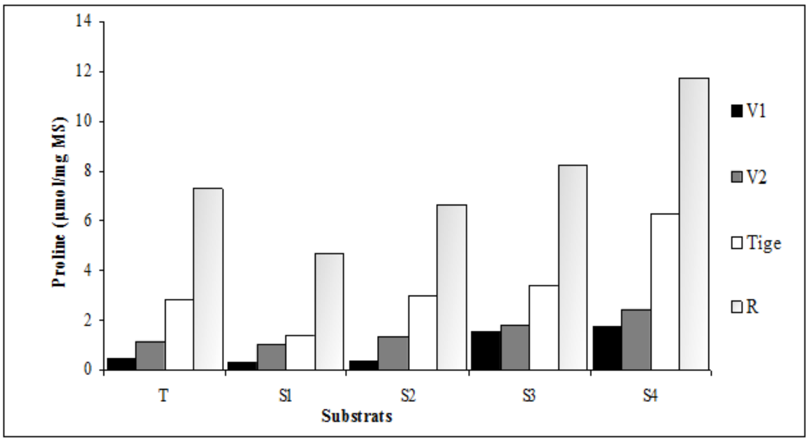

Figure 1. Proline content in different seedling organs from cork oak to different substrates.

According to the results obtained, the proline content of the roots increases in cork oak seedlings with increasing doses of sludge. The analysis of variance indicated that there is a highly significant difference on the one hand between the different substrates and on the other hand between the different organs (Table 2)

Table 2. Proline variance analysis.

Source	**DL**	**SC**	**CM**	**F**	**P**	**Signification**
Substrat	4	91.741	22.935	34.97	0.000	highly significant***
Organes	3	422.635	140.878	213.70	0.000	
Interaction	12	36.954	3.080	4.67	0.000	
Erreur	40	26.370	0.659			
Total	**59**	577.701				

The Newman and Keuls test at the 5% threshold for the substrates revealed three homogeneous groups (Table 3), it was noted that the substrate "S4" is assigned to the group (A) recording the highest values, the substrate "S3" was assigned to group (B) and the substrates "T, S1, S2" were assigned to the group (C) by registering the lowest value.

Table 3. Average ranking of different substrates according to proline contents.

Substrates	The average	Groups		
4	4	A		
3	3		B	
T	12			C
2	40			C
1	**59**			C

The proline content is lower in the plants of the substrates S1, S2 and the control also, this result was also confirmed by [9] who reported that the proline content is low under normal conditions. Different result was obtained from the S3 and S4 substrates where the maximum values were recorded, that is possible due to the adaptive strategy of cork oak seedlings.

For all the substrates, the accumulation of proline is lower in the leaves of the first wave of growth and it is higher in the leaves of the second wave, this is confirmed by [9] which shows that the accumulation of proline is influenced by internal factors: the age and arrangement of the leaf at plant level. The production of proline is frequently associated with stress (drought, high salinity, viral attack, transition from vegetative to floral state, etc.).

3.2. Effect of sludge on the accumulation of soluble sugars

The highest soluble sugar content was recorded in the second wave (substrate S4 = 35.53µmol/mg MS), whereas the minimum value was recorded from the rod (substrate S1 =3.22 µmol/mg DM) (Figure 2).

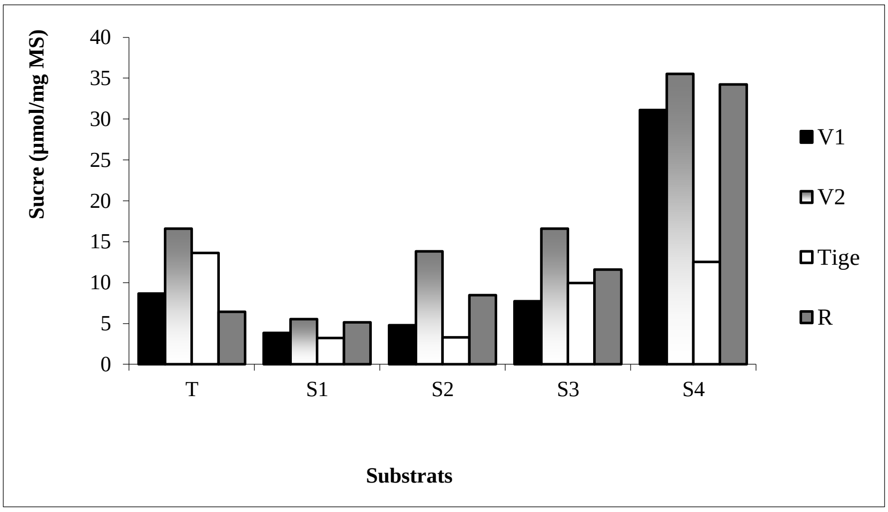

Figure 2. Proline content in different seedling organs from cork oak to different substrates.

The most important thing to note from this graphical representation is that the highest soluble sugar content was recorded for substrate S4 issue from different organs and leaves. This content is higher in the second wave than in the first, and it is equivalent for all the substrates. These results were confirmed through variance analysis which reveals highly significant differences between the different substrates and between the different organs (leaves, stem and root) (Table 4).

Table 4. Analysis of variance for soluble sugars.

Source	**DL**	**SC**	**CM**	**F**	**P**	**Signification**
Substrate	4	4112.29	1028.07	55.19	0.000	highly significant***
Organs	3	658.4	219.5	11.78	0.000	
Interaction	12	901.9	75.2	4.03	0.000	
Error	40	745.1	18.6			
Total	**59**	6417.8				

The Newman and Keuls test at the 5% cutoff shows three groups, where we note the assignment of substrate S4 to group (A) and the substrates T, S3 in group (B) the substrates S1 and S2 in the last group (C), recorded the lowest content (Table 5).

Table 5. Classification of the means of different substrates according to the soluble sugar contents

Substrates	**The average**	**Groups**		
4	28.348	A		
3	11.462		B	
T	11.324		B	
2	4.436			C
1	**7.589**			C

The comparison of means for the different organs brings out two homogeneous groups (Table 6). The first group includes the leaves and the stem and the second represented by the roots, where the soluble sugars are accumulated in the same way in the leaves and the stem. In contrast to the accumulation of proline, soluble sugars were less accumulated by the roots than by leaves and stems.

Table 6. Ranking of the average of different organs according to the soluble sugars content

Organs	**The average**	**Groups**	
Wave1	11.225	A	
Wave 2	17.604	A	
Root	13.177	A	
stem	8.521		B

Comparison of the effect of different substrates on the soluble sugar content in the different organs of cork oak seedlings at the resting stage apparent from the second wave of growth indicates that the highest soluble sugar contents are recorded in the second wave of growth and decrease in the other organs (leaf of the first wave, stem and root). This confirms that the young assimilator leaves are considered as sources of metabolites and not places of storage.

According to El-Iklil *et al*. [10]; sugars are transported to other growing organs at apparent rest, such as the apical meristem, between nodes being elongated while the roots behave like "wells". For the substrates S1, S2 and S3, we recorded low contents of soluble sugars, these levels can tell us that this metabolite could have been used by degrading himself, available and rapid source of carbon or reducing equivalents during stress [11].

Therefore, cork oak seedlings in these substrates do not appear to accumulate these sugars as a stress resistance metabolite. For the S4 substrate, very high levels were recorded compared to the control. This increase is actually a parameter of adaptation to stress conditions, allowing to constitute a guarantee for the maintenance of a high cell integrity [12]. The accumulation of soluble sugars is a parameter of adaptation to stress conditions. This type of adaptation allows the plant to perform its physiological functions normally despite a deterioration of its internal water state [13]. As with proline, sugars are accumulated in high quantities in the substrate S4 this confirms the results of [14] which notes that many cases have detected accumulations of sugars or their alcohol derivatives, also accompanied by an increase in one of the amino compounds (proline or glycinebetaine).

3.3. Effect of sludge on total chlorophyll (a+b)

The total chlorophyll content is the most used criterion to quantify the general condition of the plant, it is an excellent bio indicator of pollution and stress [15].

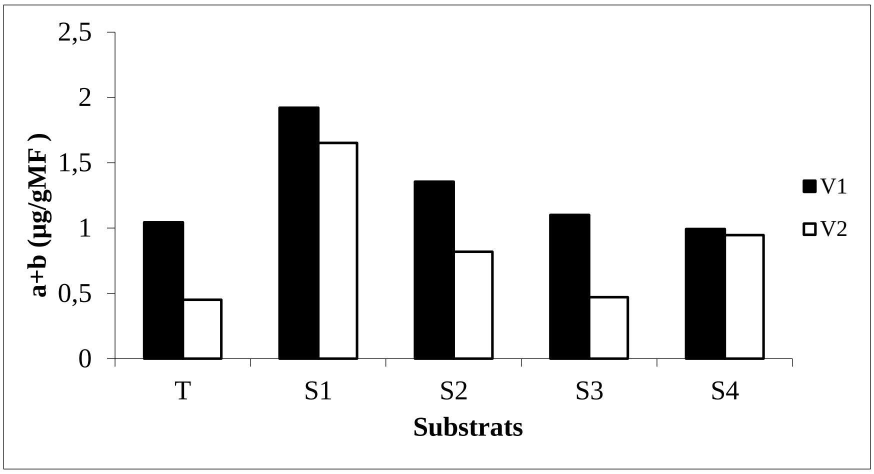

Figure 3. Chlorophyll content (a + b) in the different organs of cork oak seedlings on different substrates

The lowest value was recorded for the second wave in the S4 substrates and the maximum value was recorded from the first wave in the control. The total chlorophyll contents differ from one substrate to another (Figure 3); this is confirmed by means of the two-way controlled analysis of variance which reveals a highly significant difference between the substrates. Total chlorophyll contents for cork oak plants show a very highly significant difference between waves (Table 7).

Table 7. Analysis of variance for Cha and Chb

Source	DL	SC	CM	F	P	Signification
Substrate	4	4.2525	1.0631	21.59	0.000	highly signifi-
Organs	1	1.2863	1.2863	26.12	0.000	cant***
Interaction	4	0.3730	0.0932	1.89	0.151	N.Significant
Error	20	0.9850	0.0462			
Total	29	6.8968				

Cha: Chlorophyll a ,Chb: Chlorophyll b

It is noted that the total chlorophyll for the first wave follows the following decreasing order: S1>S2>S3>T>S4. For the second wave, it follows the following kinetics:

S1>S4>S2>S3>T

The maximum total chlorophyll contents for the two waves were recorded for substrate S1. The adding of slurry increases the total chlorophyll contents for all substrates except for substrate S4 where the addition of slurry decreases the total chlorophyll content in the first wave.

For the witness, this could be explained by the deficiency in mineral and fertilizer elements. [16] found a decrease in the rate of chlorophyll biosynthesis following a deficiency or Fe or Mg. A decrease in the synthesis of chlorophyll pigments following nitrogen deficiency is found by [17]. [18] find that heavy metals can interfere with the photosynthetic process through inhibition of chlorophyll synthesis enzymes, this confirms our results for substrate S4 where we recorded the lowest total chlorophyll rate for the first wave. An amendment in soybeans by residual sludge inhibits photosynthesis and nitrogen fixation [19]. The reduction in photosynthetic activity is observed in different cases of stress by several authors, saline [20,21], hydric [22], and oxidative [23].

5. Conclusions

The accumulation of chemicals as a response to stress induced by the addition of sludge differs from substrate to another. The Proline was accumulated in a large quantity in plants of the substrate S4 and it was lowest in the control. Soluble sugars accumulate in a proportional manner with the contribution of the sludge and this accumulation remains minimal for the witness. Chlorophylls (a) and (b) were mostly present in S1 and S2 substrates.

References

1. Ademe, A. Les boues d'épuration municipales et utilisation en agriculture. 2001. http://www.ademe.fr/collectivites/Dechets-new/mots-chiffres/chiffres-cles/dec.pdf.
2. Tahri, E. H.;Belabed A.; Sadki, K. Effet d'un stress osmotique sur l'accumulation de proline, de chlorophylle et des ARNm codant pour la glutamine synthétase chez trois variétés de blé dur (Triticumdurum). *Bul.I.S.Rabat*, 1998, 21, 81-87.
3. BoitardRaffaud, M. Les végétaux et les basses températures. DEUG Science et Vie. Univ. Pierre et Marie Curie, Paris6, 23p, 2002.
4. Harfouche, A.;Bekkar, H.; Belhou, O.; Graine, M. Quelques résultats à l'états juvénile sur la variabilité géographique du chêne liège (Quercus suber L) et stratégie d'amélioration génétique. An. Rech. For, Algérie, 2004, 37-58.
5. Argilier, C. ; Flaconnet, G.;Gruez, J. Production des plants forestiers. Guide de technique du forestier méditerranéen, CEMA-GREF, 1990,pp 85-95.
6. Troll, W.;Lindsley, G. *Aphotometric method for de determination of proline*. Biol. Chem., 1955., 215.
7. Dubois, M.; Gillesk, L.; Hamilton, J.K.; Reberg, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances . Analytical Chemistry, 1952, 28 (3).
8. Chaib, G. Teneur en proline des différents organes de blé dur : Essai d'explication de conditions d'accumulation sous manque d'eau. Biotechnologie végétales. Univ. Constantine, 30p, 1997.
9. Aissani, R.;Bousba, M. Croissance rythmiques de deux chênes méditerranéen : le chêne liège (Quercus suber L) et le chêne zeen (Quercus mirbeckiiDurieu.), 1992.
10. Hellmann, H.; Funck, D.; Rentsch, D.; Fommer, W.B. Hypersensivity of an arabidopsis sugar signaling mutant toward exogenous proline application. Plant. Physiologie., 2000, pp. 357-368.
11. Bensalem, M. Etude comparative de l'adaptation à la sécheresse du blé, de l'orge et du triticale, Tolérance à la sécheresse des céréales en zone Méditerranéenne. Diversité Génétique et Amélioration Variétale. INRA, 1993, pp 275-298.
12. De Raissac, M. Mécanismes d'adaptation à la sécheresse et maintien de la productivité des plantes cultivées. *Agronomie Tropicale*, 1992, 46(1), 29-37.
13. Levigneron, A.; Lopez, F; Varisuyt, G; Berthomien, P; Casse Delbar, T. Les plantes face au stress salin. Cahier d'agriculture, 1995.Thripathi, A.K.; Tripathi, S. Change in some physiological and biochemical characters in Albizialebbek as bioindicateurs of heavy metal toxicity .J. Environ. Biol, 1999, 20(2), 93-98.
14. Thripathi, A.K.; Tripathi, S. Change in some physiological and biochemical characters in Albizialebbek as bioindicateurs of heavy metal toxicity *. J. Environ. Biol*, 1999, 20(2), 93-98.
15. Ouzounidou, G.; Eleftheriou, E.P; Karataglis, S. Ecophysiological and ultrastructural effects of copper in Thlaspiochroleucum (cruciferae).*Can.J.Bot.* 1992. 70, 947-959.
16. Zhao, D.; Reddya, K.; Raja, ;Kakania, V.G.; ReddybEurop, V.R . Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *J .Agronomy*, 2005, 22, 91-403.
17. Chugh, L.K.; Sawhney, S.K. Photosynthetic activities of Pisumsativum seedlings grown in presence of cadmium. *Plant. Physiol. Biochem*, 1999, 37(4), 297-303.
18. Heckman, J.R.; Angle, J.S.; Chaney, R.L. Residual effects of sewage sludge on soybean : II.Accumulation of heavy metals *.J.Environ.Qual*, 1987,16(2), 113-117.
19. Almeidaviégas, R.; Gomes da silveira, J.A. Ammoniac assimilation and praline accumulation in young cashew plants during long term exposure to NaCl-salinity. *RevistaBrasileira de Fisiologia Vegetal*. 1999, 11(3), 153-159.
20. El-Iklil, Y.;Karrou, M.; Mrabet, R.; Benicho, M. Effet du stress salin sur la variation de certains métabolites chez *Lycopersiconsheesmanii.Can. J. Plant .Sci*. 2002, 82, 177-183.
21. Tabaeizadeh, Z. Drought-Induced responses in plant celles*. Int Rev Cytol*, 1998, 182,193 247.
22. Luna, C.M.; Gonzalez, C.A.; Trippi, V.S.; Oxidative damage caused by excessof copper in oat leaves. *Plant Cell Physiol*. 1994,35, 11-15.