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RESEARCH ARTICLE

Effects of leonardite on the coastal saline soil improvement

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ABSTRACT

This study investigated the influence of leonardite on coastal saline soil YX1 (very slightly saline), YX2 (slightly saline) and YX3 (strongly saline) with 0/10/30/50 g/kg addition dosage during the 81-day incubation. Changes of pH, electrical conductivity, soil texture, micromorphology, functional groups and mineral constituents of soil were discussed. Results showed that the application of leonardite enhanced the urea hydrolysis, increased the activities of urease and phosphatase in the coastal saline soil. The urease and phosphatase activity reached the maximum on the 8th incubation day. Leonardite raised C and N content in the soil colloid as well as the C/N value. However, leonardite had limited effects on the soil texture and mineral components during the incubation period. The application of leonardite would promote the activity of soil micro-organisms and relative enzyme to improve the soil ecosystem. It could benefit the amelioration of coastal saline soil, and provide the theoretical direction for agricultural production.

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Coastal saline soil; leonardite; amelioration; incubation; Yellow River Delta

Introduction

The coastal saline soils have been widely distributed on a global scale. About 1100 million hectares of land in the world have been affected by salinity to varying degrees according to a recent estimation [1]. The area of coastal saline soils is expanding and soil salinisation is getting aggravated due to global climate change, seawater intrusion and unreasonable irrigation [2]. The excessive salt causes osmotic stress and ionic stress to decline crop yield so as to constraint the utilisation of coastal saline soils. The coastal saline soils have great potential for utilisation and development to alleviate the problem of land shortage if these soils get effective reclamation and remediation.

Coastal saline soils are generally barren with limited nutrients such as N, P and K. The growth of crop is reduced due to the restriction of nutrients. Therefore, fertilizers are often

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applied excessively in order to ensure grain yield. However, more nutrients get lost thereby, and cause waste of resources due to the lower fertilizer utilisation rate in coastal saline soils. Many studies have demonstrated that increasing the content of soil organic matter not only contributes to nutrient retention, but also improves the soil structure and increases productivity [1,3]. Therefore, the application of organic materials is potential for the amelioration of coastal saline soil.

Among series of organic amendments, leonardite attracts much attention because of its high carbon content, rich functional groups, ubiquitous and cost-effective. Leonardite is an oxidized shallow coal with low combustion value. Therefore, it has no use in the industrial field. However, leonardite is a potential amendment for the coastal saline soil. Studies have demonstrated that the application of leonardite can improve the soil structure, promote the growth of microbial population, and increase crop yield [4]. The abundant humic acids can exchange with soil, subsequently affecting the ions component in the soil [5].

As a cheap and easily-available material, leonardite had the potential to get wide application in the Yellow River Delta. However, the application of leonardite in the study area was scarce. Further study of coastal saline soil using leonardite is needed before wide application. Therefore, commercial leonardite was used to explore the effects on coastal saline soil through the short-term soil incubation. Theoretical basis of leonardite utilisation would be also provided for local saline soil amelioration. It could benefit the sustainable use of other similar organic amendments in the Yellow River Delta area.

Materials and methods

Soil sampling and leonardite

The sampling site was located in the Yellow River Delta (N37°55′14.17″, E118°48′59.14″), China. Over 78% of annual rainfall concentrates from June to September and the average annual precipitation is only 564 mm [6]. The top tilled land soil samples (20 cm depth) coded as YX1, YX2 and YX3 were collected before winter wheat sowing in October of 2017. The salt content in YX1, YX2 and YX3 was determined as 1.3‰, 2.6‰ and 9.4‰ respectively. Soil samples (YX1, YX2 and YX3) were classified as very slightly saline soil, slightly saline soil, and strongly saline soil [7,8]. The soil samples were crushed to pass through the 1-mm sieve after air drying. The soils used in this study were silty clay according to taxonomy of the United States Department of Agriculture (USDA) [9]. The basic properties of the soil were present in Table 1.

Leonardite used in this study was purchased from Shandong Innovation Company. Functional groups carboxyl (–COOH) and phenolic (–OH) was 2.01 and 1.75 mol/kg, respectively. The pH of leonardite in water extraction was 3.84 while the electrical conductivity (EC) was 7.84 dS/m at 1:5 solid–liquid ratio (Table 1). The acid leonardite might potentially benefit to neutralize high pH.

Incubation experiment

Four different amendment dosages (CK of 0%, T1 of 1%, T2 of 3% and T3 of 5%, ratio of ameliorator: soil) were set by considering the economic cost and results of previous studies in the Yellow River Delta. Leonardite was added to three soils (YX1, YX2, and

Table 1. Basic properties of coastal saline soil and leonardite.

(a) Basic properties of coastal saline soil												
Soil	pH	EC (dS/m)	CEC cmol/kg	Bulk (g/cm ³)	Salinity	Organic matters	Total N	PO ₄ ³⁻	Soluble N	Sand	Silt	Clay
						g/kg		mg/kg			(%)	
YX1	7.72	0.42	15.13	1.31	Very slightly	9.54	0.70	0.43	3.22	15.72	39.61	44.67
YX2	7.65	0.89	16.22		Slightly	11.01	0.97	0.40	0.53	15.01	39.99	44.99
YX3	7.43	2.89	14.09		Strongly	4.26	0.94	0.74	34.34	15.08	34.95	49.96
(b) Basic properties of leonardite												
Amendment	pH	EC (dS/m)	CEC cmol/kg	-COOH (mol/kg)		phenol-OH			Ash		C (%)	N
Leonardite	3.84	7.84	163.72	2.01		1.75			23.70		44.21	0.91

Note: Electrical conductivity (EC) and pH were determined at the liquid-soil ratio of 5:1. CEC (cation exchange capacity) was determined using a sodium acetate - flame photometry method; the organic matter in the soil was by potassium dichromate heating method [12]. The machinery composition (sand, silt and clay) was performed by a combination of wet sieving method [15]. -COOH and phenol-OH were determined through titration method provided by the International Humic Substances Society (<http://humic-substances.org/>).

YX3), and each treatment had three replicates. Urea ($\text{CO}(\text{NH}_2)_2$) and KH_2PO_4 (N and P was equivalent to 0.16 mg/kg) were also fully mixed with the coastal saline soils at the beginning of experiment. The prepared soils (1 kg) were placed in the sealed bag, and sprayed by deionized water (DI-water) to keep the soil moisture content to 20%. The incubation experiment was conducted in dark under temperature ranging from 23 to 28°C. Considering the hydrolysis of urea and growth of major local crops, the sampling time was set on the 1st, 8th, 19th, 32nd, 49th and 81st day. Fresh soil with weight of 100 g in each treatment was collected at each sampling event. EC, pH, soluble N (N-NH_4 and N-NO_3), soluble PO_4^{3-} , urease, and phosphatase were determined. Raman spectrum and X-ray diffraction analysis (XRD) were used to explore the possible functional groups and mineral composition of soils, respectively.

Soil colloid preparation

Soil colloid extraction experiment was conducted after incubation. In this study, the colloid (particle-size $< 2 \mu\text{m}$) fraction of soil samples was separated from the soils by sedimentation without chemical pretreatments according to the previous reports [10,11]. The settling velocity of a sphere in a medium was proportional to the square of the particle radius but inversely to the viscous coefficient of the medium based on the Stokes' Law. A 3% (wt/wt) suspension of the air-dried incubated soil was firstly placed in an ultrasonic cleaning tank for a 20-min dispersion to fully resuspend the soil. The upper 10-cm suspension was removed by siphoning after setting for 7 h at $25 \pm 1^\circ\text{C}$. Water removed during the siphoning was replenished, and the dispersion-sedimentation-siphoning process was completed until the upper suspension was clear. Most of particle size below $2 \mu\text{m}$ in the soil was assumed to be removed during this process, and the particle size of the rest was over $2 \mu\text{m}$. The separated soil particles were preserved and frozen-dried for further analysis (C, N and FTIR). The separated soil was saturated in HCl (1 mol/L) for 8 h to remove the CaCO_3 in the soil before analysing C and N.

Analysis methods and data processing

Soluble salt content of the soil was determined before experiment based on the residual drying method, and the organic matter in the soil was measured by potassium dichromate heating method [12]. EC was measured by a Rex DDS-11A conductivity meters (Shanghai INESA Scientific Instrument Co., Ltd, China). Contents of soluble $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and PO_4^{3-} were determined by continuous flowing analyzer (Seal-Branlubbe AA3, German). Total C and N was determined by high temperature combustion using the elemental analyzer Vario Macro (Elementar, German). The cation exchange capacity (CEC) of soil samples was measured by NaOAc exchange [13]. Soil urease and alkaline phosphatase activities (buffer pH of 8) were determined according to Soil Enzyme and Analysis Methods [14]. The soil texture was analysed according to the procedure previously reported [15]. A scanning electron microscope (SEM) (JSM-5610LV, JEOL, Japan) was applied to investigate the surface morphology of soil samples. Fourier transform infrared (FTIR) spectra were obtained by FTIR Nicolet-iS5 (Thermo Fisher Scientific, USA) to analyse the stretching vibration of functional groups of organic matters. The resolution of the scanning spectrum was 4 cm^{-1} with the sensitivity of 5%, and the measurement range was $400\text{--}4000 \text{ cm}^{-1}$.

Raman spectrum spectra of all incubation samples were obtained by a Microscope Raman Spectrometer (DXR, Thermo Fisher Scientific, USA). The mineral constituents of incubation samples were determined by X-ray diffraction (XRD) spectrometry (Axios, PANalytical, The Netherlands).

Origin 8.1, Excel 2003 and SPSS 19.0 was used for data analysis. One-way ANOVA was performed to determine the difference of the relative parameters, followed by Duncan's multiple comparison test ($P < 0.05$).

Results and discussion

Effects on EC and pH

EC, positively related to the concentration of soluble ions, reflects the salinity of coastal saline soil. EC can also become an indicator of biological activity [16]. EC of soil fluctuated during the incubation period (Figure 1(a–c)). EC of different soils under various treatments changed around 10% compared with the corresponding initial EC value (Figure 1(a–c)). Moreover, the soil EC value showed an increasing inclination with the addition dosage, which might be ascribed to the soluble ions that induced from leonardite.

The background pH of YX1/YX2/YX3 was 7.65/7.56/7.02 (Table 1). The pH peaks (about 8.2) of all soil samples appeared at the initial incubation period, and then pH dramatically decreased to a stable state (Figure 1(d–f)). Urea ($\text{CO}(\text{NH}_2)_2$) was added based on the local culture fertilisation in this study. The higher pH on the 1st day demonstrated the possible N

Figure 1. Variation of EC and pH during incubation time. (a: Variation of EC in YX1; b: Variation of EC in YX2; c: Variation of EC in YX3; d: Variation of pH in YX1; e: Variation of pH in YX2; f: Variation of pH in YX3). Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage.

transformation process ($\text{Urea} \rightarrow \text{NH}_4^+ \rightarrow \text{NO}_3^-$). When the NH_3 (hydrolysis products of urea) reacted with the soil water ($\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_3 \cdot \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^-$), the soil pH increased correspondingly. As ammonium nitrogen converted into nitrate through nitrification, the pH of the coastal saline soil decreased gradually, and reached the equilibrium. The variation trend of pH in soil samples with different salinity was similar through the incubation (Figure 1). However, the salt in soil delayed the decrease of pH. The pH of YX1 reached the lowest on the 19th day while pH of YX2 was the lowest on the 32nd day. The pH of YX3 showed a decreasing trend during the whole incubation. The pH of soil treated by leonardite was higher than that of CK, which illustrated the increased capacity of $\text{NH}_3\text{-N}$ by leonardite. Therefore, the N loss declined. The relatively higher pH demonstrated that urea hydrolysis was more intense and leonardite acted an important role in promoting microbial activity to some extent since soil microorganisms participated in urea degradation [17].

Effect on mineral N ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$) and urease activity

The peak content of $\text{NH}_4\text{-N}$ appeared within a week or so in YX1/ YX2/ YX3 (Figure 2). The hydrolysis of urea occurred and $\text{NH}_4\text{-N}$ was gradually converted to $\text{NO}_3\text{-N}$ during the incubation process. Consequently, $\text{NO}_3\text{-N}$ dominated the soluble N in the soil at the end of incubation. The content of $\text{NH}_4\text{-N}$ in CK was lower than that treated by leonardite on the whole. Soil salinity might affect the ammonium nitrogen content. Ammonium nitrogen in YX3 (strong salinity) was higher than that of YX1 (very slight salinity) and YX2 (slight salinity). Ammonium nitrogen content in YX3 reached 99.27 mg/kg, which was 3 and 5 times higher than that of YX2 (max 29.57 mg/kg) and YX1 (max 18.09 mg/kg) respectively. This result might be explained by that microbial activity tended to be reduced at higher salinity to subsequently inhibit the nitrification [3]. The nitrification process of YX1 and YX2 reached the balance on the 8th incubation day (Figure 2(d–f)) while nitrification balance in YX3 was delayed due to the inhibition of high salinity. The results indicated that the addition of leonardite accelerated the transformation of nitrate nitrogen, which was consistent with the change of $\text{NH}_4\text{-N}$ (Figure 2(a–c)). Moreover, leonardite had limited effect on the $\text{NO}_3\text{-N}$ content of coastal saline soils.

The variety of urease activity was divided into two stages including the initial rapid increase of urease activity in coastal saline soils with application of urea and the following gradual decrease period due to the consumption of urea (Figure 2(g–i)). The urease activity followed the trend of $\text{YX2} > \text{YX1} > \text{YX3}$ during the whole incubation process. The urease activity in soil YX1 increased with the leonardite dosage and kept the peak from the 8th to 19th day. The highest urease activity of CK/T1/T2/T3 treatment in YX1 was 1.11/1.27/1.48/1.48 mg/(g·24 h). Compared with CK, the urease activity with T1, T2, and T3 treatment increased by 9%, 24%, and 33%, respectively. The urease activity of CK/T1/T2/T3 treatment in YX2 reached 1.45/1.54/1.62/1.89 mg/(g·24 h). High salt content limited the activity of urease to cause lower urease activity. The urease activity of CK/T1/T2/T3 treatment in YX3 was 0.68/0.73/1.04/0.97 mg/(g·24 h), which was lower than that in YX1 and YX2.

Urea was added to the coastal saline soil as the nitrogen source to simulate the practical farming conditions in this study. Its degradation products ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) are regarded as the main inorganic nitrogen sources for plant growth and crop yield [18].

Figure 2. Variation of mineral N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) content and urease activity in the soil during the incubation. (a: Variation of $\text{NH}_4\text{-N}$ in YX1; b: Variation of $\text{NH}_4\text{-N}$ in YX2; c: Variation of $\text{NH}_4\text{-N}$ in YX3; d: Variation of $\text{NO}_3\text{-N}$ in YX1; e: Variation of $\text{NO}_3\text{-N}$ in YX2; f: Variation of $\text{NO}_3\text{-N}$ in YX3; g: Variation of urease activity in YX1; h: Variation of urease activity in YX2; i: Variation of urease activity in YX3).

Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage.

Results showed that leonardite could provide abundant organic matter for microbes and promote the hydrolysis of urea [17]. The main loss way of urea is ammonia volatilisation in practice [19]. The carboxyl functional groups in leonardite could chemically and physically adsorb NH_4^+ (the formation of ammonium humate) to immobilize ammonium nitrogen in soil and then reduce the volatilisation loss of ammonia [20]. It could also explain why $\text{NH}_4\text{-N}$ in the leonardite group was higher than that of CK [21]. $\text{NO}_3\text{-N}$ is reported to serve as the main nitrogen source for plants [22]. It was the dominant form of ionic N at the end of the experiment. Results indicated leonardite could not affect the content of $\text{NO}_3\text{-N}$ in the soil. Therefore, leonardite was recommended to be applied with urea and other $\text{NH}_4\text{-N}$ fertilizer. Urease activity directly reflects the supply capacity of nitrogenous nutrient elements and transformation of mineral fertilizer (urea) in soil [23]. The application of leonardite would have an impact on promoting the soil urease activity which is highly correlated to soil organic matter [24].

Effect on PO_4 -P and soil phosphatase activity

The concentration of phosphorus which is reported to be one of the necessary nutrients in the soil will be essential for plant growth [25]. The concentration of phosphate during the incubation period changed with the similar trend in the soils (Figure 3(a–c)). KH_2PO_4 was introduced as the P fertilizer to simulate the practical situation before incubation. The highest phosphorous concentration was observed on the 8th incubation day instead of the 1st day. This result was similar to the previous observations [26]. Soluble phosphate would react with irons (e.g. calcium) in the soil, and the fixation took place soon after application. Mineral P was solved and released in soils on the 8th day due to the vigorous activity of microorganisms. A sharp decrease in soluble phosphorus took place subsequently and the concentration of phosphorus remained at the low level to the final incubation. Soil salinity could affect the concentration of soluble phosphorus due to the general order of $YX3 > YX2 > YX1$. In addition, soluble phosphate in soil decreased with the addition of leonardite. It was inferred that leonardite not only could promote the retention of phosphate and reduce the risk of leaching, but also would decrease the absorption of phosphorus by crops. Considering this situation, a certain extra amount of phosphate fertilizer should be added combining with leonardite.

The phosphatase activity in all soils was similar (Figure 3(d–f)) during the incubation period (1–81 d), which synchronized with the urease activity (Figure 2). The highest phosphatase activity in YX1 with CK/T1/T2/T3 was 0.20/0.23/0.43/0.31 mg/(g·day) while that in YX2 was 0.46/0.49/0.64/0.79 mg/(g·day) and in YX3 was 0.65/0.53/0.60/0.68 mg/(g·day).

Figure 3. Variation of PO_4 -P content and phosphatase activity in the soil during the incubation. (a: Variation of PO_4 -P in YX1; b: Variation of PO_4 -P in YX2; c: Variation of PO_4 -P in YX3; d: Variation of phosphatase activity in YX1; e: Variation of phosphatase activity in YX2; f: Variation of phosphatase activity in YX3).

Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage.

Phosphatase activity decreased continually after the 8th day. Moreover, phosphatase activity in soils with leonardite addition treatments was higher than that of CK. Higher leonardite dosage led to higher enzymic activity, indicating that the addition of leonardite provoked the mineralisation of organic phosphorus and solubilisation of the fixed inorganic phosphorous. Soil phosphatase activity was reported to be positively related to soil C and N availability [27]. Therefore, the production and release of phosphatase were affected by leonardite.

Effect of leonardite addition on soil texture, morphology and mineral composition

The soil texture referred to the size and proportion of mineral particles in the soil. It has an important influence on the physio-chemical properties, the aeration, water holding capacity, and fertilizer conservation of soils [28]. The main components of all soil were silt and clay (Table 1), accounting for more than 80% of soil texture. Previous studies have reported that the leonardite contributed to the formation of soil aggregates to cause changes in soil particle-size fraction [29]. However, the application of leonardite had no obvious effect on the soil texture due to the short-term incubation (Figure 4). The sand percentage in YX1/YX2/YX3 was (12.20–20.62%)/(7.75–14.88%)/(5.44–17.82%)

Figure 4. Variation of soil texture (YX1/YX2/YX3) during incubation. (CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage. YX1 was very slightly saline soil; YX2 was slightly saline soil; YX3 was strongly saline soil.)

and silt percentage in YX1/YX2/YX3 was in the range of (29.76–49.71%)/(29.55–44.97%)/(29.93–39.88%). Clay in YX1/YX2/YX3 accounted for (42.82–54.56%)/(44.97–59.37%)/(44.88–54.95%) during the incubation period.

The micromorphology of coastal saline soils added with leonardite was exhibited by SEM images (Figure 5). The micromorphology of coastal saline soils did not change during the 81-d incubation process with the addition of leonardite. Leonardite was distributed evenly among soil particles.

Raman spectroscopy was used to examine the possible changes of soils during the short incubation (Figure 6) since it was very sensitive to alteration of the carbon structure particularly aliphatic and aromatic fractions of soil components. Fluctuations observed at low shift intervals (below 1000 cm^{-1}) reflected soil mineral compositions [30]. The interval ($1000\text{--}2000\text{ cm}^{-1}$) corresponded precisely to the characteristic of the fulvic and humic molecular fractions of soils [31]. The peaks in this interval were small and dense, indicating that the complex organic might exist in the coastal saline soils. There was a short but wide peak in $2500\text{--}3000\text{ cm}^{-1}$ during the incubation period. The broad band was observed in the range of $0\text{--}3400\text{ cm}^{-1}$ and the spectra were similar in appearance for all soils. Leonardite failed to affect the carbon structure in soil during the short-term incubation. It implied the effects of leonardite would last for years after application, as only a fraction was initially degraded. Therefore, leonardite had the potential to be a cost-effective material for amelioration of coastal saline soils.

X-ray diffraction analysis was used to identify and to make semiquantitative measurements of the crystalline mineral components of clay fraction. Figure 7 showed the XRD spectra of soil incubated on the 1st and 81st day. A narrow and intense peak was observed at $2\theta = 26.62^\circ$, demonstrating the presence of large and ordered crystal particles [32]. The main mineral components of soil were quartz (44–55%), Albite (12–24%) and muscovite (18–24%) which accounted for nearly 90% of total minerals (Figure 7). The XRD patterns of all soils were similar no matter leonardite added or not. And no significant variation was found between the 1st and 81st day of incubation for all soils, indicating that no precipitates or minerals were formed during the incubation.

Effects of leonardite addition on content of C and N distribution among different soils

Soil colloid is considered to be the smallest and most active part of the soil, which is essential for physio-chemical properties [33]. Soil colloid will play an important role in soil water and fertility retention ability since it has the ability to absorb cations from soil solution [34]. The content of C and N in the separated soil (particle diameter above $2\text{ }\mu\text{m}$ was denoted by S_A ; particle diameter below $2\text{ }\mu\text{m}$ was denoted by S_B) as well as C/N values were shown in Table 2. The addition of leonardite increased the content of C, N and C/N values. In addition, the content of C and N in S_B (the colloid with particle size below $2\text{ }\mu\text{m}$) was significantly higher than that in the S_A (with particle size above $2\text{ }\mu\text{m}$). Value of C/N showed the opposite trend. The C/N value of YX1/YX2/YX3 in S_A under T3 treatment (5% of addition amount) reached 25.15/50.04/53.68 while that in S_B was 18.30/6.81/13.33. Soil colloid possessed a huge specific surface area, which led to the adsorption of organic matter and nutrients. The addition of leonardite with abundant C (44.21%) and N (0.91%) significantly increased the content of organic carbon in soil colloid, and would benefit the formation of micro-aggregates in the long term.

Figure 5. Morphologies of incubated soils in 1st day and 81st day. (CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage. YX1 was very slightly saline soil; YX2 was slightly saline soil; YX3 was strongly saline soil).

The FTIR spectral signals of all soils were similar in shape (Figure 8). The intension of signals in $S_{B1}/S_{B2}/S_{B3}$ (S_{B1} was the soil particles below 2 μm in YX1, and the same to S_{B2} and S_{B3}) was higher than that in $S_{A1}/S_{A2}/S_{A3}$ (S_{A1} was the soil particles larger than 2 μm

Figure 6. Raman spectra of incubated soil in 1st day and 81st day. (a: the soil of YX1 with CK/T1/T2/T3; b: the soil of YX2 with CK/T1/T2/T3; c: the soil of YX3 with CK/T1/T2/T3).

Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage. YX1 was very slightly saline soil; YX2 was slightly saline soil; YX3 was strongly saline soil.

Table 2 C, N content and C/N value in particle-size separated soil

Soil	Treatment	C%		N%		C/N	
		Patical size <2 μm	Patical size >2 μm	Patical size <2 μm	Patical size >2 μm	Patical size <2 μm	Patical size >2 μm
YX1	CK	1.51 $\pm$ 0.02a	0.93 $\pm$ 0.10a	0.24 $\pm$ 0.00a	0.07 $\pm$ 0.00a	6.28 $\pm$ 0.10a	14.32 $\pm$ 1.49a
	T1	1.79 $\pm$ 0.02b	1.23 $\pm$ 0.06b	0.22 $\pm$ 0.00a	0.08 $\pm$ 0.00a	8.13 $\pm$ 0.14b	15.47 $\pm$ 0.70a
	T2	2.77 $\pm$ 0.04c	2.37 $\pm$ 0.02c	0.20 $\pm$ 0.00b	0.11 $\pm$ 0.00b	13.85 $\pm$ 0.21c	21.54 $\pm$ 0.15b
	T3	4.21 $\pm$ 0.18d	3.27 $\pm$ 0.08d	0.23 $\pm$ 0.01a	0.13 $\pm$ 0.00b	18.30 $\pm$ 0.05d	25.15 $\pm$ 1.21c
YX2	CK	1.75 $\pm$ 0.33a	0.54 $\pm$ 0.01a	0.44 $\pm$ 0.01a	0.04 $\pm$ 0.00a	4.71 $\pm$ 0.69ab	14.30 $\pm$ 0.72a
	T1	2.02 $\pm$ 0.01a	1.04 $\pm$ 0.05b	0.45 $\pm$ 0.01a	0.06 $\pm$ 0.01ab	4.50 $\pm$ 0.07a	17.85 $\pm$ 4.08b
	T2	2.87 $\pm$ 0.02b	1.76 $\pm$ 0.04c	0.50 $\pm$ 0.01b	0.06 $\pm$ 0.01ab	5.70 $\pm$ 0.00b	30.53 $\pm$ 2.53b
	T3	3.55 $\pm$ 0.03c	2.76 $\pm$ 0.03d	0.52 $\pm$ 0.00c	0.05 $\pm$ 0.01b	6.81 $\pm$ 0.09b	50.04 $\pm$ 5.11c
YX3	CK	1.98 $\pm$ 0.09a	0.77 $\pm$ 0.02a	0.31 $\pm$ 0.01a	0.05 $\pm$ 0.01a	6.39 $\pm$ 0.12a	16.28 $\pm$ 1.03a
	T1	2.45 $\pm$ 0.03b	0.57 $\pm$ 0.05b	0.30 $\pm$ 0.01 a	0.04 $\pm$ 0.00a	8.18 $\pm$ 0.06b	16.37 $\pm$ 3.51a
	T2	3.84 $\pm$ 0.03c	1.56 $\pm$ 0.13c	0.36 $\pm$ 0.00b	0.05 $\pm$ 0.00a	10.69 $\pm$ 0.33c	28.72 $\pm$ 4.25b
	T3	4.90 $\pm$ 0.12d	1.84 $\pm$ 0.05d	0.37 $\pm$ 0.01b	0.03 $\pm$ 0.01b	13.33 $\pm$ 0.24d	53.68 $\pm$ 1.56c

Note: CK was the control; T1 presented the treatment of 10 g/kg leonardite dosage; T2 presented the treatment of 30 g/kg leonardite dosage; T3 presented the treatment of 50 g/kg leonardite dosage.

in YX1, and the same to S_{A2} and S_{A3}). Wavenumbers in the range of 500–1200 cm^{-1} generally indicate the specific absorptions of soil minerals such as clay, quartz or iron oxides [35]. Obvious peaks were detected at 996.20 cm^{-1} in YX1, YX2, and YX3 (Figure 8), indicating many minerals existed in the particles. C=O vibrational peaks at 1620 cm^{-1} were mainly from functional groups amides, carboxylic acids and aromatic [35,36]. The broad band from 3000 to 3500 cm^{-1} was probably the result of the O–H and N–H stretching vibrations of water and organic materials such as alcohols, phenols and amides. The absorbance at 1620 and 3365 cm^{-1} in S_A was obviously weaker than that of S_B , which indicated that the soil with small particle size contains more humic acids from leonardite. It is important for the formation of soil aggregates and the improvement of soil structure.

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Figure 7. X-ray diffraction of the incubated soil. Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage. YX1 was very slightly saline soil; YX2 was slightly saline soil; YX3 was strongly saline soil.

Figure 8. FTIR of particle separated soil.

Note: CK was the control; T1 was the treatment of 10 g/kg leonardite dosage; T2 was the treatment of 30 g/kg leonardite dosage; T3 was the treatment of 50 g/kg leonardite dosage. YX1 was very slightly saline soil; YX2 was slightly saline soil; YX3 was strongly saline soil.

Conclusions

The addition of leonardite increased the content of organic matter. The activity of urease and phosphatase subsequently increased with the accelerated hydrolysis of urea and the decreased content of soluble phosphate in the soil. Leonardite increased the C and N content as well as C/N in the soil colloid of the incubated soil. However, the leonardite addition had little effect on the soil texture and mineral composition during the short-term incubation. Overall, the application of leonardite would introduce organic matter to promote the activity of soil microbe and relative enzyme, and the soil ecosystem would thereby get improved.

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Disclosure statement

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