

Evaluation of mud-based culture substrate on the regeneration of verbena cuttings (*Verbena triphylla*)

Hannachi Abdelhakim
Department of Agronomy, University August 20, Skikda, Algeria
Corresponding author: hakhannachi@yahoo.fr

Received on: 19/01/2023 Accepted on: 25/04/2023 Published on: 04/05/2023

ABSTRACT

Aim: The aim of this study was to assess the quality of sludge by a physico-chemical approach and see the effects of its use in improving the culture substrate for increasing the productivity of verbena cultivation. , and find a solution to the sludge from treatment plants by replacing chemical fertilizers.

Materials and Methods: It was carried out at the level of botanical garden of the University of August 20, 1955 in Skikda (Algeria), the botanical garden with a surface of one hectare, with collections of ornamental trees and exotic plants. The test was placed in a greenhouse under uniform conditions to ensure that all containers are placed under the same conditions.

Results: Analysis of variance revealed that all significant differences in morphological point were not due to the block factor but only to different substrates. The results allow us to affirm that the culture substrate based sludge can be recovered, their characteristics acquire their potential for use. The morphological point of view, the supply of sludge used as a culture substrate was beneficial for improving plant growth verbena except for the substrate S4 90% of sludge which had opposite effects compared to other substrates.

Conclusion: It was concluded that the latter is very rich in organic matter, with a value of 55.67%, according to the AFNOR standard, a value of 40 to 65% seems very good for a recovery of this mud as a growing medium.

Keywords: Sesame, water harvesting systems, supplementary irrigation, rain fed.

How to cite this article: Hannachi A (2023). Evaluation of mud-based culture substrate on the regeneration of verbena cuttings (*Verbena triphylla*). J. Agri. Res. Adv., 05(02): 06-17.

Introduction

Substrate cultivation means a cultivation method that consists of growing plants in a mineral or organic substrate (Wieland, 2010). Among the arguments that weigh with all their weight in favor of the culture on substrate, it is undoubtedly the profitability and the durability. The possibility of supplying the market over longer periods and the assurance of regular production and deliveries are significant advantages of growing on substrate (Gricourt, 2009).

The recovery of residual sludge as a culture substrate seems the most effective way, from an economic point of view, to take advantage of the natural biological capacities of culture substrates to "digest" the sludge and reintroduce its elements into the natural cycles and enhance the fertilizing properties of residual sludge for crops (Pommel, 1974).

The beneficial effects of using sludge-based substrates in agriculture. These have been proven by many researchers. Indeed, their application improves the physical, chemical and biological properties of the soil which results in an increase in biomass and plant yield. (Benterrouche, 2007).

In this context, the objective of this work is to assess the quality of sludge by a physico-chemical approach and see the effects of its use in improving the culture substrate for increasing the productivity of verbena cultivation. , and find a solution to the sludge from treatment plants by replacing chemical fertilizers which are economically expensive with a sludge which may have the same performance, but which will remain, from an economic point of view, less expensive and available.

Materials and Methods

This work was carried out at the level of the botanical garden of the university of August 20, 1955 in Skikda (Algeria), the botanical garden with a surface of one hectare, with collections of ornamental trees and exotic plants. The test was placed in a greenhouse under uniform conditions to ensure that all containers are placed under the same conditions.

Waste sludge : Sludge is residual sediment from biological, physical or physicochemical wastewater treatment or pre-treatment facilities (Déléry et al., 2007). This treatment involves mechanical, physicochemical and biological processes, the application of which depends on both the characteristics of the water to be treated and the degree of purification desired (Jarde, 2002).

Excess sludge from the return sludge pumping station flows into two sludge thickening tanks (Bouallegue, 2011). These thickeners are of the scraper grid type. The dried sludge remains in the open air until the plant managers decide on its final destination (landfill, fertilizer for farmers) (Bouallegue, 2011).

Floor : This is an agricultural soil sampled at the level of the superficial horizon "A", just below the humus layers at approximately 10 to 15cm from the surface of the soil, at the level of the botanical garden of the university 20 august 1955 (Skikda-Algeria).

Plant material : For our experiment, we used: verbena cuttings which were harvested from the plant at the end of January 2015 in Tamalous, Wilaya of Skikda (Algeria). They have a yellow color and length varying between 15 and 20 cm and a diameter between 0.5 and 1 cm.

Types of containers : Different types of containers are used around the world (terracotta pots, polyethylene bag, buckets, WM containers, etc). In our case, we used, during our tests, plastic bags whose dimensions are as follows:

- Depth: 20cm
- Substrate capacity: 2 kg

The bottom of these containers is perforated (3 holes) to allow the drainage of water and thus avoid asphyxiation of the roots.

Methods

Substrate preparation

The preparation of the substrates is summarized in the following steps:

Sieving : The dry sludge and the soil were sieved at 2mm, in order to eliminate the unground residues and to have a homogeneous material.

Mixtures and choice of substrates : The mixtures used are composed of soil and residual sludge (Table 1). Through this test, we seek to carry out a physico-chemical characterization of the mud, and to see what the behavior of the verbena plants will be in these substrates, in order to substitute the supposedly ideal mixture (0% Mud and 100% soil, 10 % Mud and 90% Soil, 25% Mud and 75% Soil, 45% Mud and 55% Mud, 90% Mud and 10% Soil).

Table 1. Composition and denomination of the substrates tested

	Mixed	
Substrate	Soil	Mud
Witness	100%	0%
S1	90%	10%
S2	75%	25%
S3	55%	45%
S4	10%	90%

The substrates were mixed manually and put in plastic containers of about one liter. After the containers were filled, they were installed on the production tarps.

Experimental protocol

It was opted for 5 trials with 4 repetitions of each trial each corresponding to a substrate. Each 4 containers called a test (sample) and each test different from the other (the percentages of mud and soil). This gives 1×4= 4 Plants per trial and 20 plants for the entire experimental device.

Sowing

The cuttings were sown on February 10, 2015 during vegetative rest and each container containing a cutting.

Watering

Irrigation is carried out, with tap water, twice a week; when the containers in the greenhouse; which allowed us to keep the containers at their capacity in the field, the number of waterings is increased in the months of April and May to 3 times a week as soon as the surface of the container dries out due to the high heat in the greenhouse. For this reason we will take them out of the greenhouse.

Field capacity is the mass of water remaining in the soil, which resurfaces after rapid drainage for one to two days (expressed as a % of dry soil weight). This water will be gradually used by the plant and evaporated by the soil.

Weeding : Weeding of weeds was done manually.

Characteristic of solid substrates

Sludge and soil sampling

The samples brought back to the laboratory are dried in the open air for a few days, then the large part has been crushed and sieved in a 2mm sieve in order to eliminate the uncrushed residues and to have a homogeneous material to add them to the soil and form the different substrates (Tests).

Physicochemical analyzes of soil and sludge

The analysis of the samples taken will give us important information on the substrates based on sludge and soil.

The following tests were carried out on the crushed and sieved fraction:

• Electrical conductivity;
• pH;
• Particle size;
• Organic matter.

Data Analysis

As part of this study, we will determine morphological parameters (number of buds, number of leaves, number of branches and length of branches) and chemical parameters of the substrates on control plants grown in a soil-based culture substrate (100% Soil), then on plants grown in mixtures of soil and sludge, over 4 months, at different stages of development (bud burst, growth).

Wet was expected to note significant differences between the control plants (Soil) and the plants of different mixtures (Soil+Sludge).

The question was therefore whether the difference observed was sufficient to reject the hypothesis that treatments with sludge do not have an effect on the production of the biomass of Verbena triphylla plants or not. To verify this hypothesis, it was carried out the analysis of variance for all the parameters studied. The analysis of variance (ANOVA) is generally best suited for comparing experimental data. It tests the hypothesis of the equality of the means of several samples, in other words, the homogeneity of the means of these samples. The analysis of variance thus makes it possible to test the effect of one or more factors on the data studied, and to be able to compare these differences with a control. Its application is subject to several conditions, including:

• The random and independent character of the samples;
• Normal distribution;
• Equality of variances.

Practically, through ANOVA, we try to find out if the variability observed in the data is only due to chance, or if there are indeed significant differences between the samples. The highlighting of the significant differences between the treatments was carried out by means of an ANOVA, with repeated measures, with statistical software.

- Average = $\bar{X} = \sum X_i / n$

- Variance = **var** (X)= $\sum(\bar{X}_i - X)^2 / n - 1$

-Standard deviation=

$$\delta\sqrt{var}(X) = \sqrt{\sum}(X_i - X)^2 / n - 1$$

It was deemed useful to carry out physico-chemical analyzes of the residual sludge from the treatment plant and those of the various mixtures in order to deduce firstly whether the sludge can have a direct influence on the behavior of the plants and secondly time what their effect will be on the texture and structure of the soil in the different substrates.

The results of the physico-chemical analyzes of the mud and the substrates tested are carried out at the physical and soil chemistry laboratory of Skikda University.

The biochemical parameters allow us to decide on the degree of adaptability of lemon verbena plants to different substrates.

All these results will be compared to a control made up of agricultural soil, in order to be able to conclude whether the sludge has really brought us an advantage or a disadvantage and see which balance will prevail over the other.

Results and Discussion

Results of physicochemical analyzes of sludge

The results of the physical analyzes of the sludge were grouped (Table 2).

Table 2. Physico-chemical analyzes of sewage treatment plant sludge

Parameters		Waste sludge	Standards
Granulometry (%)	Clay	10,85	
	Fine silt	39,97	
	Coarse silt	17,29	
	Fine sand	7,94%	
	Coarse sand	21 ,89	
pH		6,96	
E.C		1640	
O.M		57,67%	40-65 (%)

Norm according to Lacée, (1985)

Table 3. Physical results of culture substrates tested

Parameters		Soil	S1 :10% Mud	S2 :25% Mud	S3 :45% Mud	S4 :90% Mud
Granulometry (%)	Clay	2,74%	17,93%	16,53%	15,40%	11,2%
	Fine silt	11,36%	32,97%	40,75%	49 ,43%	64, 36%
	Coarse silt	46,90%	21,45%	19,25%	19,25%	6,73%
	Fine sand	14 ,59	19, 23%	10,15%	1 ,02%	0,71%
	Coarse sand	24,41%	8,66%	13, 32%	14,9%	17%

Results of physical analyzes of culture substrates tested

Particle size : The results of the physical analyzes of the substrates tested were grouped (Table 3).

Organic matter : The results of the analyzes of the organic matter of the substrates tested were grouped (Table 4).

Table 4. Organic matter analyzes of the culture substrates tested.

Substrates	**Organic matter**
T	11,55%
S1	22,45%
S2	33,15%
S3	43,88%
S4	50,1%

Morphological characters

The relative results of the morphological characters of all the trials were presented by:

Number of buds

The number of buds for the first test

According to the results obtained (Table 5), the average number of buds for the 1st trial for all of (T1, T2, T3, T4) after 23 days of sowing was 2.5; the variance is 1.25 and the standard deviation was 1.29.

Table 5. The number of buds for the first test

Test	**Number of buds**
T_1	2
T_2	4
T_3	3
T_4	1
$\bar{x}$	2,5
VAR	1,25
δ	1,29

The number of buds for the second test

According to the results obtained (Table 6), the average number of average buds for the 2nd trial for all of (SA1, SA2, SA3, SA4) after 23 days of sowing was 5, the variance was 0.66 and l standard deviation was 0.81.

Table 6. The number of buds for the second test

Test	**Number of buds**
SA_1	4
SA_2	5
SA_3	6
SA_4	5
$\bar{x}$	5
VAR	0,66
δ	0,81

The number of buds for the third test

According to the results obtained (Table 7), the average number of average buds for the 3rd trial for all of (SB1, SB2, SB3, SB4) after 23 days of sowing was 9.25, the variance was 7, 58 and the standard deviation was 2.75.

Table 7. The number of buds for the third test

Test	**Number of buds**
SB_1	8
SB_2	12
SB_3	6
SB_4	11
$\bar{x}$	9,25
VAR	7,58
δ	2,75

The number of buds for the fourth test

According to the results obtained (Table 8), the average number of buds for the 4th test for all of (SC1, SC2, SC3, SC4) after 23 days of sowing was 15.75; the variance was 7.58 and the standard deviation was 2.75.

Table 8. The number of buds for the fourth test.

Test	**Number of buds**
SC_1	13
SC_2	14
SC_3	17
SC_4	19
$\bar{x}$	15,75
VAR	7,58
δ	2,75

The number of buds for the fifth test

According to the results obtained (Table 9), the average number of buds for the 4th test for all of

(SD1, SD2, SD3, SD4) after 23 days of sowing was 16.75, the variance was 2.68 and the standard deviation was 1.89.

Table 9. The number of buds for the fifth test

Test	Number of buds
SD_1	14
SD_2	18
SD_3	17
SD_4	18
−	16,75
VAR	2,68
	1,89

Number of sheets

The number of sheets for the first test

According to the results obtained (Table 10), the mean number of leaves for the 1st trial for all of (T1, T2, T3, T4) at 5 weeks is 36.95; the variance was 178.74 and the standard deviation was 12.18.

The number of sheets for the second test

According to the results obtained (Table 11), the average number of leaves for the 2nd trial for all of (SA1, SA2, SA3, SA4) at 5 weeks was 136.75; the variance was 56.47 and the standard deviation was 7.11.

The number of sheets for the third test

According to the results obtained (Table 12), the average number of leaves for the 3rd test for all of (SB1, SCB2, SB3, SB4) at 5 weeks was 185.45; the variance was 127.54 and the standard deviation was 10.68.

The number of sheets for the fourth test

According to the results obtained (Table 13), the average number of leaves for the 4th test for all of (SC1, SC2, SC3, SC4) at 5 weeks was 249.2 the variance was 127.96 and the difference type was 10.99.

The number of sheets for the fifth test

According to the results obtained (Table 14), the average number of leaves for the 5th test for all of (SD1, SD2, SD3, SD4) at 5 weeks was 1.65, the variance was 1.65 and the difference typical was 0.57.

Table 10. The number of sheets for the first test.

Essai	Nombre des feuilles $1^{ére}$ semaine	Nombre des feuilles $2^{éme}$ semaine	Nombre des feuilles $3^{éme}$semaine	Nombre des feuilles $4^{éme}$semaine	Nombre des feuilles $5^{éme}$semaine	Moyenne
T_1	12	18	25	35	47	
T_2	16	28	44	60	76	
T_3	24	36	48	60	72	
T_4	22	25	28	30	33	
$\bar{x}$	18,5	26,75	36,25	46,25	57	36,95
VAR	30,33	55,58	130 ,91	256,25	420,66	178,74
δ	5,50	7,45	11,44	16,007	20,51	12,18

Table11. The number of sheets for the second test.

Test	Number of sheets 1st week	Number of sheets 2nd week	Number of sheets 3rd week	Number of sheets 4th week	Number of sheets 5th week	Average
SA_1	124	136	146	158	170	
SA_2	109	118	133	147	162	
SA_3	101	114	130	147	165	
SA_4	107	119	134	148	169	
$\bar{x}$	110,25	121,25	135,75	150	166,5	136,75
VAR	95,58	94,91	49,58	28,66	13,66	56,47
δ	9,77	9,74	7,04	5,35	3,69	7,11

Table 12. The number of leaves for the third test.

Test	Number of sheets 1st week	Number of sheets 2nd week	Number of sheets 3rd week	Number of sheets 4th week	Number of sheets 5th week	Average
SB_1	136	155	178	205	232	
SB_2	139	166	191	218	241	
SB_3	125	151	176	202	229	
SB_4	165	179	193	207	221	
$\bar{x}$	141,25	162,75	184,5	208	230,75	185,45
VAR	286,91	157,58	76,33	48,66	68,25	127,54
δ	16,93	12,55	8,73	6,97	8,26	10,68

Table 13. Number of leaves for the fourth trial

Test	Number of sheets 1st week	Number of sheets 2nd week	Number of sheets 3rd week	Number of sheets 4th week	Number of sheets 5th week	Average
SC_1	182	212	238	268	298	
SC_2	190	220	252	284	315	
SC_3	188	213	241	271	301	
SC_4	215	240	263	283	310	
$\bar{x}$	193,75	221,25	248,5	276,5	306	249,2
VAR	212,25	168,91	129,66	67	62	127,96
δ	14,56	1299	11,38	8,18	7,87	10,99

Table 14. The number of leaves for the fifth test

Test	Number of sheets 1st week	Number of sheets 2nd week	Number of sheets 3rd week	Number of sheets 4th week	Number of sheets 5th week	Average
SD_1	6	0	0	0	0	
SD_2	9	0	0	0	0	
SD_3	6	0	0	0	0	
SD_4	12	0	0	0	0	
$\bar{x}$	8.25	0	0	0	0	1.65
VAR	8.25	0	0	0	0	1.65
δ	2.87	0	0	0	0	0.57

The number of vines :

The number of branches for the first attempt

According to the results obtained (Table 15), the average number of vines for the 1st test for all of (T1, T2, T3, T4), on April 5, 2015 was 2.5; the variance was 1.25 and the standard deviation was 1.29.

Table 15. The number of branches for the first test

Test	The number of vines
T_1	2
T_2	4
T_3	3
T_4	1
$\bar{x}$	2,5
Var	1,25
δ	1,29

The number of branches for the second test

According to the results obtained (Table 16), the average number of vines for the 2nd test for all of (SA1, SA2, SA3, SA4), on April 5, 2015 was 4; the variance was 0.5 and the standard deviation was 0.70.

Table 16. The number of branches for the second test

Test	The number of vines
SA_1	4
SA_2	5
SA_3	6
SA_4	5
$\bar{x}$	4
Var	0,5
δ	0,70

The number of branches for the third test

According to the results obtained (Table 17), the average number of vines for the 3rd test for all of (SB1, SCB2, SB3, SB4), on April 5, 2015 is 9.25; the variance is 5.68 and the standard deviation is 2.45.

Table 17. The number of branches for the third test

Test	The number of vines
SB_1	8
SB_2	12
SB_3	6
SB_4	11
$\bar{x}$	9,25
Var	5,68
δ	2,45

The number of branches for the fourth test

According to the results obtained (Table 18), the average number of vines for the 4th test for all of (SC1, SC2, SC3, SC4) on April 5, 2015 was 15.75; the variance was 5.68 and the standard deviation was 2.75.

Table 18. The number of branches for the fourth test.

Test	The number of vines
SC_1	13
SC_2	14
TSC_3	17
SC_4	19
$\bar{x}$	15,75
Var	5,68
δ	2,75

The number of branches for the fifth test

According to the results obtained (Table 19), the average number of vines for the 5th test for all of (SD1, SD2, SD3, SD4), on April 5, 2015 was 0; the variance was 0 and the standard deviation was 0.

Table 19. The number of branches for the fifth test

Test	The number of vines
SD_1	0
SD_2	0
SD_3	0
SD_4	0
$\bar{x}$	0
Var	0
δ	0

The length of the branches in (cm)

According to the results obtained (Table 20), the average length of the vine branches for the various trials at 5 weeks was summarized.

Interpretation of results

The texture made it possible to assess permeability, water retention, aeration and cation exchange capacity (Baize, 1988). According to data (Table 21), the particle size analysis of the sludge from the treatment plant used in our experiment consists of the following fractions:

- thin: 10.85%
- average: 57.26%
- Coarse: 31.83%

If we report these results on the American textural triangle (USDA), it can be concluded that our sludge has a silty texture.

The average pH of station sludge is around 6.95, it is less than 7, therefore, considered as weakly acidic (Leclech, 2000), this pH value was considered acceptable because it is between 5 and 8, which makes the mineral elements assimilable for the plant.

The electrical conductivity was around 1640µS/cm, which indicates that the sludge is saline (EC greater than 1000µS/cm).

After carrying out the analyzes of the organic matter, the rate of the latter was estimated at around 57.67%, this value according to Duthil, (1971) was considered high.

According to AFNOR standards. (1994), a value of 40 to 65% seems very good for recycling this sludge in culture substrates. Therefore sludge cannot be used as a culture substrate on its own, but mixing it with other substrates can give satisfactory results.

With regard to the contents of metallic trace elements, the table represents the contents of the latter according to Bensouiki and Aib; Bourekeb (2002), who worked on sludge from the same treatment plant.

According to the results (Table 22), the values both in the years 1998-2001 or the year 2002 never exceeded the norms.

The sludge from the treatment plant is far from being contaminated by cadmium and that was also the case for other metals (Cu, Zn and Pb), which makes them recoverable in culture substrate. It consists of classifying the elements of the soil according to their size and determining the percentage of each fraction. The comparison of the different fractions, with a textural triangle, defines the type of soil (Soltner, 2000 and Tessier, 2005).

The particle size analysis of the different fractions for the substrates tested was classified according to the American textural triangle (USDA). The control, consisting of agricultural soil from the botanical garden at the university of August 20, 1955, was composed of several fractions dominated much more by silt and sand, the percentage of each fraction was summarized in the table above and if we report the results of the different fractions on the textural triangle, we can only conclude that our soil has a sandy loam texture.

It can be seen from the table that the more the proportion of sludge increases, the more the fraction of fine silt and coarse sand increases, while that of clay decreases; thus if we report the results of the different fractions of the other substrates on the American textural triangle, it can that these substrates belong to the medium sandy loam textural class.

According to Roula (2005), the medium texture does not cause asphyxiation of the root system and allows good root development unlike the fine textures.

organic matter

A very high content can be a sign of remarkable fertility, and soil with a very low organic matter content is generally fragile and not very productive (Pousset, 2002).

According to the figure shows that the rate of the lowest organic matter is recorded for the control which represents the soil of the botanical garden with a value of 11.55%.

The contribution of sludge increases the organic matter content of which all the substrates (except the control), they are rich in organic matter they present values which vary between

22.45% for the S1 substrate and 33.15% for the substrate S2 and 43.88% for the substrate S3 and for the substrate S4 which presents the highest value with a rate of 50.1% of M.O. of the substrates tested was considered high.

The pH was determining factor for the availability of nutrients in the soil. The decrease in this induces a significant drop in the assimilability of the main soil fertilizing elements (Boudreault, 2010).

The substrate must have a pH between 5 and 8, outside its limits, the plants will face mineral nutrition problems (Benseghir 1996).

From the figure we can see the average pH of the different substrates tested is weakly acidic (Leclech, 2000), it is proportional to the doses of the sludge (except the control), it varies from a very low value for the control which corresponds at a value of 6.05 (weakly acidic) up to the highest value in the S4 substrate which was close to neutral and corresponds to 6.9. And according to Gagnard et al., (1988) the substrates S1, S2 and S3 were very slightly acidic.

It was with a pH close to neutrality that the maximum number of elements was available. (Callot et al, 1982).

Analysis of variance for pH indicates that there was a very significant difference for the different substrates used (Table 22).

The EC can be an indication of the availability of mineral elements in the culture medium. When the saline concentration is too high, the roots develop badly and can even be burned, consequently the growth of the plant will be slowed down according to (IPCS, 1998).

From the figure, it can be seen that the average electrical conductivity varies from 172.75 µs/cm for the control to 1239.5 µs/cm for the S4 substrate (figure 66). This conductivity was estimated at about 172.75 µs/cm and it was 6 times higher in the 90% sludge substrate (S4).

The different substrates were considered moderately salty with the exception of the S4 substrate which is highly salty according to (Baize, 2000).

Since the S4 substrate was highly salty, it can therefore pose risks to plant growth.

Salinity causes an increase in the osmotic pressure of the soil solution, thus preventing root absorption.

According to (Duchaufour, 1965) Saline soils hinder or prohibit the development of plants.

At high levels, the EC of the substrates has increased considerably, which results in an increase in salinity, this increase in soil salinity was probably due to mineralization of organic matter (Roula, 2005).

The analysis of variance for electrical conductivity indicates that there was a highly significant difference for the different substrates used (Table 23).

Effect of sludge on the morphological parameters of lemon verbena (Verbena triphylla)

The morphological results will give us an idea of the best dose for plant growth. These parameters would allow us to decide on the degree of adaptability of lemon verbena plants to different growing substrates (depending on the different doses of sludge).

Mud Effect on Bud Count : Bud burst is the first diagnosis of crop success. In most plants, buds bud when environmental conditions were favorable. Poor bud burst can have several causes related to climatic conditions (frost or cold, for example). (Geslin, 2000).

In order to determine the success rate of budburst of the plants for the different substrates tested, a count was made of the buds which were still alive after 23 days from the date of the establishment of the cuttings.

From the figure it can be seen that the addition of sludge acts differently, depending on the doses, on the average number of buds. Of which the highest average number of buds of the plants was recorded at the level of the S4 substrate corresponding to 90% of sludge compared to the control which represents a lower value than that obtained in the S1, S2 and S4 substrates.

This can be explained by the fact that the sludge will increase the number of buds and it can be deduced that all the substrates have a satisfactory number of buds. $P>3.06$ So the analysis of variance for the average number of buds indicated that there was a significant difference between the different substrates tested (table 24).

Effect of sludge on the number of vines (The number of branches)

It was represented the number of branching plants for the different types of culture substrates tested. The number of ramifications was a good indicator of a good supply of water and mineral salts and a good biomass production by the plant. (Bouafia, 2012).

The average number of vine branches was different from one substrate to another, the maximum average number was recorded in the S3 substrate, then this number decreases with the decrease in the amount of sludge in the S1 and S2 substrates up to the control, however the S4 substrate because of its high electrical conductivity (a heavily salted substrate) gave a zero result.

P>3.06. Therefore, the analysis of variance for the number of vine branches indicated that there was a significant difference between the different substrates tested (Table 25).

Source of variances DL SCE CM P F

Between groups 4 6 14.5 153.12 43.89

3.06

Within groups 15 52.5 3.5

Total 19,667 35.10

Effect of sludge on the number of leaves

The number of leaves was a good indicator of a good supply of water and mineral salts and a good biomass production by the plant (Fiscchesser and Dupuitate, 1996). The data representd the number of leaves of Lemon Verbena plants for the different types of substrates.

It can be observed that the number of leaves of the Verbena triphylla plant is variable between the substrates tested, of which the greatest number of leaves was recorded in the substrate S3, with a value of 1224 it was higher by compared to the control which was recorded a value of 228, then come the plants lower in S1 and S2 substrates with values ranging from 666 for S1 and 923 for the S2 substrate. However the S4 substrate gave a zero result perhaps due to other factors such as the high concentration of salts.

P>3.06, Therefore, The analysis of variance for the number of leaves indicated that there was a significant difference for the different substrates used (table 26).

Table 20. The average length of the branches of the different trials.

Tests	T	S1	S2	S3	S4
The 1st wee	1,43	0,71	1,94	1,78	0
The 2nd week	3,68	1,95	4,44	4,02	0
The 3rd week	5,17	3,25	7,13	6,12	0
The 4th week	6,55	6,67	9,66	8,74	0
The 5th week	8,75	5,67	10,58	11,31	0
$\bar{x}$	5,11	3,65	6,75	6,39	0
VAR	7,72	6,22	12,93	14,16	0
δ	2,78	2,49	3,59	3,76	0

Table 21. Heavy metal content of sludge from the treatment plant

Heavy metals (ppm)	Mean values for years* 1998-2001-2002	Average values** 2002	Standards AFNOR
Cu	149	28,01 ± 0,025	1000
Pb	185	2,325 ± 0,01	800
Cd	3,13	<0,001	20
Zn	850	100 ± 0,01	3000

Table 22. Analysis of pH variance for the different substrates tested

Source of variances	DDL	SCE	CM	P	F
Between groups	4	0,29	0,07	140	3,06
Inside groups	15	0,008	0,0005		
Total	19	0,30	0,015		

Table 23. Analysis of variance of electrical conductivity for the different substrates tested.

Source of variances	DDL	SCE	CM	P	F
Between groups	4	2720594	680149	19994	3,06
Inside groups	15	510	34		
Total	19	2721104	143,216		

Table 24. Analysis of variance for the number of buds of the different substrates tested

Source of variances	DL	SCE	CM	P	F
Between groups	4	6 14,5	153,12	43,89	3,06
Inside groups	15	52,5	3,5		
Total	19	667	35,10		

Table 25. Analysis of variance for the number of vines of the different substrates tested

Source of variances	DL	SCE	CM	P	F
Between groups	4	6 14,5	153,12	43,89	3,06
Inside groups	15	52,5	3,5		
Total	19	667	35,10		

Table 26. Analysis of variance for the number of leaves of the different substrates tested

Source of variances	DL	SCE	CM	P	F
Between groups	4	120124 ,9	30031,2	28,42	3,06
Inside groups	15	15849,88	1056,65		
Total	19	135974,78	7156,56		

Mud effect on the length of the vine branches

From the data, there was a difference in the average length of the substrates used, from which it differs from one substrate to another, the maximum length is recorded in the substrate S3, then this length decreases with the decrease in the amount of sludge in the substrates but the S1 substrate has a lower value compared to the control may be due to the high number of ramifications (figure 68). However, the S4 substrate presents zero results because According to (Bouafia, 2012) the different doses of sludge with an electrical conductivity was highly salty which risks causing salt stress which affects the growth of the stem.

Conclusions

The results of the analysis of the sludge from the urban effluent treatment plant showed that the latter is very rich in organic matter, with a value of 55.67%, according to the AFNOR standard, a value of 40 to 65% seems very good for a recovery of this mud as a growing medium.

With regard to heavy metals, namely Zn, Cu, Pb, and Cd, the sludge contains levels much lower than those indicated by the AFNOR standard and which make them usable in the culture substrate technique.

The mud has a pH close to neutrality (6.96), its contribution to the soil does not present any risk as regards the absorption of the elements available to plants. Our mud (with an electrical conductivity value of 1640 µS/cm) is highly salty.

Mixing this sludge with the soil (10%, 25%, 45% and 90% sludge) makes it possible to produce substrates (S1, S2, S3 and S4) which have the following characteristics:

- Medium sandy loam texture which allows good root development,
- Weakly acidic pH which allows good bioavailability of mineral elements.
- Moderately salty substrates
- Average levels to be raised in organic matter.

The addition of sludge in well-determined proportions allowed us to increase the chances of budburst of verbena plants and the number of leaves and vines; and thus their growth in length, which currently presents in cultivation in the soil growth difficulties such as bud burst.

Although this work was carried out in the greenhouse, whose conditions are more or less constant throughout the test, it was considered useful to develop a complete random block work for the sole purpose of verifying the isotropy of our environment (the greenhouse).

This detail has been formally proven because the analysis of variance revealed that all the significant differences from the morphological and biochemical point of view are not due to the block factor but only to the different substrates.

The results obtained throughout this work allow us to affirm that the culture substrate based on residual sludge from the treatment plant can be valued, their physico-chemical characteristics give them a potential for use, not as substrate but rather as one of the constituents of our mixture which is based on sludge and agricultural soil.

The physico-chemical analyzes of the sludge from the treatment plant and the various mixtures allowed us in the first place to ensure that the latter are not toxic for the fragrant verbena plants because they contain a very small quantity of heavy metals. , and this quantity is well below AFNOR standards.

Analyzes of the different mixtures confirmed that the sludge improves soil texture.

The sludge gradually increases the proportions of silt and sand and therefore the water will be less retained, something which could be beneficial for the plants from the assimilability point of view.

The addition of sludge has allowed us to have substrates rich in organic matter.

The analyzes have shown that the addition of sludge increases the organic matter of the growing medium without modifying the pH of the medium too much, therefore the availability of nutrients in the soil will not be hindered. This richness has brought a plus for the substrate and therefore can subsequently be beneficial for the plants.

From the morphological point of view, the addition of sludge used as a culture substrate is beneficial to improve the growth of verbena plants except for the S4 substrate with 90% mud which has the opposite effects compared to the other substrates.

The culture substrate increases the number of leaves compared to the control, the growth in the number of vines is stimulated by the presence of sludge if they are compared to those obtained in the control (agricultural soil).

The S3 substrate at 45% sludge allowed the verbena plants to have the greatest number of buds and branches. For this same substrate, the plants that are there have the greatest number of leaves, the greatest biomass.

All these results remain at the experimental stage, but still remain significant because they have brought a plus compared to those obtained in the control, something that could be beneficial and at the same time encouraging for researchers, who want to find a way to recycle sludge on one side and on the other side improve the profitability of verbena by culture substrate technique.

So the mud-based culture substrate can, in a way, have an important role, which boils down to improving the texture of the substrate and the production of verbena, and an economic role because this residual sludge does not are inexpensive compared to fertilizers.

References

Accevedo E (1989). Improvement of winter wheat crops in Mediterranean environments, use of yield, morphological traits. Ed.InraF. The conferences. 55 : 273-305.

AFNOR (1994). Soil quality. Environment. Collection of French standards. A.F.N.R., Ed. Paris, P.77-97.

Anderson WK (2010). Closing the gap between actual and potential yield of rainfed wheat. The impacts of environment, management and cultivar, Field Crops Research, 116 :14-22

Baize D (1988). Guide to current analyzes in pedology. Ed. Paris: INRA, 172P.

Baize D (2000). Guide to analyzes in pedology. 2nd ed. Paris: INRA, 256P.

Bajji M (1999). Study of the mechanisms of resistance to water stress in durum wheat: characterization of cultivars differing in their levels of resistance to drought and of clonal soma variants selected In vitro. Doctoral thesis. Univ. Leuven.

Benbelkacem A, Mekni MS and Rasmusson DC (1984). Breeding for high tiller number and yield in barley. Crop. Sci., 24 : 968-972.

Benseghir LA (2002). Improvement of above-ground cork oak production techniques: container- substrates- mineral nutrition. Memo. Mag: Plant Ecotoxicology: Univ. Badji Mokhtar: Annaba (Algeria), 91P.

Bensouiki A and Aib M (2002). Development of a technique for extracting heavy metals from sediments. Evaluation of the level of contamination by Cd, Pb, Zn and Cu of sewage sludge and the impact of the STEP Ibn Ziad on the Rhumel wadi. Same. Ing: Agronomy (plant science): Univ. Mentouri: Constantine (Algeria), 76P.

Bhati M and Singh G (2003). Growth and mineral accumulation in Eucatyptus camaldulensis seedlings irrigated with mixed industrial effluents. Bioresources Technology. 88: 221-228.

Bouafia DJ (2012). The effect of residual sludge on some phenological parameters of alfalfa (Medicago sativa L.). Memo. Ing: crop science (Saharan Agronomy): Univ. Kasdi Merbah: Ouargla (Algeria), 53P.

Bouallegue MM (2011). Technical summary: Metals in sludge from wastewater treatment plants? Consequences, origins and prevention. Institute of sciences and industry of life and environment. Ed. Paris, 17P.

Boudreault S (2010). Effects of physical and chemical properties of substrates on the growth and development of white spruce seedlings in containers after one growing season. Memo. Mag. Master's Program in Soils and Environment (Soils and Agrifood Engineering): Univ. Lavel: Quebec, 90P.

Bourekeb A (2002). Possibility of phytoremediation of soils amended with sewage sludge by spinach and lettuce: Agronomy production system, 100P.

Callot G, Chamayou H, Maertens C and Saalasac L (1982). Better understanding soil-root interactions: Mineral nutrition index. Ed.Paris: INRA, 325P.

Calvet R (2003). The soil, properties and functions: Constitution and structure, interface phenomena. Ed. Paris: DUNOD, Volume I, 455P.

Chenini F, Trad M, Rejeb S, Chaabouni Z and Xanthoulis D (2002). Optimization and sustainability of wastewater treatment and use in agriculture. Faculty of Agricultural Sciences, Tunisia, 71p.

Choukr-Allah R (2009). Recycling of unconventional waters as potential water saving resources for Mediterranean countries. AGDUMED. Rabat, Morocco.

Choukr-Allah R, Hamdy A and Young T (2003). Wastewater treatment technology adapted to small and medium community in the Mediterranean region. In: Hamdy A. (edt). Water resources management and water saving in irrigated agriculture (Wasla project). Mediterranean Options, ser. B 44: p. 103-111.

Djenane AM (1992). Some results of the cereal intensification extension program in the Hautes Plaines Sétifiennes region. Maghreb Seminar Agricultural extension in the Maghreb: theory and practice, April 1992. Algiers.

DSA, 2013. Directorate of Agricultural Services, Statistical Services, Algeria, 2013.

Duchaufour P (1965). Précis de pédologie. 2nd ed. Paris: Masson, 481p.

Duthil J (1971). Elements of ecology and agronomy. Volume I, 343P.

FAO (2005). Current trends in the world fertilizer market and outlook to 2009/10.60 p.

Fiscchesser B and Dupuitate T (1996). The Illustrated Guide to Ecology. Ed. Mornings, 319P.

French RJ and Schultz JE (1984). Water use efficiency of wheat in a Mediterranean-type environment. I. The relationship between yield, water use and climate, Australian Journal of Agricultural Research., 35, 743-764. Gagnard G., Huguet C. and Ryser J P., 1988. Soil and plant analysis in the conduct of fertilization. 83P.

Jarde E (2002). Organic composition of residual sludge from Lourraine wastewater treatment plant: molecular characterization and effects of biodegradation. Doctoral thesis: Science: Univ. Henri Poincaré, Nancy 1: Lourraine, 283P.

Lacée C (1985). Sludge analysis. Volume 2,127P.

Latiri K, Lhomme JP, Annabi M and SetterTL (2010). Wheat production in Tunisia: Progress, Inter-annual variability and relation to rainfall, European Journal of Agronomy, 33 : 33-42.

Leclech B (2000). Agronomy "from the basics to new orientations". Ed. Maroon, 260P.

Mahi M (2008). Current national status of treatment and reuse of treated wastewater and sludge recovery. The Third Workshop And Training Session For Sowamed Project In Rabat. Rabat, Morocco.

Pousset J (2002). Green manures and soil fertility. 2nd ed. France: Agridecisions, 303P.

Ripert C, Tiercelin JR, Navarot C, Klimo E, Gajarszki G, Cadillon M, Tremea L and Vermes L (1990). Agricultural and forestry use of domestic wastewater. Cemagref Technology Bulletin. 79, 18-19.

Roula S (2005). Physico-chemical characterization and recovery of urban residual sludge for the production of culture substrates in aboveground nurseries. Mémo.mag: Agronomy (forest and soil conservation): Univ. Batna (Algeria), 84P.

Steduto P, Hsiao TC, Fereres E and Raes D (2012). Crop yield response to water. paper 66, FAO Rome, 500p.

****