



Research article

The vermicomposting for agricultural valorization of sludge from Algerian wastewater treatment plant: impact on growth of snap bean *Phaseolus vulgaris L.*Hayet Belmeskine^{a,b,*}, Wissam Ait Ouameur^a, Nora Dilmi^a, Ali Aouabed^b^a Saad Dahleb University-Blida 1, Faculty of Nature and Life Sciences, Soumaa Road, BP270, Blida, 09000, Algeria^b Saad Dahleb University-Blida 1, Faculty of Technology, Laboratory of Functional Analysis of Chemical Processes (LAFPC), Soumaa Road, BP270, Blida, 09000, Algeria

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ABSTRACT

In the present study, vermicomposting of sewage sludges and their application in agriculture have been investigated. The impact of *Eisenia fetida* earthworms on the stabilization of these sludges during 7, 14 and 21 days was assessed. The final product was applied as soil amendment (set-1) to evaluate the effects on vegetative parameters (Chlorophyll, carotenoid, number of nodules, leaf weight, and plant height) of *Phaseolus vulgaris L.* bean. In the second test (set-2), the amendment was made with raw sludge. In the third test (set-3), the worms were added to the raw sludge at the time of spreading and left during the whole period of beans growth. In order to identify and better understand the behavior and role of worms with regard to sludge, their growth (weight and length), some pathogens and physico-chemical parameters were assessed during the vermicomposting. Results showed significant increase of earthworm weight of 10.62, 23.89 and 35.72 % after 7, 14 and 21 days of sludge vermicomposting, respectively, which explain their acclimatization. The vermicomposting caused a decrease of fecal coliforms number and organic matter (OM), an increase in electrical conductivity (EC) and stabilization in pH. Furthermore, significant differences were obtained for all plants vegetative parameters in soil amended with vermicomposts, compared to the control (unamended soil). Indeed, a significant increase of the chlorophyll level (a, b) was noted in the beans having undergone sludge spreading. However, worm-stabilized sludge for 7 days (set-1) showed the highest chlorophyll a and b levels about 16.1 and 24.64 µg/g, respectively, against 10.13 and 4.38 µg/g in the control. For the carotenoid, a significant decrease was observed compared to the control. Our experiments have also showed that the application of vermicomposted sludge significantly increases the number of nodules, leaf weight and plant height of *Phaseolus Vulgaris L.* snap beans.

1. Introduction

In Algeria, more than seventy per cent of the lands are arid, marked by irregular rainfall and poor soil organic matter. This fragility of soils due mainly to climatic conditions is accentuated by anthropic practices which can be destructive. All these disadvantages considerably limit agricultural production, and impose soil quality improvement. If manure is the traditional organic amendment, the decline in livestock production, the increase in area under cultivation and the need for organic matter means that manure production is insufficient to restore and maintain the soil humic stock. The agronomic valorization of sewage sludge of wastewater treatment plant is an alternative that enables agriculture to serve the community. At the same time, it takes advantage

of these organic products by improving the fertility of cultivated soils. It is generally admitted that sewage sludge improves the physical, chemical and biological properties of soils (Alvarenga et al., 2015). However, it can exhibit metal traces, pharmaceutical derivatives, hormones, etc., which are undesirable and potentially toxic constituents. Thus, it was suggested that sludges' stabilization is therefore essential before their use in agriculture (Sharma and Garg, 2018). Since then, numerous studies have been conducted to evaluate the use of vermicomposting for sludge reduction and stabilisation. It is an environment-friendly technology in which earthworms interact with microorganisms, under thermophilic and aerobic conditions, allowing the stabilisation of organic matter and modifying its physical and biochemical properties (Singh et al., 2020; Liu et al., 2012; Dominguez, 2004). Huang and Xia (2018) have studied the

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performance of vermicomposting and demonstrated the enhancing effect of earthworms on excess sludge treatment. It was also indicated that the application of a vermicomposted sewage sludge in the agriculture would not have any negative impact on fields as long as the concentrations of heavy metals (Cu, Mn, Pb and Zn) still in the limits allowed for sludges (Taki et al., 2019; Khwairakpam and Bhargava, 2009). Despite the rapid growth of these technics in the world, the sludges, composted or vermicomposted, are little used in Algeria. So, the purpose of this study is to investigate the possibility of agricultural valorization of sludges provided from waste water treatment plant of Chenoua, district of Tipaza (Algeria) and evaluate the effects of their vermicomposting, using *Eisenia fetida* (*oligochaeta*), on growth of snap bean *Phaseolus vulgaris* L. This common bean is one of the most widely cultivated and consumed vegetable in the world. According to the Algerian Technical Institute of Vegetable and Industrial Crops (ITCMI), the bean is a plant cultivated throughout the Algerian territory. It is placed in the 13th position of vegetable crops, represents 2.16% of total production and is very important for local consumption. Based on previous information about its cultivation, the period for producing green pods is short which lead us to get results rapidly (Abu Seif et al., 2016). So, our experiments were conducted during the period from March to June. To the best of author's knowledge, there are no studies in Algeria that investigated the impact of vermicomposted sewage sludge on growth parameters of snap bean. For this reason, the main aims of our study were, first; to assess the earthworms' acclimatization in the sludge during its vermicomposting, secondly, to evaluate some vegetative growth parameters such as; plant height, leaf weight, number of nodules and photosynthetic pigments of the snap bean having undergone amendments with, i) vermicomposted sludge, ii) non-vermicomposted sludge, iii) sludge-worms combination at the time of spreading.

2. Materials and methods

2.1. Sewage sludge

The sludge was obtained from the municipal waste water treatment plant in Chenoua, district of Tipaza (North West of Algeria) (Figure 1). This WWTP was implemented in 2008, certified according to the international standard ISO 14001–2004. Its area is 40819 m² and capacity of 13817 m³/d for 70000 eq/Hab. It was designed to purify urban wastewater to protect Nador River and the Mediterranean Sea. The Chenoua WWTP produces 1300 tons per year of sludge at 24.4% of dryness (Office National de l'Assainissement (ONA), 2014). The waste water treatment system is summarized in the flowchart below (Figure 2).

2.2. Soil

Agricultural soil was collected from the 0–20 cm layer of domestic garden with no history of pesticide exposure within the last 10 years. It was also far from industrial areas. Samples collected were sieved to recover the fraction ≤ 2 mm that was used in our bean culture (OECD, 2004). The physical and chemical analyses are as follow: pH (H₂O) = 8.58; maximum water holding capacity (WHC) = 68.85%; clay = 5.69 %, silt = 6.02%, sand = 88.29%; organic matter content = 3.86 %; phosphorus = 76.57 ppm; potassium = 2.58 ppm; electric conductivity (EC, 25 °C) = 144.76 μ S/cm. The soil was air dried before its use.

2.3. Earthworms

The earthworms *Eisenia fetida* used in our study were cultured according to the OECD guide line. Only adults with well-developed Clitellae are used in our experiments. Their weight was between 300 and 500 mg. The age of individuals in the same test group did not deviate more than 4 weeks. The earthworms are acclimatized 24 h before each test. During this period, the worms receive the same food as during the test. The selected worms were removed from culture, washed with de-

ionized water and then deposited in Petri dishes on damp filter paper for 24 h, in the dark at 22 ± 2 °C, to void the gut contents (OECD, 2004).

2.4. *Phaseolus Vulgaris* L. beans

The plant used in our experiment is the green bean *Phaseolus Vulgaris*.L for which we chose the variety DJADIDA whose seed was obtained from the Technical Institute of Vegetable and Industrial Cultures (ITCMI, Algeria). Sowing time of the experiments was done on the first week of March in the spring season. The seeds of the beans were soaked in water for 2 h; the pre-germination was carried out in boxes of glass for 4 days. Germination was carried out in plastic cells filled with potting soil at the rate of 1 seed per cell. At the second stage, after germination, the bean plants were transplanted into plastic pots 14.5 cm high and 12 cm in diameter with holes at their bases for water drain. The pots already contained the soils that underwent the various treatments.

2.5. Experimental design

Earthworms dried on filter paper were individually weighed, length measured and randomly divided into groups of six earthworms. Subsequently, twelve (12) experimental plastic containers containing about 400 g of sludge each were prepared. Then, six (6) earthworms were released into each container. All containers were covered by porous membrane, in which the holes are narrow to prevent earthworms from leaving the containers, and kept in the dark room at temperature of 22 ± 2 °C (Rorat et al., 2016). The time of Sludge-*Eisenia fetida* contact was for three different periods; 7, 14 and 21 days. The experiment was carried out in 4 replications for each exposure time. After one week, the worms were handily removed from their containers, rinsed with de-ionized water and placed on moist filter paper to remove gut content before to be weighed and measured. Then, the 7-day sludge (S7) was mixed with 75% of soil (by mass) (Rorat et al., 2016) to be used as soil amendment for the bean crop. We repeat the same stage for the 14-day (S14) and the 21-day (S21) sludges. This assay aimed the assessment of vermicomposting as a process of sewage sludge valorization.

In another experiment, we cultivate bean with spreading of SS alone (without contact with earthworms) and a 5th experiment with SS plus earthworms; in addition to the control set (unamended soil). In total we obtain six sets of bean cultures with 4 replications for each experiment:

1-Bean culture without sludge (**control**); 2- Bean culture with sludge 7 days-vermicomposted (**S7**); 3- Bean culture with sludge 14 days-vermicomposted (**S14**); 4- Bean culture with sludge 21 days-vermicomposted (**S21**); 5- Bean culture with raw sludge (S),6- Bean culture with sludge + earthworms (**SE**).

2.6. Data recorded

2.6.1. Sludge composition

It is well known that the determination of the exact sludge composition is more time-consuming and delicate because it varies according to the origin of the wastewater, the period of the year and the type of treatment and conditioning practiced in the treatment plant (Koller, 2009). In general, three kinds of elements are present in the sludge; useful elements (C, N, P, K...), undesirable elements (inorganic and organic chemical contaminants) and pathogenic microorganisms. It should be noted that the two major constraints that must be managed, when opting for agricultural recovery of sludge, are heavy metals and pathogens. Concerning the heavy metals, data provided by the chenoua WWTP laboratory are illustrated in Table 1. In our study, to clarify the role of *E. fetida* earthworms to change in microbial parameters during the vermicomposting of sewage sludge, some pathogenic bacteria were assessed in the raw sludge and after 21 days of vermicomposting. So, total and faecal coliforms were determined according to the method ISO 9308-1, *Streptococcus* according to ISO/FDIS 7899-2, *Syaphylococcus* with ISO 6222. As well, *Salmonella* and *Vibrio cholerea* were determined as

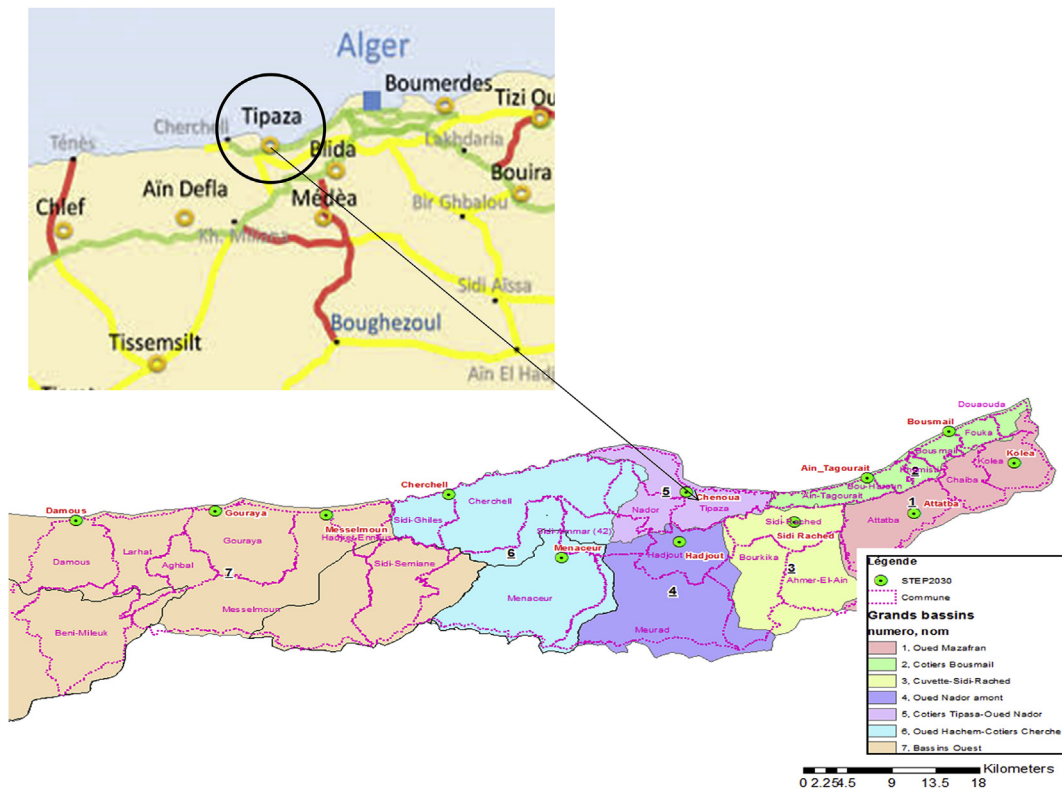


Figure 1. Localisation of the municipal WWTP in Chenoua (Tipaza, Algeria).

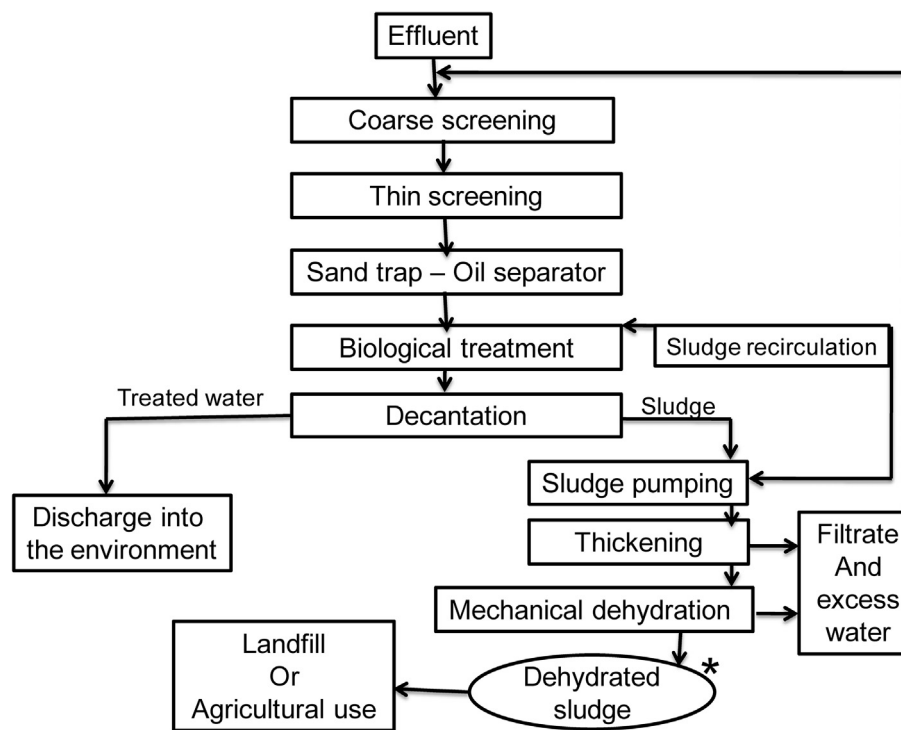


Figure 2. General scheme of the treatment process at Chenoua WWTP. The asterisk (*) represents the point of sludge samples collection.

Table 1. Variations in the concentrations of heavy metals in the sludge from the Chenoua WWTP.

Elements	average concentration (mg/Kg)	maximum concentration (mg/Kg)	regulation Limit value (mg/Kg)*
Cd	8–13.4	8.1–26.8	20–40
Cr	64.8–435.3	109–1876	NC
Cu	115.7–126.9	150.4–292.2	1000–1750
Ni	27.2–79.2	35.2–140	300–400
Pb	110.4–212.8	222–333	750–1200
Zn	363–592	455–1245	2500–4000
Hg	0.15–1.62	0.18–4.6	16–25

NC: Not cited in [Official Journal of the European Union \(OJEU\) \(2019\)](#)

* Official Journal of the European Union (OJEU), L170, 25th June 2019.

described by ISO19250 and ISO/TS 21872-1, respectively. In addition, a not exhaustive analysis of chemical parameters (pH, Electric conductivity, total organic matter) was carried out to characterize the vermicompost.

2.6.2. Earthworm growth parameters

In order to know whether or not earthworms be able to acclimatize to the experimental conditions of the sludge vermicomposting, growth parameters (weight and length) are assessed during the three periods of 7, 14 and 21 days. Thus, the growth rate was calculated using [Eq. \(1\)](#) of [Martin \(1986\)](#), where W_0 is the weight at the beginning of vermicomposting and W_t is the weight after t days of vermicomposting. Also, the percentage of growth was determined according to the [Eq. \(2\)](#):

$$\text{Growth rate} = \text{Ln} (W_t/W_0) \times 100 \quad \text{Eq. (1)}$$

$$\% \text{ growth} = (W_t - W_0/W_0) \times 100 \quad \text{Eq. (2)}$$

2.6.3. Vegetative growth parameters of *Phaseolus vulgaris* L.

The fresh leaves weight and the plant aerial part height were measured at the onset of flowering stage. While, the number of nodules and the length of the underground part of the plant were measured at harvest stage.

2.6.4. Chemical analysis of *Phaseolus Vulgaris* L.

At the onset of flowering stage, leaves were picked up from plants of each pot and subjected for determining Chlorophyll a/b and Carotenoids.

To measure the chlorophyll, 100 mg of leaves of each pot of each experiment are taken with a mortar and then ground with 40 ml of acetone. The sheets are then placed in the centrifuge for 10 min at 3000 rpm ([Amiri et al., 2017](#)). After the chlorophyll separation, the tanks are passed through a spectrophotometer with three different wavelengths; 645 nm for chlorophyll a, 663 nm for chlorophyll b and 470 nm for Carotenoids. Their quantification was done according to the following equations ([Tan and Francis 1962](#)):

$$\text{Chl (a)} (\mu\text{g/g}) = [12.7 \times \text{OD} (663) - 2.59 \times \text{OD} (645)] \times V/1000 \times W \quad \text{Eq. (3)}$$

$$\text{Chl (b)} (\mu\text{g/g}) = [22.9 \times \text{OD} (645) - 4.68 \times \text{OD} (663)] \times V/1000 \times W \quad \text{Eq. (4)}$$

$$\text{Carotenoids (mg/ml)} = [(1000 \times \text{OD} (470) - (1.82 \times \text{Chl b}) + (85.02 \times \text{Chl b}) / 198 \quad \text{Eq. (5)}$$

where OD is optic density, V is the volume of extracted solution and W is the weight of fresh material.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad version 6.01 (Software, San Diego, California, USA). All data are presented as means \pm standard deviation (SD). Significant differences between treatments were analyzed by one way analysis of variance (ANOVA) at 95% confidence level.

3. Results and discussion

The agricultural valorization of sewage sludge can be considered as the most suitable recycling method to rebalance the biogeochemical cycles, for the protection of the environment and of great economic interest. It aims to conserve natural resources and avoid any waste of organic matter due to incineration or burial in landfills ([Lambkin et al., 2004](#)). However, the reuse of sludge is managed by constraints and limits linked to, i) its quality (heavy metals, pathogens, pesticides...), ii) farmers' perception, iii) soil quality.

3.1. Sludge composition

In sludge, a variety of organic and inorganic pollutants can be found in varying concentrations and toxicity. For example, some metallic trace elements are essential for the development of plants and animals such as Zn, Cu, Cr and Ni, but can be toxic at too high doses. While, Cd, Hg and Pb are potentially toxic. Most of the chemical contamination comes from industrial waste and to a lesser extent domestic waste. Knowing that our study area is tourist, non-industrial and mainly agricultural, we predicted a low pollution load. In fact, as shown in [Table 1](#), the average concentrations of heavy metals in the residual sludge comply with Algerian (NA 17671 2010) and European (NF U44-041 1985) limit values ([Official Journal of the European Union \(OJEU\), 2019](#)). This allows suggesting that the agronomic application of this sludge after vermicomposting may be realized without any negative effects on soil characteristics, as long as the concentrations remain within the authorized range ([Khwaïrakpam and Bhargava, 2009](#)).

Otherwise, as illustrated on [Table 2](#), we noted very weak increase of the pH between the initial (raw sludge) and the final product (vermicomposted sludge). [Suthar et al. \(2015\)](#) reported that an intense microbial activity and decomposition of organic matter in the first weeks

Table 2. Selected physico-chemical parameters in raw and vermicomposted sludges.

	pH	EC ($\mu\text{S/cm}$)	OM (%)
S	8.30	563	65.0
S21	8.36	1914	59.0

S: raw sludge, S21: 21days-vermicomposted sludge.

Table 3. Analysis of pathogenic bacteria in raw sludge (S) and vermicomposted sludge during 21 days (S21).

Microorganisms	Soil amendments	
	S	S21
Total coliforms*	3666.0	3600.0
Faecal coliforms*	50.0	7.2
Total streptococcus*	366.0	3.0
<i>Staphylococcus</i>		
<i>Staphylococcus haemolyticus</i>	+	-
<i>Staphylococcus aureus</i>	+	-
<i>Salmonella</i>	-	-
<i>Vibrio colerea</i>	-	-

* MPN/g dry weight.

resulted in the formation of ammonium and increased pH in the compost. Thus, our study suggested that the addition of the earthworms allowed the stabilization of pH.

Another parameter of interest was the electrical conductivity (EC) whose analysis showed an increase during the vermicomposting, from 593 to 1914 $\mu\text{S}/\text{cm}$. Our findings may be attributed to the mineralization of organic matter and release of different minerals salts, such as phosphate, ammonia and potassium (Ozdemir et al., 2019; Suthar, 2010; Jadia and Fulekar, 2008). It should be noted that despite the increase in EC, its value remains lower than the maximum tolerance limit of plants 4.0 mS/cm, as suggested by Dede and Ozdemir (2015).

For the effects of earthworms on sludge's organic matter, data in Table 2 showed a reduction in the vermicomposted sludge compared to the raw one. This decrease was due to the mineralization and decomposition of organic matter by earthworms-microorganisms combination in the substrate material and the loss of carbon compounds in CO_2 (Ozdemir et al., 2019; Amouei et al., 2017).

Concerning the bacterial analysis, the counts of total and faecal coliforms during the vermicomposting of sewage sludge as most probable number (MPN) are shown in Table 3. At the beginning, the average number of total and faecal coliforms was 3666 and 50 MPN/g dry sludge, respectively. After 21 days time-vermicomposting, the number of total coliforms decreased slightly to 3600 MPN/g dry compost. However, the number of faecal coliforms decreased to 7.2 MPN/g dry compost. It is well known that coliform organisms are good indicator of the overall sanitary quality of water and soil (Khalil et al., 2011). They are used as opposed to the actual disease-causing organisms and occurred at higher frequencies than the pathogens and are simpler and safer to detect (Hassen et al., 2001; Rodier, 1996). It was also shown that coliforms are more resistant to inactivation than *Salmonella* sp. (Kuhlman, 1990). Another study determined that the faecal coliforms concentrations of less than 1,000 MPN/g enhanced the probability of destruction of bacteria and parasitic and viral pathogens (Hay, 1996).

Otherwise, *Staphylococcus*, *Salmonella* and *Vibrio colerea* determination was only qualitative (presence or absence). As shown in Table 3, *Staphylococcus* were present in the beginning of vermicomposting but disappeared by the end of the 21 days. While, *Salmonella* and *Vibrio colerea* were not detected.

From the results obtained, it can be seen that earthworms had an effect on decreasing microbial biomass. This agree with other studies which mentioned that earthworms can modify microbial biomass,

activity and structure through burrowing, digesting and dispersing microbes and casting behaviour (Hait and Tare, 2011; Liu et al., 2012). Huang and Xia (2018) have demonstrated that the mucus of *Eisenia fetida* could accelerate the mineralization and humification of vermicomposting materials. Also, the bacterial community structure was modified by the addition of mucus, with showing the greater increase abundances of proteobacteria and a decrease in the Firmicutes.

In addition, with regard to the effect of earthworms on bacteria, the final material of vermicomposting smelt less and presented dark color, compared to initial substrate. These results agree with those of Khalil et al. (2011).

3.2. Effect of sludge on earthworms' acclimatization

Data in Tables 4 and 5 reveal that earthworms in sludge exhibited an increase of individual weight and length, respectively. The weight and length increased with increasing duration of vermicomposting, compared to their initial stage. Xing et al. (2016) reported that a vermifiltration of sewage sludge caused a significant increase of the earthworms' average weight from 0.32 to 0.46 g and therefore an increase of the total biomass. In the same way, according to our results (Table 4), during the experimental periods of 7, 14 and 21 days, the percentage of growth was 10.62, 23.89 and 35.72 %, respectively. Similarly, the growth rate of earthworms increased during these periods of 11.22, 27.30 and 44.20 %, respectively, but it was significantly different at 14 and 21 days. Otherwise, the measurement of the length showed 9.12, 9.84 and 27.6% increase than initial worms' length, respectively (Table 5). Also, it was indicated that the gain in both weight and length was more significantly different ($p = 0.0001$) at 21 days vermicomposting period. This difference could be due to the more time taken by earthworms to ingest and digest the nutrients especially organic matter mostly present in sewage sludge. In addition, Huang and Xia (2018) suggested that 20 days are sufficient for earthworms to promote humification of substrates, because their mucus significantly accelerated the transformation of organic matter and could enhanced production of humic and fulvic-like substances.

Taken together, our results showed that the earthworms *E. fetida* were able to acclimatize to our lab-experimental conditions of the sludge vermicomposting.

3.3. Vegetative growth parameters of *Phaseolus vulgaris* L.

These experiments involved the impact of sludge, vermicomposted or non-vermicomposted, on some growth parameters in the aerial and underground parts of *Phaseolus vulgaris* L. plant. The results are presented in Figures 3 and 4, respectively.

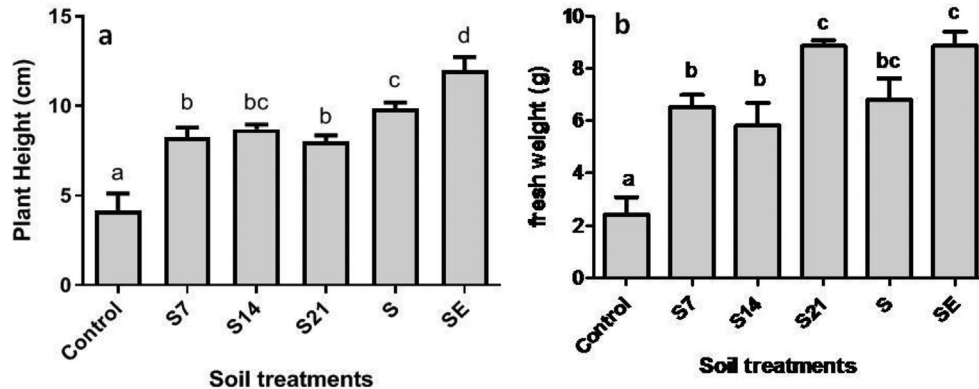
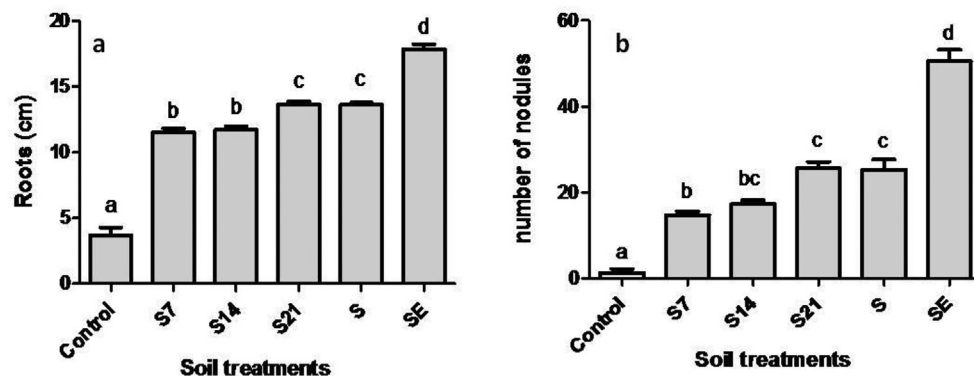
For the aerial part (Figure 3), the plant height and the fresh leaves weight were measured. Compared to the control (unamended soil), all soil treatments with sludge (vermicomposted or non-vermicomposted) displayed a significant increase ($p < 0.05$) of plant height (Figure 3a). This difference might be attributed to the additives which have enhanced nutrient supply capacity for soils, especially in organic matter and trace elements essential for plant growth. Song et al. (2015) reported that plants showed a higher growth rate because the vermicompost contains plant growth hormones and humic acid which increases root hair proliferation and mineral nutrient release and is involved in oxidative phosphorylation, cellular respiration, photosynthesis, protein synthesis and several enzymatic reactions.

Table 4. Effect of sludge on earthworm's weight at different treatment periods.

Treatment time (days)	Wo (mg)	Wt (mg)	% growth	growth rate (%)	p-value
7	466.67 \pm 70.71	522.11 \pm 76.06	10.62	11.22	0.1003
14	472.72 \pm 131.59	621.14 \pm 191.56	23.89	27.30	0.0032
21	425 \pm 68.31	661.25 \pm 143.67	35.72	44.20	0.0001

Table 5. Effect of sludge on earthworm's length at different treatment periods.

Treatment time (days)	L ₀ (cm)	L _t (cm)	% increase	p-value
7	7.75 ± 1.31	8.53 ± 1.65	9.12	0.1410
14	8.58 ± 1.6	9.46 ± 1.62	9.84	0.1588
21	7.05 ± 1.62	9.73 ± 2.12	27.6	0.0001

**Figure 3.** Assessment of vegetative parameters in aerial part of *Phaseolus vulgaris* L. at different soil treatments. a: the plant height (cm), b: The leaves fresh weight (g). Bars with different letters show significant difference at $p < 0.05$.**Figure 4.** Assessment of vegetative parameters in underground part of *Phaseolus vulgaris* L. at different soil treatments. a: the roots' length (cm), b: the number of nodules (unit). Bars with different letters show significant difference at $p < 0.05$.

As indicated in the same figure, the highest height was noted after spreading sewage sludge with addition of worms at the same time (SE treatment). This can be explained that the sludge used (raw sludge) contained higher content of organic matter compared to vermicomposted sludge where organic matter varied depending on ingestion and digestion pattern of earthworms-microbes. In another hand, the implication of soil microbes should be considered. In fact, previous studies have demonstrated that earthworms interact intensively with microorganisms to accelerate the stabilization of organic matter and modify its physical and chemical properties (Liu et al., 2012). This meant that insoluble organic materials were transformed into soluble forms easily assimilated (Xing et al., 2016).

As shown in Figure 3.b, the average weight of leaves was also increased significantly ($p < 0.05$) in all treatments with sludge compared to control. As can be seen, there was no significant difference between S21, S and SE soil treatments. But, both S21 and SE gave the higher weight than S, which could be due to the activities of both earthworms and microorganisms involved in the two treatments (SE and S21). Numerous studies on the interactions between earthworms and soils' microbial communities have demonstrated the beneficial role of earthworms on

pedogenesis and soil quality and its implications on the increase in plant biomass (Jacquiod et al., 2020; Liu et al., 2019; Rodriguez-Campos et al., 2014). Recently, Guhra et al. (2020) observed a preferential adsorption of the organic carbon and organic phosphorus compounds in the earthworms' mucus on soil minerals which could enrich the newly-formed organo-mineral associations with biogenic nutrient elements. These findings, suggest that earthworm mucus contributes to nutrient redistribution throughout the soil profile and implies a biogeochemical mechanism to retain the phosphorus secreted in earthworm mucus.

Concerning the underground part, the length of roots and number of nodules were determined and the results were reported in Figure 4. It was shown that these two parameters present the same pattern in term of evolution depending on soil treatments. In contrast to the control, the significant increases ($p < 0.05$) in roots length at different treatments indicated that the soil was enriched with nutrients provided by these amendments (Figure 4a). This could be explained that the plant has developed its root system and had better explore the soil and use the nutrients it needs. Effectively, Alvarenga et al. (2017) when tested sewage sludge (SS), a mixed municipal solid waste compost (MMSWC) and a compost produced from agricultural wastes (AWC), found that

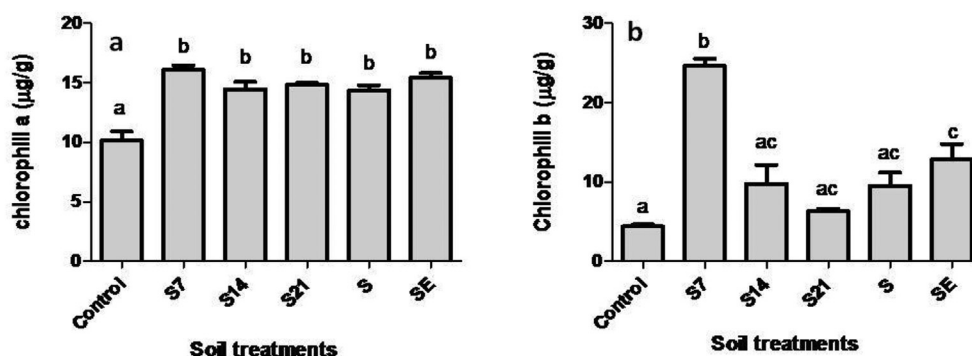


Figure 5. Leaf chlorophyll content of *Phaseolus vulgaris* L. at different soil treatments. a: chlorophyll a; b: chlorophyll b. Bars with different letters show significant difference at $p < 0.05$.

these amendments had significant beneficial effects on the increase of soil parameters such as; organic matter, phosphorus and potassium, and plant biomass. It was also shown that the effects were more pronounced for SS than for both compost applications, because it presents the greater capacity to provide Nitrogen to the plant in assimilable form. The Vermicompost has been shown to increase biological yield with improvement in growth parameters such as root and shoot length (Khan et al., 2015).

Similarly, we noted a significant increase ($p < 0.05$) in the number of nodules in the plants submitted to the soil treatments, comparing to the control (Figure 4b). Legumes, like beans, have the ability to fix nitrogen in their nodules. Thus, they engage in root **nodule** symbioses with nitrogen-fixing soil bacteria known as rhizobia. In nodule cells, bacteria are enclosed in membrane-bound vesicles called symbiosomes and differentiate into bacteroids that are capable of converting atmospheric nitrogen into ammonia (Wang et al., 2017). Another remarkable difference observed in Figure 4.b was the lower number of nodules in soils amended with vermicomposts. These results are in agreement with those of previous studies in which the sludge treated with vermifiltration had lower Nitrogen. It was suggested that microorganisms convert a part of N into new cellular material for energy and growth, and transform another part to nitrate. So, this referred to assimilation-dissimilation phenomena (Fu et al., 2015; Liu et al., 2012).

3.4. Chemical analysis of *Phaseolus vulgaris* L.

The effects of vermicomposted sludges on photosynthetic pigments (i.e., chlorophyll a, b and carotenoids) of the plant leaves were assessed.

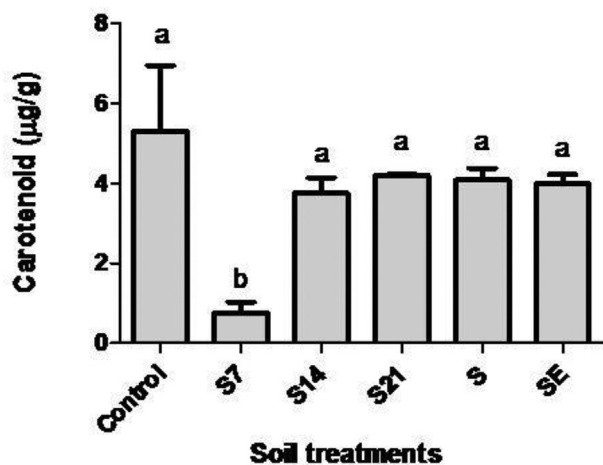


Figure 6. Leaf carotenoid content of *Phaseolus vulgaris* L. at different soil treatments. Bars with different letters show significant difference at $p < 0.05$.

Analysis of chlorophyll content is considered as a measure of physiological stress and a marker to describe photosynthetic ability of plants (Sharma et al., 2018).

As indicated in Figure 5.a, the application of sludge (vermicomposted or non-vermicomposted) as soil amendment caused a significant increase ($p < 0.05$) of leaf chlorophyll a of *Phaseolus vulgaris* L., comparing to the unamended soil (control). The obtained results may be attributed to the presence of nutrients such as nitrogen and magnesium which enter in the structure of chlorophyll molecule with, obviously, the contribution of the sunlight which plays the main role in chlorophyll molecule formation and therefore increases chlorophyll pigment accumulation (Abu Seif et al., 2016). Furthermore, as shown in Figure 5.b, the amendment with 7 days-vermicomposted sludge (S7) gave, significantly, the highest value of chlorophyll b followed by the sludge-earthworms combination (SE), in contrast of the control. However, we noted an increase at the other treatments (S14, S21 and S) but not significantly different. Rekha et al. (2018) observed that vermicompost improved plant nutrition, growth, photosynthesis and chlorophyll content of leaves. Lung et al. (2013) suggested that the reduced chlorophyll biosynthesis or increased degradation of chlorophyll could be a result of plant stress or environmental factors.

Otherwise, the determination of carotenoids content in leaves revealed a decrease for all soil treatments, based on the comparison to control (see Figure 6). The obtained results agree with those of Sharma et al. (2018) in which they reported that the application of soil amendments like sewage sludge or a mixture of sewage sludge and fly ash have caused a decrease in Carotenoid content of Palak plants. Also, Behera and Choudhury (2003) found a significant decrease in carotenoid contents in the leaves of soybean plants in soils treated with organic manure. They reported that carotenoids are non-enzymatic antioxidants, which protect the chlorophyll molecules against oxidative stresses.

4. Conclusion

Globally, we found that the application of vermicomposted sewage sludge as soil amendment positively enhanced the vegetative parameters of snap bean *Phaseolus vulgaris* L. and our final product presents an environmental acceptability in term of heavy metals and pathogens. However, we recognize that there are still some physico-chemical and microbiological (Ex.: viruses, parasites) parameters to determine, in addition of phytotoxicity assays. This will allow a better appreciation of the agronomic quality of final product. But this does not prevent that municipal sewage sludge vermicomposting is promising approach of waste management and can reduce the excessive use of chemical fertilizers. It is a concept that requires more attention due to its economic, social and environmental interest hence its contribution to sustainable agriculture.

Declarations

Author contribution statement

Hayet Belmeskine: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wissam Ait Ouamer, Nora Dilmi: Performed the experiments; Analyzed and interpreted the data.

Ali Aouabed: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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