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ORIGINAL RESEARCH

Aquacultural sludge recovery and vermicomposting for soil amendment: A useful strategy for sustainable agriculture

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Abstract

Purpose Our study concerned the recovery and reuse of sludge from aquaculture system implemented in Ain Defla District situated in the North-West of Algeria. As a biotreatment and ecological stabilization technique, vermicomposting of aquacultural sludge with *Eisenia fetida* earthworms has been advocated.

The main goals were to assessthe impact of vermicomposting on the quality of aquacultural sludge in terms of stabilization and hygienization, and to investigate its potential use in agriculture as a biofertilizer without compromising the quality of agricultural products.

Method The vermicompost was used to amend the soil and assess its impact on some growth parameters of *Phaseolus vulgaris.*L snap bean was considered**.** The physical and chemical parameters of sludge were also evaluated.

Results Significant increase of earthworms'weight and length by more than 27 and 22%, respectively, after 21 days of sludge vermicomposting was obtained. Also, significant differences were noted for selected plants' agronomic parameters in soils amended with aquacultural sludge, compared to the control (unamended soil). In fact, beans with vermicomposted sludge had substantial increases in plant height, leaves weight, and chlorophyll (a) level of 29 cm, 3.6 g, and 0.8 g/g, respectively, compared to 20 cm, 1.5 g, and 0.46 g/g in control.The results also showed that the vermicomposting process allowed for decreased faecal coliforms and *streptococcus* in the aquacultural sludge.

Conclusion The end-product was a safe biofertilizer for use in agriculture.

Keywords Biofertilizers, Bioremediation, Dissolved organic matter, Faecal coliforms, Snap bean, Waste management

Introduction

Aquaculture is an important food producing sector for millions of people in the world. In Algeria, the development of aquaculture activity, at sea or in freshwater, was a strategic choice recently adopted by farmers to absorb the fish deficit in the national market. The current production in Algeria varies between 104,000 and 120,000 tons/year (APS 2021; ONS 2019). However,

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the intensification of these practices induces the production of organic wastes and toxic compounds that present negative impact on to the environment. In order to overcome their dangerous effect, the solid waste management has received wide attention with economic, social and environmental aspects in all countries (Jasmin et al. 2020).

The aquaculture sludge is a solid part of waste containing nitrogenous compounds and phosphorus at high concentrations. It also contains different forms of dissolved organic carbon which can be harmful to the environment if their concentration is higher than usual. This solid waste is mostly formed due amounts of excessive feed and organic degradation matters such as; particulate organic matter (POM), dissolved organic matter (DOM), total suspended solids (TSS) and dissolved metabolites (Jasmin et al. 2020; Gómez et al. 2019; Piedrahita 2003).These residues of organic matter constitute a source of fertilizers whose impact is beneficial for agricultural soils.

Following the objectives of sustainable development, and like it was explained in a circular economy, the organic wastes may be recycled into high-value products and used as substrates or fertilizers in agriculture. Recovery, bioremediation and reuse of aquaculture wastes in agriculture have attracted considerable attention in recent years and have become one of the most important current challenges.

However, in the aquaculture system, the suspended solids mineralize to produce ammonia which is highly toxic to fish (Chen et al. 1993). The sludge may also contain various pathogens and toxic matters which can be harmful to aquatic animals and limit its direct use as soil amendment. It was recommended that sludge dewatering and stabilization were therefore essential before such application on agricultural lands (Sharma and Garg 2018). Extractive species, such as oligochaetes were used in the bioremediation process of aquaculture sludge because of their ability to acclimate and survive in organic enrichment conditions and assimilate particulate organic wastes from intensive aquaculture (Gómez et al. 2019).In this way, Kouba et al. (2018) used *Eisenia andrei* in vermicomposting andquality assessment of end-products from recirculating aquaculture system. They recommended vermicomposting as an eco-friendly and sustainable process transforming the aquacultural sludge into a value-added product.

The main objectives of this study were first, to assess the influence of vermicomposting, using *Eisenia fetida*, on the quality of aquaculture sludge (AS), in term of stabilization and hygienization, and secondly, to explore its use in agriculture as fertilizer without influencing the quality of agricultural products. The hypothesized benefit was that using the application of vermicomposted aquaculture sludge (VAS) as fertilizer would be a useful approach in Integrated Agriculture and Aquaculture (IAA) systems.In this context, the technological feasibility was tested on *Phaseolus vulgaris*.L snap bean crop, in which some growth parameters were assessed.

Materials and Methods

Aquaculture Sludge

Samples of aquacultural sludge were obtained from the fish farm of Hariza in Ain Defla District (NW-Algeria), at GPS coordinates of 36°11’08.3’’N, 2°06’01.3’’E (Fig. 1**)**. It was commissioned in 2017 and ensures an annual production of 5 tonnes of fish, 50 tonnes of fish feed, and 10 million multi-species fry.

In this aquaculture system, sludge was collected manually from sedimentation zones. The samples were left for two (02) hours on polyamides meshes for gravitational dewatering (Kouba et al. 2018). Then, they were air dried for four (04) hours, sieved at mesh size ≤ 2 mm;

and were further pooled before being subdivided into two parts; one of which was vermicomposted for 21 days (VAS) and the second remained as the raw aquaculture sludge (AS) (Kouba et al. 2018). Both types of sludge were used later as soil amendments. The chemical composition of raw sludge (AS) is shown in Table 1. These chemical analyses were carried out in the accredited laboratory of the Research Center for Chemical and Physical Analysis (CRAPC), Ministry of higher education and scientific research, Algeria. In addition,thepH, Electric conductivity (EC) and total organic matter (OM) were measured in AS and VAS. Some pathogenic bacteria were also assessed. Thus, faecal coliforms, *Streptococcus and Staphylococcus* were evaluated following the methods of ISO 9308-1, ISO/FDIS 7899-2, and ISO 6222, respectively. While*Salmonella*and *Vibrio cholerea*were determined according to ISO19250 and ISO/TS 21872-1, respectively (Table 2).

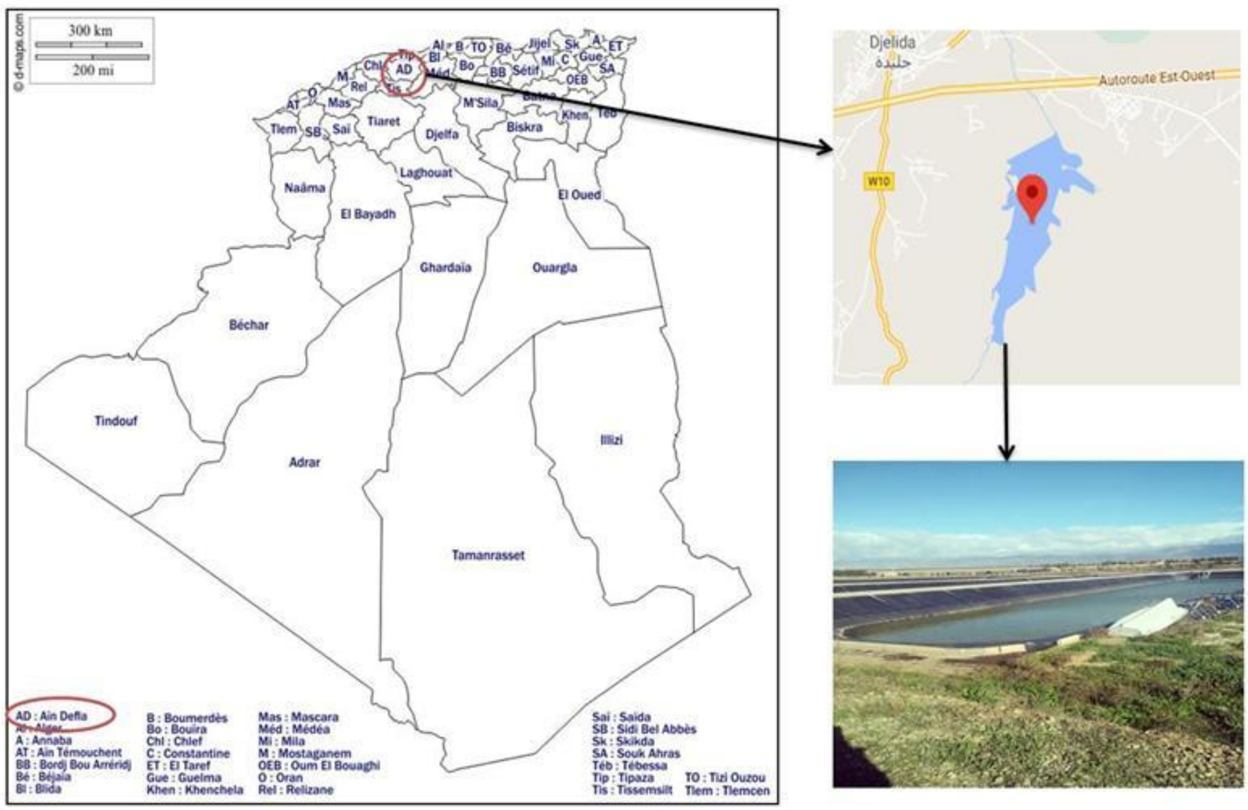

Fig. 1 Localization of Aquaculture farm of Hariza in Djelida (Ain Defla, Algeria)

Earthworms and sludge vermicomposting

Eisenia fetida is a common epigeic earthworm species typically involved in vermicomposting. The worms used in our study were obtained from laboratory culture carried out as described in OECD guidelines (OECD 2004). Adult earthworms were acclimatized 24 hours before tests. The sludge vermicomposting test was carried out following the protocol described in Belmeskine et al. (2020). Briefly, selected earthworms were weighed, length measured and divided into groups of six earthworms. Each group was then put in a glass container containing about 400 g of sludge. All containers were kept in a darkroom at a temperature of 22± 3°C (Rorat et al. 2016). The experiment was carried out in four (04) replications. After 21 days-contact times, the worms were handly separated from the resulting vermicomposts, rinsed and left on wetted filter paper to allow defecation. After three hours, the earthworms were weighed and length measured. The growth percentage was evaluated according to the Eq. (1), where W_0 is the earthworm's weight at the time **0** day of vermicomposting and Wt is the weight after **t** days.

$$\% \text{ growth} = (W_t - W_0) \times 100 / W_t \quad \text{Eq. (1)}$$

Table 1 Elementary composition, within oxides, of aquacultural sludge obtained from Hariza fish-farm (Algeria) and regulation limit values for selected heavy metals in sludge used in agriculture

Element	Concentration	Regulation limit value (mg/kg)*	Oxyde	Concentration (% in mass)
B (g/kg)	11.1		B2O3	3.5858
C (g/kg)	68.1		CO2	24.9697
Mg (g/kg)	1.54		MgO	0.2557
Al (g/kg)	62.7		Al2O3	11.8541
Si (g/kg)	199		SiO2	42.6022
P (g/kg)	0.178		P2O5	0.0408
S (g/kg)	0.833		SO3	0.2079
K (g/kg)	6.74		K2O	0.8116
Ca (g/kg)	72.9		CaO	10.1989
Ti (g/kg)	3.39		TiO2	0.5648
Cr (mg/kg)	167	200-1200*	Cr2O3	0.0244
Mn (g/kg)	0.447		MnO	0.0577
Fe (g/kg)	33.1		Fe2O3	4.7379
Ni (mg/kg)	35	300-400*	NiO	0.0044
Cu (mg/kg)	20	1000-1750*	CuO	0.0024
Zn(mg/kg)	121	2500-4000*	ZnO	0.015
As (mg/kg)	19	30*	As2O3	0.0025
Br (mg/kg)	15		/	/
Rb (mg/kg)	54		Rb2O	0.0059
Sr (mg/kg)	208		SrO	0.0246
Y (mg/kg)	14		Y2O3	0.0018
Zr (mg/kg)	209		ZrO2	0.0282
Nb (mg/kg)	14		Nb2O5	0.002

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

Table 2 Assessment of selected physico-chemical parameters and pathogenic bacteria in raw sludge (AS) and vermicompost (VAS)

Physico-chemical parameter	AS	VAS
pH	7.93	8.36
EC (µS/cm)	522	1062
OM (%)	72	25
Microorganisms		
Feacal coliforms*	20	3
Total *streptococcus**	740	6.1
Feacal *streptococcus**	6.2	1
Staphylococcus	+	-
Salmonella	-	-
Vibrio colerea	-	-

*MPN/g dry weight
AS: aquacultural sludge at t=0 day, VAS: vermicomposted sludge at t=21 days

Soil amendment

The substrate (vermicompost or AS) was applied as an amendment for the bean crop with 75% of soil (by mass) (Rorat et al. 2016). The soil used in this study is the same used in previous study on the agricultural valorisation of vermicomposted-sewage sludge of waste water treatment plant (WWTP) (Belmeskine et al. 2020). It was clean soil without a history of pesticide exposure.

Phaseolus Vulgaris L. Beans

Seeds of snap beans, more precisely DJADIDA variety, were obtained from the Technical Institute of Vegetable and Industrial Cultures (ITCMI, Algeria). The experimental work was carried out during the spring. As described in Belmeskine et al. (2020), after the pre-germination and germination steps of the beans' seeds; the seedlings were transferred into plastic pots containing the soils with the various treatments (soil alone; soil+AS and soil+VAS).At the onset of the flowering stage, some growth parameters of *Phaseolus vulgaris.*Lwere assessed. Thus, the number of leaves, leaves fresh weight, leaf area, chlorophyll a, plant height and weight and roots length were evaluated following the protocol described in Belmeskine et al. (2020), since this work is the second part of our study concerning the impact of vermicomposting on hygienization and stabilisation of sludge to its recovery and reuse in agriculture as soil fertilizers.

Statistical analyses

The statistical analyses were done with Graph Pad Prism Software (Ver.9.2.0.332 for Windows, San Diego, California, USA). A student's *t*-test was applied to compare differences in weight and length of earthworms between initial time (t0) and 21days-vermicomposting (t21). The one -way analysis of variance (ANOVA) was used to analyse the differences between control and treatments in vegetative parameters assessment, followed by Tukey's HSD *post hoc* tests. All data were presented as means ± SD and differences were considered significant when $p< 0.05$.

Results and discussion

Aquaculture sludge composition

The reuse of sludge in agriculture as a soil amendment is subjected to constraints concerning its quality in term of heavy metals and pathogens. The elementary composition, within oxides, of original aquaculture sludge (AS) obtained from Hariza fish-farm (Algeria) and regulation limits of selected heavy metals are illustrated in Table 1.Selected heavy metal concentrations (Cr, Ni, Cu, Zn, As) of original sludge (AS) are by following Algerian (NA 17671, 2010) and European (NF U44-041,1985) limit values (OJEU 2019). Nevertheless, the results showed a lack of Cd and Pb which reflect that their concentrations were below the detection limit. As expected, carbon and many macro and micro nutrients (Ca, Mg, P, K...) were present in AS (Table 1) contributingto soil fertilization when applied as an amendment. Otherwise, as shown in Table 2, the pH increased slightly in the end-product (vermicompost) compared to the original (raw) sludge (AS). This may be attributed to the microbial activity and organic matter decomposition in the first weeks of vermicomposting which allowed ammonium formation and increased pH (Suthar et al. 2015). Our results also showed an increase in the electrical conductivity (EC), from 522 to 1062 µS/cm. As reported in numerous studies, the increase of EC may be caused by the transformation of organic matter into minerals and different minerals salts, such as potassium, phosphate and ammonia (Ozdemir et al. 2019; Suthar 2010; Jadia and Fulekar 2008). Despite its increase, EC remains below the tolerance limit for plants estimated to be 4000 µS/cm (Dede and Ozdemir 2015).

For the organic matter (OM), we noted a reduction during the vermicomposting process, from 72 (AS) to 25% (VAS), due to the biodegradability in the presence of earthworms-microorganisms combination, and consequently a mineralization and carbon lost as CO_2 (Ozdemir et al. 2019; Amouei et al. 2017).

Regarding the effects of earthworms on the behaviourof the bacterial community in the vermicomposting process, data illustrated in Table 2 showed a decrease in the counts of faecal coliforms, as the most probable number (MPN/g dry weight), from 20 to 3 in AS and VAS, respectively. Usually, the coliform organisms are known to be good indicators of the water and soil sanitary quality (Khalil et al. 2011), because of their simple and safe detection and considered as opposed to the actual disease-causing organisms and operate at higher frequencies than pathogens (Belmeskine et al. 2020; Hassen et al. 2001; Rodier 1996). In addition, it was demonstrated that levels of less than 1,000 MPN/g of faecal coliforms increased the probability of the destruction of other pathogenic microorganisms (Hay 1996). Similarly, we noted a decrease in faecal *streptococcus* counts during the vermicmposting process, from 6.2 to 1 MPN/g dry weight, in AS and VAS, respectively. Concerning *Staphylococcus*, *Salmonella* and *Vibrio colerea*, only qualitative evaluation was carried out. As indicated in Table 2, *Staphylococcus* was detected in the original sludge (AS) but disappeared by the end of three weeks-vermicomposting processes. However, the detection tests of *Salmonella* and *Vibrio colerea* indicated their absence. These changes in the bacterial community during the vermicomposting process werein accordance with the results of other studies which confirmed that earthworms could change microbial activity and structure through burrowing, digesting and dispersing microorganisms and casting behaviour (Hait and Tare 2011; Liu et al. 2012**)**. Another study reported that the presence of earthworm's mucus caused a modification of bacterial structure (Huang and Xia 2018). It is important to note here that the influence of the earthworms-bacteria combination on the sludge aspect was apparent since the end-product smelts less and presented dark color. The same observations were reported by Khalil et al. (2011).

Earthworm growth assessment in vermicomposting process

As shown in Table 3, both the weight and length of earthworms were increased significantly ($p< 0.05$) after 21 days of vermicomposting compared to the initial state (at t=0 days). It was observed that the average weight increased from 0.29 ± 0.09 to 0.41 ± 0.1g. Similarly, the average length increased from 7.16 ± 0.73 to 9.23 ± 0.87 cm. In the same way, the percentage of growth enhanced to 27.51% and 22.36 % in term of weight and length, respectively. So, it can be deduced *Eisenia fetida* earthworms were acclimatized during the aquacultural sludge vermicomposting process under the lab-experimental conditions. Our aquacultural sludge is nutrients-rich and humidity, aeration and temperature were maintained favourable. The same pattern of results was obtained in our previous study concerning the vermicomposting of sewage sludge in wastewater treatment plant (WWTP) (Belmeskine et al. 2020), and we agree with the results of Xing et al. (2016).They also found a significant increase in the average weight of earthworms during a vermifiltration of sewage sludge. Otherwise, Kouba et al. (2018) provides in their study that the vermicomposting influenced positively the weight of earthworms in aquacultural sludge and their production system exceeding 90% during six weeks.

Table 3 Variaton of earthworms' mean weight and length after 21 days-vermicomposting in aquacultural sludge.

	t-0 days	t-21 days	% growth	p-value
Weight (g)	0.29± 0.09	0.41± 0.1	27.51± 9.69	0.015
Length (cm)	7.16± 0.73	9.23± 0.87	22.36± 3.8	0.011

Influence of aquacultural sludge on growth parameters of *Phaseolus Vulgaris* L.

Expanding on our previous study about the impact of the reuse of vermicomposted sewage sludge, as soil bio-fertilizer, on some agronomic growth parameters of *Phaseolus vulgaris* L. (Belmeskine et al. 2020), the present study provides an evaluation of a successful vermicomposting of aquacultural sludge and its use as fertilizer. So, our experiments involved its influence on plant height and weight, roots length, the number of leaves and their fresh weight, leaf area and chlorophyll **a**. As shown in Fig. 2,the evaluation of growth parameters of *Phaseolus vulgaris* L plant at selected soil amendments indicated a significant increase ($p<0.05$) in plant height (Fig. 2a) and weight (Fig. 2b), and roots length (Fig. 2c) when the VAS was applied, compared to the control. The aquaculture sludge provided particulate organic matter (POM) accumulation. It was composed essentially of organic compounds of carbon (C), phosphorus (P) and nitrogen (N) present in the fish diet such as minerals, carbohydrates, lipids, proteins and vitamins (Herath and Satoh 2015). Its application as soil fertilizer caused organic enrichment nutrients and water content. It was demonstrated that some growth hormones and humic acids were found in the vermicompost which might increase root hair proliferation and mineral nutrient release. They also contributed to oxidative phosphorylation, cellular respiration, photosynthesis, protein synthesis and several enzymatic reactions(Song et al. 2015). Concerning the leaves parameters presented in Fig 3, there was no significant difference between treated soils and control for the number of leaves in plants (Fig.3a), this can be attributed to leaves falling. Nevertheless, for the other leaves' parameters; fresh weight, area and chlorophyll a, illustrated in Figs 3b, 3c and 3d, respectively, we obtained significant differences ($p<0.05$) for all treatments compared to untreated soil. As shown in Fig. 3b, the higher fresh weight of leaves was obtained when the vermicompost (VAS) was applied as a soil amendment. Similarly, we noted a significant increase in leaf area (Fig. 3c) when AS and VAS were applied with greater effects under VAS. This rise in plant biomass could be linked to the beneficial role of vermicompost and its implication in soil enrichment with nutrients (Song et al. 2015). Otherwise, the evaluation of chlorophyll **a** level in leaves (Fig. 3d) indicated a significant increase for the treatment with VAS, compared to the control. This could be due to the presence of nitrogen and magnesium and other nutrients implicated in the structure of chlorophyll molecules and increase its accumulation (Abu Seif et al. 2016). Generally, all results revealed that the effects of aquaculture sludge on agronomic parameters of *Phaseolus vulgaris* L. plant were more pronounced when the vermicomposted aquacultural sludge was applied. Our observations were by following those of Rekha et al. (2018) who described the important role of the vermicompost in the plant nutrition and growth.

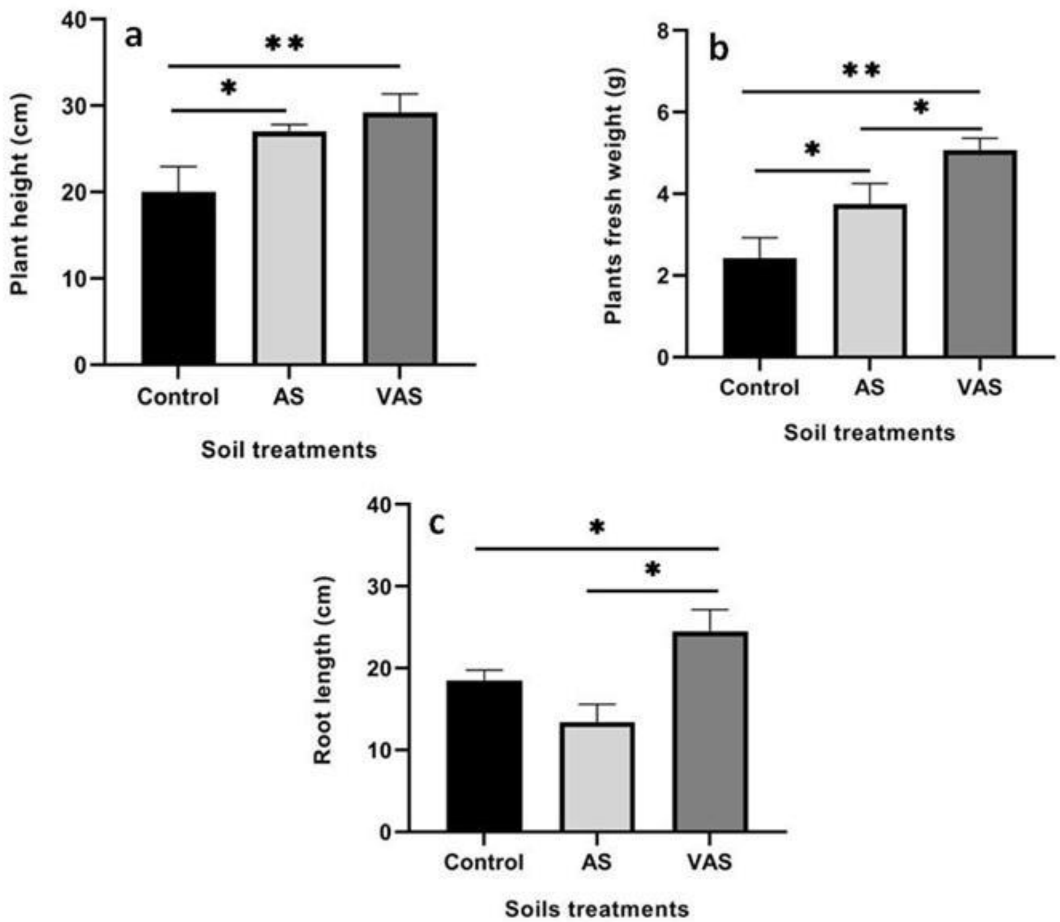

Fig. 2 Evaluation of growth parameters *Phaseolus vulgaris* L. plant at different soil amendments. **a**: the plant height (cm), **b**: the plant fresh weight (aerial part) (g) and **c**: the roots' length (cm)

Asterisks represent a statistically significant difference compared with control (* $p < 0.05$; ** $p < 0.01$).

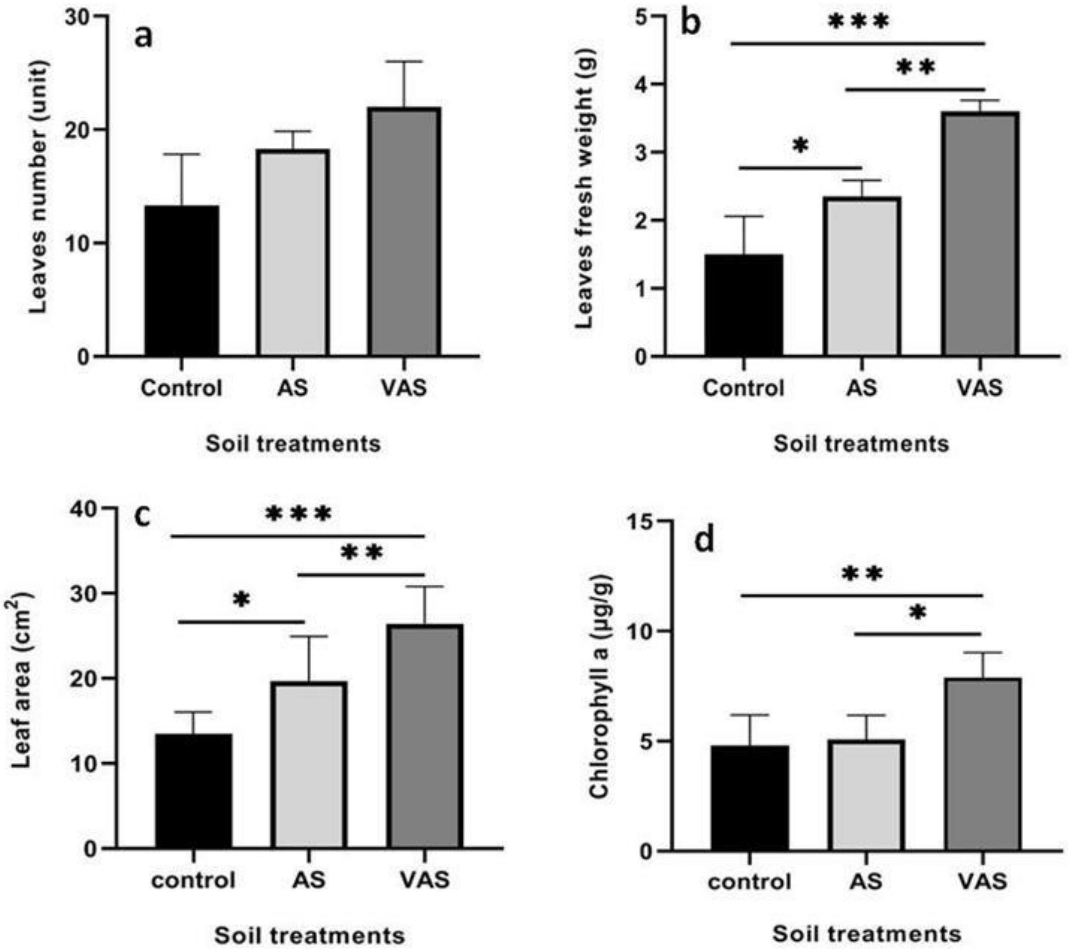

Fig. 3 Leaves' parameters of *Phaseolus vulgaris* L. plant at different soil amendments. **a**: the mean number of leaves (unit), **b**: the leaves' fresh weight (g), **c** : Leaf area (cm^2) and **d**: chlorophyll **a** content (µg/g)

Asterisks represent a statistically significant difference compared with control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Conclusion

It was indicated that the vermicomposting of aquaculture sludge could be a suitable biotreatment before its use in agriculture as a soil amendment. *Eisenia fetida* earthworms contribute positively to the stabilization and hygienization of the sludge which has a significant effect on the growth parameters of snap bean (*Phazeolus vulgaris*).The use of vermicomposted aquacultural sludge as soil fertilizer is a promising approach in Integrated Agriculture and Aquaculture (IAA) systems and represents an important interest in sustainable agriculture. Future research is needed to explore the technological feasibility of the integration of vermiculture with aquaculture.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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