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ORIGINAL RESEARCH PAPER

The ability of layered double hydroxides for nitrate absorption and desorption in crop and fallow rotation

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ABSTRACT

BACKGROUND AND OBJECTIVES: This study aims down to evaluate the ability of chloride magnesium- aluminium- layered double hydroxides (4:1) for nitrate adsorption from the soil solution in successive cropping periods.

METHODS: The study was conducted under long-term cropping periods, including first crop): bell pepper; second crop: mentheae; third crop: cherry tomato; and fort h crop: wheat), absorption of soil mineral nitrate in fallow periods and nitrate absorption from plants by layered double hydroxides. The effect of layered double hydroxides on qualitative and quantitative characteristics of plants was also studied.

FINDINGS: Results indicated that layered double hydroxides were able to induce long-term nitrate exchange in crop and fallow sequences. Layered double hydroxides can adsorb soil excessive nitrates in cropping periods and reduce nitrate concentration in the soil solution. Compared to control, the treatment with 16 gram layered double hydroxide/kilogram soil could reduce nitrate concentration in the soil solution by 95%. During two-week fallow periods, the amount of nitrates mineralized in the soil solution was increased, but layered double hydroxides treatments could adsorb them well and maintained the N-nitrate concentration in the soil solution at a low level. Additionally, Results indicated that application of 2, 4, 8 and 16 gram layered double hydroxides/ kilogram soil led to 34%, 44%, 58% and 69% reduction in N-nitrate concentration of soil leachates, respectively, compared to control. By increasing nitrogen availability, layered double hydroxides improved the quantitative and qualitative properties of plants. Application of 2, 4, 8 and 16 gram layered double hydroxides/ kilogram soil increased the plant height (cherry tomato) by 14%, 26%, 50% and 80%, respectively.

CONCLUSION: It is concluded that the layered double hydroxides has a potential to be used as a long-term nitrate exchanger to control the movement of nitrate in soil, and thereby reduce risks of nitrate leaching in crop production in sensible areas.

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INTRODUCTION

Nitrogen is one of the plant macronutrients playing a crucial role in improving the yield of most plants. Nitrogen fertilizers are used to fulfil the plant nutritional demand (Haider *et al*., 2017). The rates of nitrogen fertilizers applied in farmlands are usually more than the real nitrogen need of crops (Canter, 2019). More than 90% of the world's urea production is used as nitrogen fertilizers. When urea fertilizer is used in the soil, urea is first converted to ammonia (NH_3) or ammonium ion (NH^{4+}) and bicarbonate ion (HCO_3^-) by urea enzyme. A group of soil microorganisms called Nitrosomonas converts ammonium to nitrite, and then nitrite is converted to nitrate by Nitrobacter. The process of converting ammonium to nitrate is called nitrification. This process is relatively quick and takes a few days (Overdahl *et al*., 1991). Nitrate leaching in crop fields depends not only on the amount of inorganic nitrogen at the harvesting time but also on the subsequent mineralization of nitrogen. To reduce inorganic nitrogen of soil at the harvesting time, the N_{min} nitrogen fertilizer method recommended by Wehrmann and Scharpf, (1986) was used during the plant growth period; in this method, nitrate leaching was observed from late fall to early spring. Nitrate leaching occurs during this period by the large amount of soil water and the mineralization of nitrogen (Köhler *et al*., 2006). To reduce the amount of inorganic nitrogen in this period, cover crops are used, which again this depends on the date of cultivation, adaptation, crop type and its root system capacity to reach deeper layers of the soil. Nevertheless, implementation of ecological farming methods may not reduce nitrate leaching (Torres-Dorante and Lammel, 2009; Köhler *et al*., 2006). However, nitrate leaching control in the soil by adsorbing it from the soil solution, in the same way that clay minerals such as vermiculites and smectites adsorb potassium and ammonium cations from the soil solution, can be an appropriate option to reduce nitrate leaching in the soil (Torres-Dorante and Lammel, 2009; Elmi *et al*., 2019). The use of a special exchanger with nitrate adsorption ability is the best way to reduce soil leaching in the soil (Torres-Dorante and Lammel, 2009).Recently, it has been suggested to apply layered double hydroxides (LDHs) or anionic clay minerals as anionic exchanger in order to increase both the efficiency of nitrogen application and the soil capacity to buffer nitrate (Halajnia *et al*., 2016; Torres-Dorante *et al*., 2008). LDHs are a group of non-silicate layered compounds with positive charges. Hydrotalcite is the main and the most dominant LDH mineral, which can be called the hydrotalcite-type compound (Berber *et al*., 2014; Bernardo *et al*., 2017). Having brucite-like layers with positive charges and relatively weak interlayer bonds, LDHs exhibit an excellent ability to adsorb inorganic anions (Halajnia *et al*., 2013; Benicio *et al*., 2018; Chubar, 2018). Koilraj *et al*. (2013); Berber *et al*. (2014) and Everaert, *et al*. (2017) mentioned LDH as a slow-release fertilizer under laboratory conditions. Halajnia *et al*. (2016) suggested LDH as a nitrate exchanger to reduce the risk of nitrate leaching when growing corn in the soil column. in preliminary experiments were conducted Mg-Al-LDH (4:1) with the highest nitrate adsorption capacity in aqueous and soil solutions of 188.67 and 107.52 mg/g (Milligram per gram) respectively, was selected for proceeding studies. Furthermore, Mg-Al-LDH (4:1) could remove nitrate preferentially in the presence of other anions. In addition, nitrate exchanged nitrate 20 times in different concentrations while its adsorption capacity was not decreased (Mohammadi *et al*., 2019). Proceeding studies were conducted to evaluate the nitrate adsorption capacity of chloride Mg-Al-LDH (4:1) under long-term cropping periods, the mineral nitrate adsorption during fallows, the plant nitrate absorption from LDH, and the effects of LDH on quantitative and qualitative characteristics of plants.

The objective of the present study was to evaluate a) the nitrate adsorption capacity of chloride Mg-Al-LDH (4:1) under long-term cropping periods, b) the mineral nitrate adsorption during fallows, c) the plant nitrate absorption by LDH, and d) the effect of LDH on quantitative and qualitative characteristics of plants. The present study was conducted during 2018 - 2019 in Tehran, Iran, under greenhouse conditions.

MATERIALS AND METHODS

Synthesis and properties of LDH

Chloride Mg-Al-LDH with the M^{2+}/M^{3+} ratio of 4:1 was synthetized by the co-precipitation method as explained in the previous study by Mohammadi *et al*. (2019). The maximum capacities of LDH for nitrate adsorption in aqueous and soil solutions were measured as 188.67 and 107.52 mg/g, respectively (Mohammadi *et al*., 2019).

Preparation of pots and treatments

This study was conducted in a 16-month period under laboratory environmental conditions at the mean daily temperature of 26±3 Centigrade (°C) and the mean might temperature of 21±3 °C. The laboratory was located in the Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. The study was conducted in the form of completely randomized design with 5 treatments and 21 replicates. Treatments included 0 (control), 2, 4, 8 and 16 g LDH/kg soil. The soil used was collected from Maraveh Tappeh, Golestan, Iran (40° 82´ 01´´ N, 41° 94´ 77´´ E). The soils were air dried, passed through a 2 mm sieve and kept for testing. Some properties of the studied soil are presented in Table 1. Sandy soil was used for the experiment. It should be noted that the soil was poor in organic matter, and the amount of its nitrate was zero and its acidity was slightly alkaline. The soil had a sandy texture. Soil bulk density value was 1.32 g/cm^3.

First crop (bell pepper) and first fallow

To provide green bell pepper, transplant trays (55×27 centimetre (cm)) were filled with peat moss and perlite in May 2018, and Traviata variety bell pepper seeds were planted in regular distances. The seeds were covered with 0.5 cm soil on the top and irrigated uniformly. When the plants reached a height of 10 cm in June 2018, 3 of them were transplanted in each pot. Additionally, 5 kg air-dried soil, nitrogen fertilizer and LDH treatments were mixed and filled in the pots. LDH was applied as a dry powder. In preliminary trials (data not shown), this amount was found to supply 200% of the total N requirement of the vegetable. This rate was selected in order to supply "an excess" of nitrate to the soil allowing therefore to evaluate the ability of the LDH to adsorb nitrate, and its influence on plant uptake. Furthermore, 756 milligram (mg) of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 5 kg of soil). to support the plant growth and emphasize the plant nutrient needs and soil nutrient content, Basic fertilizers rates of 150 mg Mg, 300 mg P, 500 mg K and 150 mg S from magnesium sulphate ($MgSO_4$), superphosphate (KH_2PO_4), and potassium sulphate (K_2SO_4) sources were added to the liquid form, respectively. Micronutrients were added using a solution of 5 g Hortimicro-2 in 1 L water. The fertilizer contained 7% iron chelated with EDTA, 1.7% zinc chelated with EDTA, 3% manganese chelated with EDTA, 0.25% copper chelated with EDTA, 0.25 soluble Boron and 0.35 soluble Molybdenum. There was 20-cm space between the pots. Irrigation was carried out according to the plant water requirements and retaining the soil moisture content at 65% water holding capacity. Plant height was measured when plant growth was completed (September 2018) using a ruler, and weights of plant shoots and roots were determined using a weighting scale. The plant shoots and roots were oven-dried at 65 °C for 72 hours, and then their dry weights were determined. Chlorophyll values were measured using an OS-30 chlorophyll fluorescence-meter (made in England). Number of fruits was recorded in each treatment and replicate separately. Fruit length and diameter were determined using a calliper (Extra Strong model). Measurement of vitamin C value in bell pepper fruits was performed according to the study by Ting and Rouseff, (1986) through titrating the fruit extract with indophenol. Values of total suspended solids (TSS%) were calculated using a refractometer (ATAGO Brixo-32%), and N amounts were measured in plant shoots and roots. Soil samples were collected from 3 of 21 replicates to determine N-nitrate concentration in the soil solution and N-nitrate adsorbed on LDH. After harvest, to achieve good aeration, types of soil in 18 pots remained in each treatment were mixed separately, and then the pots were filled with 4 kg of the mixed soil. During the fallow period, the pots were covered with black plastics for two weeks and placed under the conditions of 10-hours light daily, mean day temperature of 20 °C and mean night

Table 1: Soil specifications used in the study

Sand	Silt	Clay	Texture	Bulk density	Particle density	Electrical conductivity	pH	Nitrate	Total nitrogen	Lime
%	%	%	-	g/cm^3	g/cm^3	dS/m	-	mg/kg	%	%
91	2	7	Sandy	1.322	1.65	0.452	7.8	0	0.019	31

temperature of 15 °C. Soil moisture content retained at 65% of soil water holding capacity. After the fallow period, soil samples were collected from 3 of 18 replicates to determine the N-nitrate concentrations in the soil solution and LDH.

Second crop and second fallow

For the second crop, two mentheae plants (*M. spicata*) with a height of 15 cm were transplanted in each pot, and 15 replicates were rendered in the pot experiment. No nitrogen fertilizer was added. Moreover, basic and micronutrient fertilizers and irrigation were applied in the same way explained in the first crop. After completion of the plant growth period (Jan 2019), soil samples were collected from 3 of 15 replicates and used to determine the N-nitrate concentrations in the soil solution and LDH. All experiments were performed in a pot tested with the same soil and initial LDH treatments, and no soil was added. As at each stage of cultivation and fallow, 3 samples were taken from replicates to measure the concentration of N-Nitrate in the soil solution and LDH, the number of replicates of each treatment was reduced in each step. The methods explained in the first crop (bell pepper) were used to measure the number of leaves, number of side branches, plant height, and wet and dry weights of shoots and roots. Moreover, Clevenger Apparatus (Goldis Ltd., Iran) was used to measure the mentheae leaves essence by the water distillation process. Amounts of flavones and flavonols were measured using a spectrophotometer (UV/Vis T90 Company PG) at a wavelength of 420 nm as explained by Popova *et al*. (2014). Anti-oxidant activity was determined by spectrophotometry at a wavelength of 517 nm as explained by Oke *et al*. (2009). To measure the values of chlorophyll-a, chlorophyll-b and carotenoid according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at wavelengths of 653, 666 and 470 nm, respectively. Total phenolic compounds were evaluated according to Folin-Ciocalteu method (Folin-Ciocalteu). After harvest, to achieve good aeration, types of soil in 12 pots remained in each treatment were mixed separately, and then the pots were filled with 3 kg of the mixed soil. Additionally, 453 mg of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 3 kg of the soil). After harvesting the mentheae, the results of experiments indicated that the amount of N-Nitrate in the soil solution and LDH treatments was low. For this reason, fertilizer was added again before the second fallow, when the soil of the repeated pots for each treatment was individually mixed to allow aeration, and no soil was added. The fallow period was repeated in the same moisture and heat regime of the first fallow. After the fallow period, soil samples were collected from 3 of 12 replicates to determine the N-nitrate concentrations in the soil solution and LDH. LDH absorbed only nitrate, and we measured the N-nitrate of the soil solution and LDH structure in all experiments.

Third crop (cherry tomato), leaching periods and third fallow

For the third crop, in March 2018, 3 plants of 15 cm high cherry tomato of Belize variety were transplanted in each pot in 9 replicates, and no nitrogen fertilizer was added. The basic and micronutrient fertilization schedules were the same as the schedules explained in the first crop. In this period, the ability of LDH to reduce nitrate leaching at plant growth period was investigated. Before irrigation and collecting the leachates, a container was placed at the bottom of each pot. In this experiment, irrigation practices were repeated four times, and the leachates from each irrigation practice were transferred to the laboratory for leachate tests. Pot weights at saturation and field capacity points were used to determine the water requirement. For this purpose, a pot filled with soil - considered the moisture control - was placed in a water basin for 24 hours to become water saturated from its bottom. Afterward, by weighting the saturated pot, the percentage of gravimetric soil water content was calculated. To prevent volatilization, the pots were covered with plastic covers and subjected to gravity force for 48 hours, and then the soil samples were collected. Since the soil gravimetric water content evaluated at field capacity for a sandy soil was 18% and there was 3 kg soil in each pot, 540 mL water was required for every irrigation practice to obtain 20% of field capacity water as leachates. During the plant growth period, soil moisture (water content) was calculated using gravimetric (mass) methods (65% of water holding capacity). After completion of the plant growth period (May 2019), soil samples were collected from 3 of 9 replicates to measure the N-nitrate concentrations in the soil solution and LDH. Methods explained in the first crop (bell

pepper) were used to measure plant height, wet and dry weights of shoots and roots, number of fruits, fruit length and diameter and TSS%. To evaluate the amounts of chlorophyll-a and chlorophyll-b according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at wavelengths of 653 and 666 nm respectively. The value of vitamin C in cherry tomato fruits was evaluated according to Hernandez *et al*. (2006) by titration with Dichloroindophenol (DCIP). After harvest, the 6 pots remained in each treatment were mixed separately to achieve good aeration, and then the pots were filled with 2 kg of the mixed soil. The fallow period was repeated in the same moisture and heat regime of the first fallow. After the fallow period, soil samples were collected from 3 of 6 replicates to determine the N-nitrate concentrations in the soil solution and LDH.

Fourth crop (wheat)

In the fourth crop, 15-cm-high wheat plants, Chamran cultivar, were transplanted in pots in June 2019. Five plants were transplanted in each pot and no nitrogen fertilizer was added. The basic and micronutrient fertilizers and irrigation practices were applied as explained in the first crop. After the plant growth period (September 2019), plant height, dry weights of shoots and roots, 1000-seed weight, and number of seeds per spike were measured. To evaluate the amounts of chlorophyll-a and chlorophyll-b and carotenoid according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at the wavelengths of 653, 666 and 470 nm, respectively. Soil samples were collected from pots to evaluate the N-nitrate concentrations in the soil solution and LDH.

Nitrate measurement methods

To measure N-nitrate concentration using the soil extraction method, 140 g wet soil (at 65% water holding capacity) was centrifuged for 25 minutes at 3500 rpm (revolutions per minute). Then, N-nitrate concentration in soil extract was evaluated using a spectrophotometer (UV/Vis T90 Company PG) at a wavelength of 535 nm (Adams *et al*., 1980). To measure the total N-nitrate, (N-nitrate adsorbed on LDH and N-nitrate in soil solution), 500 ml 0.1 M $KHCO_3$ solution was mixed with 50 g wet soil and the obtained mixture was shook for 2 hours and finally filtrated. N-nitrate concentration of the obtained extract was measured using a spectrophotometer at a wavelength of 210 nm (Dorante, 2007). N-nitrate adsorbed on LDH was obtained from the difference between total soil N-nitrate and soil solution N-nitrate. According to Keeney and Nelson, (1982), nitrate concentration in leachates was evaluated using a spectrophotometer at a wavelength of 410 nm. The plant total nitrogen was measured according to the Kjeldhal method at a wavelength of 660 nm (Holz and Kremers, 1971).

Analysis of data

Statistical data analysis was performed using the SPSS and MSTAT software programs, and the Tukey test with 1% error level was used to compare the means. The Excel software was used to draw the diagrams.

RESULTS AND DISCUSSION

Ability of LDH in adsorbing soil excessive nitrate in bell pepper plantation

Fig. 1 indicates the average effects of applying LDH to the N-nitrate concentration in the soil solution, N-nitrate adsorbed on LDH, and the total N adsorbed by bell pepper plants. Results indicated that an increase in the LDH application rate increased N-nitrate adsorption on LDH, thereby decreasing the N-nitrate concentration in the soil solution. Compared to the control, treatments with 2, 4, 8 and 16 g LDH per kg soil could reduce the nitrate concentration in the soil solution by 31%, 51%, 79% and 95%, respectively. LDHs are anionic clay minerals which have anionic exchange capacity and have a layered structure with positive charge. In octahedron structure, when bivalent cations isomorphic ally substitute trivalent cations, the formed layer indicates the remaining positive charge. Thus, for electrical neutralization, presence of anions among the layers is crucial. LDH has a high selectivity for nitrate adsorption. Soil solution nitrate replaces LDH interlayer chloride and soil solution nitrate concentration is decreased after chloride adsorption by LDH. Thus, LDH controls nitrate movement in soil. LDHs have a significant anion exchange capacity, and this characteristic contributes to their good ability in nitrate adsorption (Halajnia *et al*., 2013; Torres-Dorante *et al*., 2008). Lower availability of nitrates can decrease the risk of nitrate leaching in farmlands during the winter and

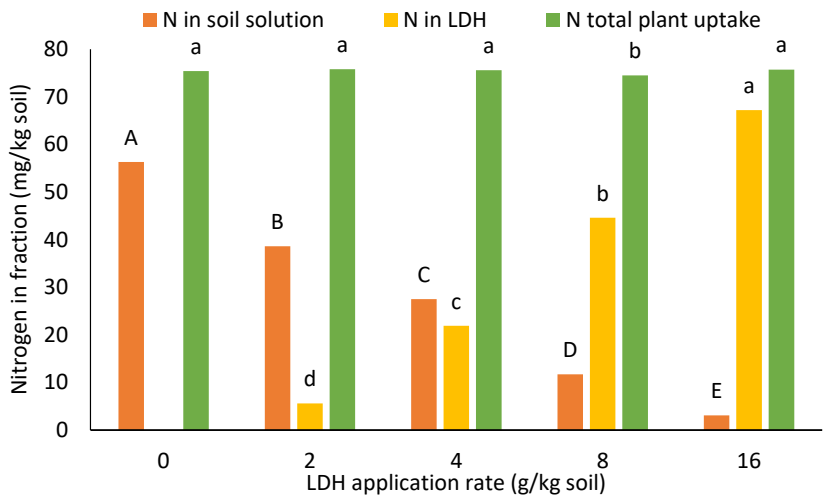

Fig. 1: Effect of LDH application rate along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N adsorbed by bell pepper plant. Vertical bars indicate standard deviation (n=4). Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

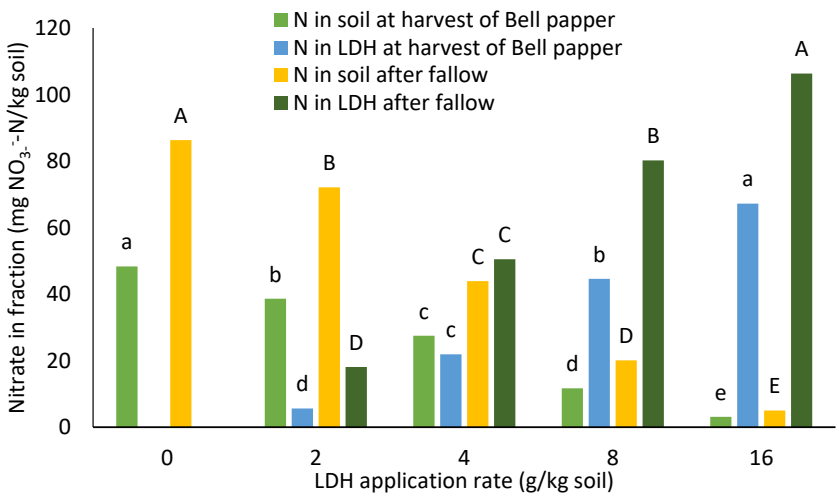

Fig. 2: Effect of LDH application rate along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N adsorbed by bell pepper after a two-week fallow period. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.

in farmlands with a sandy soil texture. No significant difference was detected in the N-nitrate adsorbed by plants in treatments, LDH and control treatment (Fig. 1).

Effects of LDH on adsorbing soil mineral nitrates

Fig. 2 indicates the amounts of the N-nitrate adsorbed on LDH and N-nitrate content in the soil solution before fallow and after a two-week fallow period. During the fallow period, the immobilized (mineralized) nitrate in the soil was increased, LDH treatments adsorbed it well, and the N-nitrate concentration in the soil solution was decreased. However, in the control, the N-nitrate concentration in the soil solution was increased (86.3 mg NO_3^- N/kg soil). Treatments with 8 and 16 g/LDH kg soil adsorbed

almost the whole nitrate mineralized during the fallow period. This could reduce the nitrate leaching occurred owing to nitrogen mineralization during the fall and winter (with high rainfall) in the farmlands of humid and tropical areas. Higher LDH application rates led to higher N-nitrate adsorption on LDH, and the highest N-nitrate adsorption was related to the treatment with 16 g/LDH kg soil (114.9 mg NO_3^-N/ kg soil).

Fig. 3 shows the bell pepper plant with different LDH treatments and the control. Comparison of the means showed significant differences in LDH



Fig. 3: Bell pepper plants with different LDH treatments (2, 4, 8, and 16 g LDH/kg soil) and control treatment

Table 2: Effect of LDH application rate along with urea fertilization (46.5%) on the qualitative and quantitative properties of bell pepper plants

Qualitative and quantitative properties of plant	LDH application rate (g/kg soil)				
	0	2	4	8	16
Plant height(cm)	31.5 d	35.6 c	39.5 b	42.4 a	42.9 a
Plant wet weight(g)	58.1 e	64.4 d	72.8 c	86.5 b	93.3 a
Plant dry weight(g)	10.4 e	12.5 d	15.1 c	17.4 b	18.7 a
Root wet weight(g)	33.2 e	38.1 d	40.5 c	42.3 b	44.8 a
Root dry weight(g)	8.0 e	10.1 d	12.1 c	13.2 b	15.3 a
Fruit wet weight(g)	81.3 e	115.6 d	127.4 c	135.4 b	138.6 a
Fruit dry weight(g)	14.4 e	22.8 d	26.2 c	29.5 b	32.7 a
Fruit total weight(g)	829.2 e	1629.9 d	2242.2 c	3046.5 b	3534.3 a
Fruit length(mm)	65.6 c	69.3 b	69.5 b	84.1 a	85.3 a
Fruit diameter(mm)	127.3 d	142.4 c	161.7 b	173.6 a	176.2 a
Number of fruits	10.2 e	14.1 d	17.6 c	22.5 b	25.5 a
TSS (%)	6.4 d	6.5 cd	6.7 bc	7.3 ab	7.4 a
Fruit vitamin C (mg per 100 ml fruit juice)	88.4 d	119.4 c	138.1 b	156.1 a	157.6 a
relative chlorophyll content (chlorophyll meter)	33.1 e	38.1 d	40.3 c	45.7 b	46.6 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

treatments in terms of number of fruits, wet and dry weights of shoots, roots and fruit, total weight of fruits, and relative chlorophyll. Increase of LDH application improved these traits (Table 2). Treatments with 16 and 8 g LDH/kg soil did not show significant differences in terms of plant height. The lowest plants were seen in the control (31.5 cm), and the highest plants were seen in treatments with 16 and 8 g LDH/kg soil (42.5 cm). The maximum and minimum weights of wet and dry shoots were related to the treatment with 16 g/LDH kg soil and the control, respectively. Treatments with 2, 4, 8 and 16 g/LDH kg soil led to 14.1%, 17.6%, 22.5% and 25.5% increase in the number of fruits. The least number of fruits was seen in the control (10.2). Comparison of the means demonstrated a significant difference in different treatments in terms of leaf area, but no statistically significant difference was observed between the treatments with 2 and 4 g LDH/kg soil. Maximum and minimum leaf areas were related to 16 g LDH/kg soil (54.2 cm^2) and control (40.4 cm^2) treatments, respectively. With the increase of LDH application rate, wet and dry weights of fruits increased compared to the control, so that 16 g LDH/kg soil resulted in 70% and 127% increase in wet and dry weights, respectively. Furthermore, appropriate plant nutrition is highly important. N compounds comprise 40%-50% of protein dry matter as the living material of plant cellules (Togun *et al*., 2003). For this reason, higher N uptake plays an important role in plant growth processes, and without sufficient N supply, the plant cannot grow well (Aminifard *et al*., 2018). LDH contributes to higher nitrogen and availability of other nutrients for plants when necessary; therefore, it plays an important role in better growth of roots and shoots (height, leaf area, number of leaves, etc.). Many researchers reported that higher plant N uptake significantly increased bush height, number of branches, number of leaves, wet and dry weights of plant shoots and roots and ripe fruit yield (number and wet and dry weights) of bell peppers (Ayodele *et al*., 2015; Aminifard *et al*., 2018). Compared to the control, application of 2, 4, 8 and 16 g LDH/kg soil increased plant chlorophyll content up to 15%, 19%, 38% and 41%, respectively, due to increases in the nitrogen absorption (uptake) by the plant. Nitrogen influence on the plant chlorophyll content might be due to nitrogen role as a chlorophyll component. Furthermore, nitrogen is the main component of all amino acids of proteins and lipids as the structural components of an active chloroplast (Ouda and Mahadeen, 2008). Aminifard *et al*. (2018) reported increases in the chlorophyll content of bell peppers due to higher plant nitrogen absorption. Higher LDH application rates resulted in longer fruits, but there was not a significant difference between treatments with 16 and 8 g LDH/kg soil and between

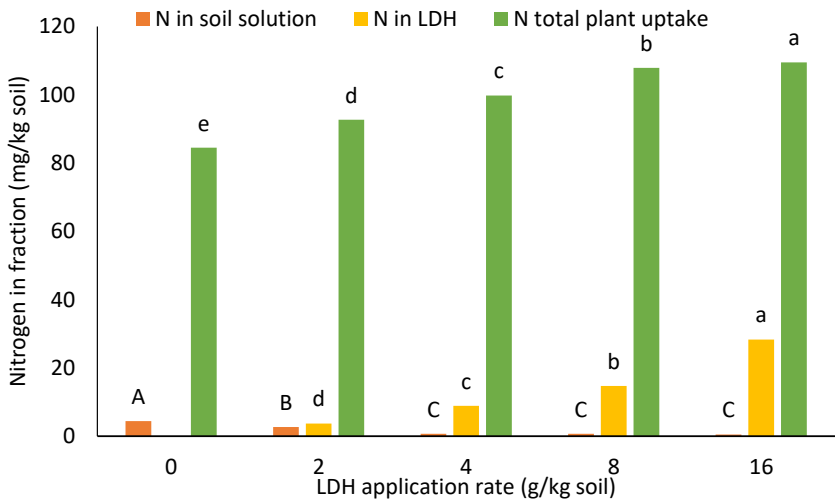

Fig. 4: The effect of LDH application rate along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N absorbed by mentheae plant. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

treatments 2 and 4 g LDH/kg soil. Comparison of means indicated a significant effect of LDH application rates on fruit diameter, but no significant difference was observed between applying 8 and 16 g LDH/kg soil. LDH treatments had a significant effect on vitamin C content. However, there was no significant difference between treatments with 8 and 16 g LDH/kg soil. Higher vitamin C contents can be attributed to increase of plant nitrogen absorption, being consistent with the results reported by Aminifard *et al*., (2012) suggesting that increase of plant nitrogen absorption (uptake) affects vitamin C content in bell pepper plants. Comparison of the means suggested a significant difference in terms of TSS%; however, there was no significant difference between applying 8 and 16 g LDH/kg soil, 4 and 8 g LDH/kg soil, 2 and 4 g LDH//kg soil.

Reversibility of nitrate adsorption process from LDH during mentheae growth period

In order to study the nitrate exchange capacity of LDH in the long term, successive crop and fallow periods were established. For this reason, the second crop was planted in previous pots. During the second crop growth period, plants could make use of the N-nitrate adsorbed on LDHs and absorb (uptake) the nitrates remained in the soil solution. Therefore, the N-nitrate concentration in the soil solution remained low (Fig. 4). At the end of the second crop's growth period, values of the N-nitrate adsorbed in treatments with 2, 4, 8 and 16 g LDH/kg soil reached 3.7, 8.9, 14.7 and 28.3 mg N-Nitrate/kg soil, respectively. Comparison of the means revealed the significant effect ($p \leq 0/05$) of LDH treatments on the nitrogen values of the plant biomass (Fig. 4). Application of 16 g LDH/kg soil had the largest effect on the nitrogen amount in plants, increasing the availability of nitrogen in plants to 109.5 mg N-Nitrate/kg soil and resulting in 30% increase in the amount of plant nitrogen compared to the control treatment. In fact, LDHs act as a slowly nitrogen releasing agent, which not only satisfy plant nitrogen needs, but also prevent nitrogen loss in the soil.

LDH ability to resorb soil excessive nitrate

At the end of the second crop's growth period, the N-nitrate concentration was decreased in LDH treatments and the control. In order to investigate the ability of LDHs for resorbing mineralized nitrate from the soil solution, 453 mg of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 3 kg of the soil). Therefore, LDHs could adsorb the N-nitrate added from the urea fertilizer as well as nitrates mineralized during a two-week

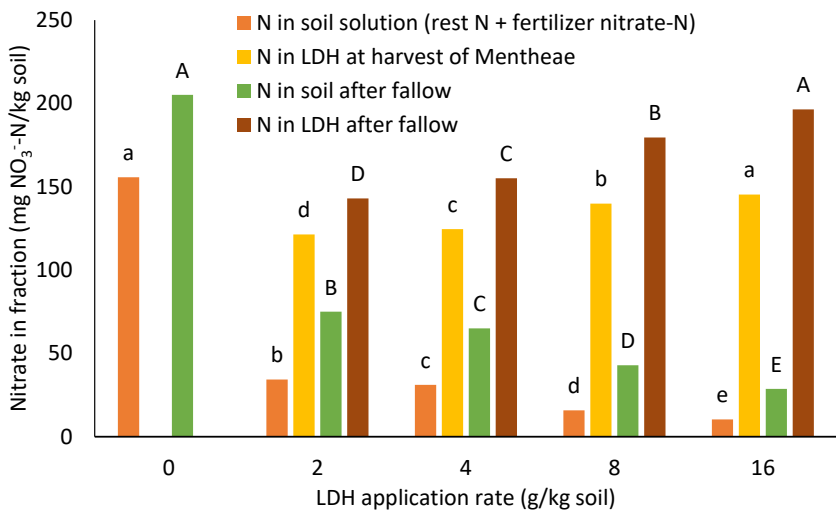

Fig. 5: The effect of LDH application rate along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N absorbed by mentheae plant after a two-week fallow at greenhouse. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.

fallow and retained the N-nitrate concentration low in the soil solution (Fig. 5). A significant difference was observed in different LDH treatments in terms of the N-nitrate adsorbed on LDH surfaces. After the fallow period, concentrations of N-nitrate adsorbed in treatments with 2, 4, 8, 16 g LDH/kg soil reached 143, 155, 179.5 and 196.38 mg N-Nitrate/kg soil, respectively. The N-nitrate concentration on the LDH surface in the treatment with 16 g LDH/kg soil was almost the same as its concentration in the soil solution of the control (205.18 mg N-Nitrate/kg soil). This suggests that LDH has a considerable ability to control nitrate movement in the soil and keep the N-nitrate concentration low. The results of Halajnia *et al*. (2016) demonstrated that application of 20 g LDH per kg soil could reduce N-Nitrate in the soil solution by 59% compared to the control. Torres-Dorante *et al*. (2009) stated that LDH could maintain N-Nitrate in its structure, and the application of 20 g LDH per kg reduced the amount of N-Nitrate in the soil solution by up to 80% compared to the control.

Fig. 6 shows mentheae pots with different LDH treatments and the control. Results of comparison of the means were significantly different in terms of plant height, but there was no significant difference between the treatments applying 2 and 4 g LDH/kg soil (Table 3). The highest plants were seen in 16 g LDH/kg soil treatment (58.2 cm), and the lowest plants were seen in the control (28.3 cm). Compared to the control, the lowest rate of LDH application (2 g LDH/kg soil) resulted in 53% increase in plant height. Apparently, LDH treatment leads to better plant growth by reducing nitrogen loss and making more nitrogen available. Considering the mean number of side shoots, comparison of the means showed a significant difference between control and LDH treatments, but treatments with 8 and 16 g LDH/kg soil were not statistically different. The most and the least numbers of side shoots were related to 16 g LDH/kg soil treatment and the control, respectively. Although, number of leaves was increased with applying higher LDH rates, comparison of the means did not show a statistically significant difference between treatments with 8 and 16 g LDH/kg soil and between the control and 2 g LDH/kg soil treatment. The least number of leaves in the control (510.4) was probably owing to lower availability of nitrogen in plants, since increase in nitrogen availability increases photosynthate content and improves plants vegetative traits, including the number of leaves (Ardalani *et al*., 2017). More leaves in LDH treatments suggests the positive role of LDH in plant nitrogen uptake. Regarding the mean wet and dry weights of roots and shoots, comparison of the means shows that LDH treatments improved these traits compared to the control. However, treatments with 8 and 16 g LDH/kg soil were not statistically different in terms of wet and dry weights of roots, and treatments with 8 and 16 g LDH/kg soil were not statistically different in terms of wet weight of root. Comparison of the means of LDH treatments showed significantly different results for wet and dry weights of plant shoots. However, the



Fig. 6: Mentheae plants in different LDH treatments (2, 4, 8, 16 g LDH/kg soil) and control

Table 3: Effect of LDH application rate along with urea fertilization (46.5% N) on the qualitative and qualitative traits of mint plants

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Plant height(cm)	28.3 d	43.4 c	43.6 c	48.5 b	58.2 a
Shoots wet weight (g)	54.3 e	60.4 d	76.1 c	83.4 b	90.6 a
Shoots dry weight(g)	10.3 d	21.2 c	21.5 c	25.7 b	30.7 a
Roots wet weight (g)	9.1 c	14.5 b	14.4 b	18.2 a	18.4 a
Roots dry weight (g)	3.1 d	5.2 c	5.9 b	7.1 a	7.1 a
Number of leaves	510.4 c	513.3 c	558.2 b	662.8 a	667.3 a
Number of side branches	10.2 d	18.3 c	21.4 b	27.6 a	28.3 a
Total leaf chlorophyll (mg/g dry sample)	20.7 e	41.2 d	45.2 c	47.9 b	49.6 a
Chlorophyll-a (mg/g dry sample)	12.3 d	22.4 c	25.1 b	27.6 a	27.8 a
Chlorophyll-b (mg/g dry sample)	8.4 d	18.8 c	19.9 b	20.3 b	21.8 a
Carotenoid (mg/g dry sample)	0.68 d	4.03 c	4.4 b	4.8 a	4.8 a
Flavone and flavonol (mg/g dry sample)	10.2 c	13.3 c	18.1 bc	21.1 ab	26.5 a
Anti-oxidant (%)	77.3 e	88.1 d	93.8 c	94.5 b	98.8 a
Total phenol l (mg Galic acid/100 g dry sample)	20.4 e	29.2 d	39.4 c	40.5 b	43.4 a
Essence (%)	2.1 d	2.45 d	2.9 c	3.4 b	3.9 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

difference between treatments with 2 and 4 g LDH/kg soil was not statistically significant. Compared to the control, application of 16 g LDH/kg soil led to 3 times heavier dry shoots. Results showed that the highest and lowest wet weights were obtained by applying 16 g LDH/kg soil (90.6 g) and the control (54.3 g), respectively. Compared to the control, application of 2 g LDH/kg soil led to 11% increase in wet weight. Lighter plant wet weight in the control was probably due lower water holding capacity and lower anion exchange capacity. Growth of shoots in hydrophilic plants such as mentheae was closely associated with water availability, so that plant beds with more available water could improve plant growth and increase the weight of plant shoots (Keshavarz *et al*., 2018). It seems that LDH increases plant wet and dry weights by making more nitrogen available for plants and highlighting the role of nitrogen in the structure of macromolecules such as proteins, amino acids, and nucleic acids. Moreover, enough nitrogen resulted in more flowering branches and leaves, thereby increasing production per unit area (Kheirandish *et al*., 2016). Many studies have reported the effects of increase of plant nitrogen uptake (absorption) on improving vegetative traits of mentheae (plant height, wet and dry weight of shoots, leaves and roots, number of side branches, leaves etc.) (Keshavarz *et al*., 2018; Ardalani *et al*., 2017). Increase in the LDH application rate improved the contents of total chlorophyll, chlorophyll-a, chlorophyll-b, carotenoids and essence in plants, and comparison of the means indicated a significant difference between LDH treatments and the control. The highest and lowest chlorophyll contents were observed in treatment with 16 g LDH/kg soil (49.6 mg/g dry sample) and the control (20.7 mg/g dry sample). Treatments with 8 and 16 g LDH/kg soil exhibited no significant difference in terms of chlorophyll-a and carotenoid contents, and treatments with 8 and 4 g LDH/kg soil did not lead to a significant difference in terms of chlorophyll-b content. LDH application increased the leaf essence content (%) as in treatments with 2, 4, 8, 16 g LDH/kg soil, it reached 2.45%, 2.9%, 3.4%, and 3.9% respectively. Nitrogen, by increasing the number of leaves and leaf area, provided an appropriate bed to obtain solar energy and was a component of

chlorophyll structure. Furthermore, other enzymes involved in the photosynthetic process of carbon metabolism increased photosynthetic efficiency and played a key role in rising the essence content (Ouda and Mahadeen, 2008). Considering the considerable ability of LDH for nitrogen absorption, increase of LDH application rate improved nitrogen absorption and consequently chlorophyll and essence content. Carotenoid is one of the photosynthetic pigments, and nitrogen is a component of carotenoids. Therefore, LDH application increases the plant carotenoid content. Comparison of the results obtained for flavones and flavonols revealed that the difference between applying 4 and 8 g LDH/kg soil, 2 and 4 g LDH/kg soil, and 2 g LDH/kg soil was not significant. Increase of LDH application rate increased the total phenol content and the plant antioxidant percentage. Compared to the control, application of 16 g LDH /kg soil resulted in 112% increase in the total phenol content. The least and the most antioxidant contents were related to the control (77.3%) and the treatment with 16 g LDH/kg soil (98.8%), respectively. Phenolic compounds include flavonoids, flavonols, anthocyanin, anthraquinone, and their derivatives. Apparently, phenolic compounds, especially flavonoids and flavonols, have higher antioxidant power. Flavone and flavonole are found in flowers and leaves of green plants. Compared to anthocyanin, these compounds absorb light in lower wavelengths and act as cell protectants against harmful ultraviolet rays (Fathiazad *et al*., 2010). Phenolic content of plants depends on the genetic and environmental factors of plants. Several studies have investigated the effect of plant growth condition on second metabolites, most of which emphasize the role of habitat as an effective factor in accumulation of second metabolites. By changing the moisture condition and increasing the availability of nutrients such as nitrogen, site and crop growth, medium conditions can be effective in forming plant active ingredients (Pourmorad *et al*., 2006). LDH has a high capacity for adsorbing nitrogen and water (Berber *et al*., 2014). Bidgoli *et al*. (2018) and Keshavarz *et al*. (2018) reported that higher nitrogen content in mentheaes increased total contents of phenolic compounds, flavone content, antioxidant, and extract.

LDH ability for reducing nitrate leaching in tomato cropping condition

One of the present study objectives was to investigate the LDH ability to reduce nitrate loss in the soil and to evaluate the retention of the nitrate adsorbed on LDH under leaching conditions. For this reason, 4 phases of leaching were performed during the third crop (cherry tomato) growth period. Results indicated that application of 2, 4, 8 and 16 g LDH/kg LDH led to 34%, 44%, 58% and 69% reduction in the N-nitrate concentration of soil leachates compared to the control, respectively (Table 4). In fact, the nitrate adsorbed on LDH during crop growth was retained well against different leaching phases. This can be attributed to the structural characteristics of LDH. Some important characteristics of LDH are a layered structure for keeping interlayer anions, a significant anion exchange capacity, and a very high specific surface area (Benicio *et al*., 2018; Hu *et al*., 2017). By applying one-hour drop irrigation of 45 mL water for two days in the soil with 2500 mL N-nitrate as the initial concentration and 1.5 g Mg-Al-LDH/kg soil, Torres-Dorante and Lammel (2009)

Table 4: Effect of LDH application rate along with urea fertilization on nitrate concentration in leachates (mg/L) obtained from different leaching phases during cherry tomato growth period

LDH application rate (g/kg soil)	Leaching phases				
	First phase	Second phase	Third phase	Fourth phase	Total leachate
0	17.3 a	35.4 a	28.9 a	7.5 a	89.1 a
2	14.1 b	23.6 b	15.8 b	4.9 b	58.4 b
4	8.1 c	19.8 c	17.2 c	4.3 c	49.4 c
8	7.6 c	14.5 d	10.8 d	3.8 d	36.7 d
16	5.9 d	10.9 e	7.5 e	2.6 e	26.9 e

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

found that the synthetized LDH had a capacity to leach nitrates up to 75%. The least amounts of leached nitrates were observed in the first and the fourth phases of irrigation. Reduction of N-nitrate concentration in leachate during the first phase occurred owing to transformation of urea to nitrate whose movement in the soil profile to the deep soil is a time-consuming process. The reason for reduction of the N-nitrate concentration in leachate during the fourth phase was nitrate leaching in previous phases and reduction of the soil N-nitrate concentration (Table 4). Considering the N-nitrate absorption by plants, a significant difference was observed between control and LDH treatments. The highest and the lowest plant N-nitrate absorption belonged to the treatment with 16 g LDH/kg soil (106.5 mg N-Nitrate/kg) and the control (50 mg N-Nitrate/kg), respectively (Fig. 7). In addition, LDHs could retain the N-nitrate concentration in the soil solution at low levels. LDHs not only prevented soil nitrate loss, but also led to better conditions for plant nitrogen absorption by making nitrogen available regularly and gradually. Finally, LDHs prevented the loss of this useful nutrient by keeping nitrogen inside its structure.

Mineral nitrate adsorption capacity of LDH after leaching

To investigate the capacity of LDH to adsorb soil mineral nitrate, a two-week fallow was performed after the plant growth period. During the third fallow, the nitrate content in the control was increased owing to mineralization (immobilization) (91.4 mg N-Nitrate/kg soil), but in LDH treatments, the N-nitrate concentration in the soil solution was decreased owing to LDH adsorption on LDH (Fig. 8). There was a significant difference between all LDH treatments and the control in terms of nitrate adsorption on LDH. In treatments with 2, 4, 8 and 16 g LDH/kg soil, the rates of nitrate adsorption on LDH after fallow were 79.3, 97.01, 112.1 and 126.7 mg N-Nitrate/kg soil, respectively.

Fig. 9 shows the cherry tomato plants in control and LDH treatments. Comparison of the means showed significant differences in terms of plant height, as well as wet and dry eights of shoots and roots (Table 5). Increase of LDH application rate led to increase plant height, wet and dry weights of shoots and roots. Considering the wet and dry weights of plant roots, there was no significant difference between treatments with 4 and 8 g LDH/kg soil. Application of 2, 4, 8 and 16 g LDH/kg soil increased plant height by 14%, 26%%, 50 and 80%, respectively. Nitrate had a growth-stimulating effect on plants, and LDH made more nitrate available, thereby increasing the plant height, as well as wet and dry weights of plant roots and shoots. The effect of nitrogen on increasing the vegetative growth of stems is attributed to changes of the plant hormones balance in plant vegetative

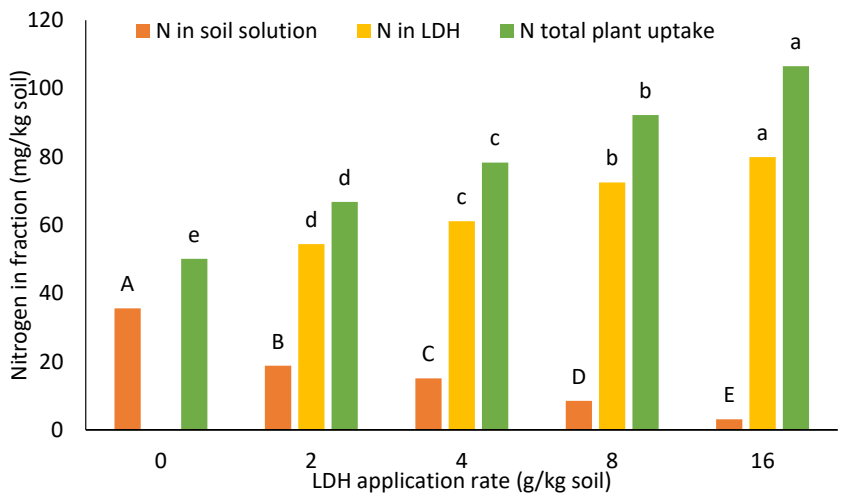

Fig. 7: Effects of LDH application along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total plant N uptake in cherry tomato plants after leaching. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

shoots (Rahman *et al*., 2007). Many researchers have demonstrated the positive relationship between increase in nitrogen availability and vegetative growth of tomatoes (height, wet and dry weight of shoots, fruit and roots, etc.) (Rahman *et al*., 2007; Li *et al*., 2017; Chen *et al*., 2016). Comparison of the means showed significant differences in terms of number of fruits, wet and dry weight of fruit, total weight of fruits, fruit length and diameter. Higher LDH application rates increased these traits. Considering the wet and dry weights of fruit, there was no significant difference between treatments with 8 and 16 g LDH/kg soil and treatments with 4 and 8 g LDH/kg soil. Additionally, fruit length and diameter were not significantly different in treatments with 8 and 16 g LDH/kg soil. Comparison of the means revealed that the treatment with 16 g LDH/kg soil had the highest number of fruits (102.7), weight of one fruit (15.3 g), and fruit dry weight (0.76 g), while the control had the lowest number of fruits (28.4), weight of one fruit (7.3), and fruit dry weight (0.34). The mean difference of total weight of fruits in the treatment with 16 g LDH /kg soil, and the control was 1300 g. Compared to the control, application of 2 g LDH/kg soil led to 85% increase. LDH made plant nitrogen availability higher, and increased the fruit length and diameter. Many studies have reported that fruit length and diameter increases with the increase of nitrogen availability (Li *et al*., 2017). Growth improvement plays an important role in tomato yields and number of fruit per bush. Nitrogen deficiency during flowering leads to flower falling and lower number of fruits, whereas appropriate nitrogen availability leads to higher nitrogen content in the plant. This nitrogen prevents abscisic acid accumulation, thereby preventing flower falling (Streeter, 1978). In fact, LDH increases the number of fruits by making sufficient nitrogen available when it is necessary for plant growth. Comparison of the means for total chlorophyll content and chlorophyll-a content in LDH treatments showed a significant difference, but there was no statistical difference in terms of chlorophyll-b content. Higher LDH application rates led to higher chlorophyll content in plants. The highest and the lowest chlorophyll contents were related to 16 g LDH/kg soil (1.42 mg/g dry sample) and the control (0.485 mg/g dry sample), respectively. This indicates that nitrogen is part of chlorophyll molecule. Nitrogen is a component of all main amino acids in proteins and lipids as parts of an active chloroplast structure (Ouda and Mahadeen, 2008). Therefore, higher nitrogen uptake by plants leads to higher chlorophyll contents in plants. TSS had an ascending trend with the increase of LDH application rate, but there was no significant difference between treatments with 4 and 8 LDH/kg soil, treatments with 2 and 4 LDH/kg soil, and treatments with 2 and 0 LDH/kg soil (control).

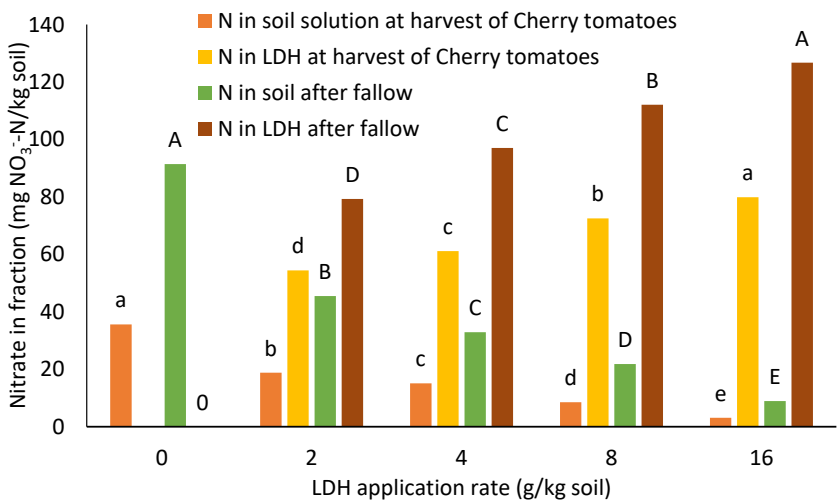

Fig. 8: Effects of LDH application along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total plant N uptake in cherry tomato plants after a two-week fallow period in greenhouse. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.



Fig. 9: Cherry tomatoes with LDH treatments (2, 4, 8 and 16 g LDH/kg soil) and control treatment

Table 5: Effects of LDH application rate along with urea fertilization (46.5%) on qualitative and quantitative traits of cherry tomato plants

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Plant height (cm)	21.2 e	24.3 d	26.5 c	31.5 b	38 a
Shoot weight(g)	68.4 e	120.3 d	147.7 c	152.2 b	168.2 a
Shoot dry weight(g)	8.7 e	18.4 d	23.8 c	25.1 b	27.8 a
Root weight (g)	10.5 d	14.3 c	16.1 b	17.8 ab	18.6 a
Root dry weight (g)	3.4 d	5.1 c	6.1 b	6.7 ab	7.3 a
Number of fruits	28.4 e	58.8 d	66.1 c	95.2 b	102.7 a
Weight per fruit (g)	7.3 d	12.8 c	14.5 b	15.2 ab	15.3 a
Dry weight per fruit (g)	0.34 d	0.5 c	0.63 b	0.74 a	0.76 a
Total weight of fruits (g)	204.4 e	742.4 d	957 c	1444 b	1560.6 a
Fruit length (mm)	0.7 d	1.3 c	1.7 b	2.6 a	2.8 a
Fruit diameter (mm)	0.8 d	1.5 c	2 b	2.8 a	3 a
Chlorophyll a (mg/g dry sample)	0.41 c	0.7 b	0.86 ab	0.89 a	0.96 a
Chlorophyll b (mg/g dry sample)	0.075 a	0.1 a	0.35 a	0.4 a	0.46 a
Total chlorophyll (mg/g dry sample)	0.485 e	0.8 d	1.21 c	1.29 b	1.42 a
TSS (%)	4.1 c	4.5 bc	5.7 ab	5.8 ab	6.4 a
Vitamin C (mg/100 ml fruit juice)	25.1 e	30.2 d	40.6 c	45.2 b	48.1 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

Comparison of the means for vitamin C content in fruits showed a significant increase. Application of 16 g LDH/kg soil led to 91% increase in fruit vitamin C content. Ochoa-Velasco *et al*. (2016) have investigated the effect of nitrate absorption (uptake) on improving vitamin C amount in tomato fruits and their TSS.

LDH ability for long-term nitrate exchange during successive crop-fallow rotations in wheat plantation

To investigate the ability of LDH to desorb the nitrates adsorbed during long-term crop-fallow rotations, the nitrate released from LDH was studied by planting wheat as the fourth crop. Wheat plants were grown without any nitrogen fertilization. Results demonstrated that after one and half a year crop-fallow rotations, LDH still had a high ability to desorb the N-nitrate for the needs of plants (Fig. 10). Wheat plants consumed the N-nitrate adsorbed by LDH and N-nitrate in the soil solution (Fig. 10). At the end of growth period, the N-nitrate concentration in the soil solution of all treatments (LDH and control) was decreased considerably. However, LDHs still contained some nitrates. Amounts of N-nitrate remained in treatments with 2, 4, 8 and 16 g LDH/kg soil were equal to 8.9, 12.8, 147 and 15.6 mg N-Nitrate/kg soil. In terms of N-nitrate uptake by plants, a significant difference was observed between

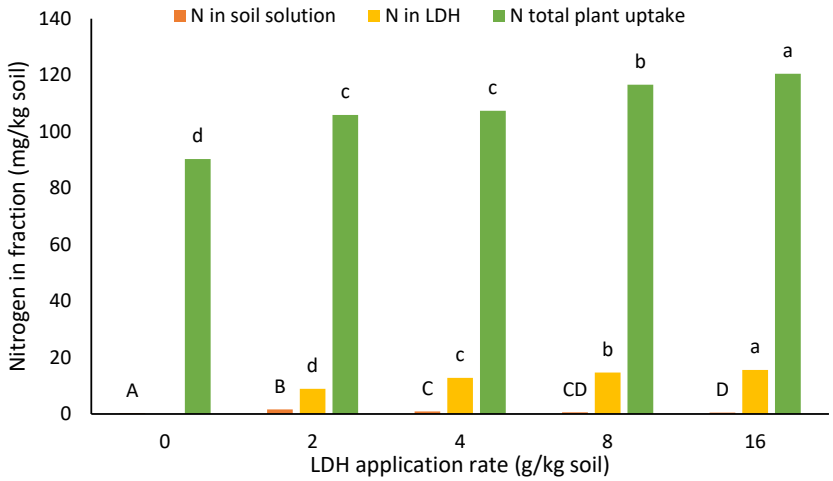

Fig. 10: Effects of LDH application along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N uptake by wheat plants. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.



Fig. 11: Wheat plants in LDH treatments (2, 4, 8, 16 g LDH/kg soil) and control

Table 6: Effects of LDH application rate along with urea fertilization (46.5%) on qualitative and quantitative traits of wheat

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Bush height(cm)	39.2 e	62.3 d	64.3c	65.2 b	68.8 a
Shoots dry weight(g)	8.1 e	14.8 d	17.4 c	22.3 b	23.6 a
Roots dry weight(g)	0.6 c	1.7 b	1.7 b	1.8 b	2.2 a
Total dry weight(g)	8.7 e	16.5 d	19.2 c	24.1 b	25.8 a
Seeds per spike	16.2 d	24.1 c	27.3 b	27.3 b	29.6 a
1000-seed weight (g)	33.1 d	42.4 c	47.1 b	47.3 b	49.8 a
Total chlorophyll (mg/g dry sample)	0.3 a	0.4 a	0.5 a	0.6 a	0.7 a
Chlorophyll a(mg/g dry sample)	0.2 c	0.2 c	0.3 b	0.4 a	0.4 a
Chlorophyll b(mg/g dry sample)	0.1 c	0.1 bc	0.2 b	0.2 a	0.2 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

LDH treatments and the control, but there was no significant difference between treatments with 2 and 4 g LDH/kg soil.

Fig. 11 indicates the wheat plants in LDH treatments and the control. Table 6 presents the effects of LDH application rate on the quantitative and qualitative traits of wheat plants. Comparison of the means showed significant differences in terms of plant height, dry weights of shoots and roots and total plant weight. However, no significant difference was seen in treatments with 2, 4 and 8 g LDH/kg soil in terms of root dry weight. The highest and the lowest plants were obtained in the treatment with 16 g LDH/kg soil (68.8 cm) and the control (39.2 cm) treatment, respectively. As explained for previous crops, LDH, by making more water and nitrogen available when necessary for plant growth, improves plants vegetative traits such as height, dry weight of roots, and plant biomass. Additionally, 1000-seed weight and seeds per spike were increased in higher LDH application rates. Comparison of the means showed no statistically significant difference between application of 4 and 8 g LDH/kg soil. The maximum number of seeds (29.6 g) and 1000-seed weight (49.8 g) were related to the treatment with 16 g LDH/kg soil, and the minimum number of seeds (16.2 g) and 1000-seed weight (33.1 g) were related to the control. Although total chlorophyll values were increased in higher LDH application rates, there was no significant difference in different LDH treatments. Chlorophyll content is nearly proportional to leaf nitrogen content (Evans, 1989). Many studies have demonstrated the effect of higher plant nitrogen uptake on wheat yield (height, dry weights of roots and shoots, 1000-seed weight, number of seeds per spike, and chlorophyll content) (Wang *et al*., 2017; Nie *et al*., 2018).

CONCLUSION

When plants require nitrate, LDH, as a useful nitrate exchanger, adsorbs nitrate if the concentration of nitrate in soil solution is high. LDH can re-adsorb nitrate If the nitrate concentration in soil solution rises again. To determine the nitrate exchange capacity of LDH in a long-term period, successive periods of crop and fallow were established. In all crop periods, LDH could adsorb soil excessive nitrate and decreased the nitrate concentration in the soil solution. Lower nitrate availability in the soil solution would reduce the risk of nitrate leaching in farmlands during the winter and farmlands with sandy-textured soil. During the two-week fallow periods, the amounts of mineralized nitrate in the soil solution were increased, and LDH treatments could adsorb them efficiently and decrease the N-nitrate concentration in the soil solution. Treatments with 8 and 16 g/LDH kg soil adsorbed almost the whole nitrate mineralized during the fallow period. This could reduce the nitrate leaching occurred due to nitrogen mineralization in farmlands of humid and tropical areas during fall and winter (with high rate of rainfall). LDHs not only prevented the loss of nitrate

in the soil, but also made it available regularly and gradually, thereby improving the nitrate uptake conditions for plants. LDHs, by making more nitrogen available, improved the quantitative and qualitative traits of medical plants (mentheae), cucurbits (cherry tomato and bell pepper) and crops (wheat). LDH contributes to increase of nitrogen and availability of other nutrients for plants when necessary; therefore, it plays an important role in improving the growth of roots and shoots (height, leaf area, number of leaves, etc.). Higher plant N uptake significantly increased bush height, number of branches, number of leaves, wet and dry weights of plant shoots and roots and ripe fruit yield of plants (number and wet and dry weights). Results demonstrated that even after 18-month crop-fallow rotations, LDH had a high ability to desorb N-nitrate to fulfil the needs of wheat. Wheat consumed the N-nitrate adsorbed by LDH and the N-nitrate in the soil solution. At the end of growth period, N-nitrate concentrations in the soil solution in all treatments (LDH and control) were decreased significantly. Considering the importance of nitrate leaching risk in greenhouses, LDHs are applied as amendments for pot crops. LDH can also be used in golf courses, public greens, and sport fields. The risk of nitrate leaching losses in these areas is of high concern because of permanent irrigation and fertilizer application required to maintain the grass quality. Greens are mainly constructed on sand-based media (>90% sand) in order to increase their resistance to traffic and to ensure water percolation. As in containerized crop production, the main problems of sand-based growing media are their low cation exchange capacity and low water retention. Therefore, the nutrients applied in these media are prone to be leached. To provide cation exchange capacity, the zeolites-based products, which are silicate minerals, are recommended as amendments to sand putting greens in golf courses and turf grasses. Thus, LDH can be easily incorporated during the construction of putting greens/growing media in order to create nitrate exchange capacity.

AUTHOR CONTRIBUTIONS

M. Mohammadi analysed and interpreted the data and finalized the manuscript for publication. A. Mohammadi Torkashvand conducted the comprehensive review. M. Esfandiari helped in the sampling experiments. P. Bi-Parva performed experimental design. All authors participated in literature review and preparation of the manuscript.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

%	Percent
A°	Angstrom
C	Carbon
CO_3^{2-}	Carbonate ion
°C	Centigrade
Cl^-	Chloride ion
G	Gram
g/cm^3	Gram per cubic Centimetre
g/L	Gram per Litre
g/mg	Gram per milligram
g/mg min	Gram per gram minute
HCO_3^-	Bicarbonate ion
kg	Kilogram
L	Litre
L/g	Litre per gram
L/kg	Litre per kilogram
L/mg	Litre per milligram
LDH	Layered Double Hydroxide
M	Molar
Mg	Milligram
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
mg/L	Milligram per Litre
min	Minute
mmol/g	Mill mole per gram
N	Nitrogen
NH_4^+	Ammonium ion
NO_3^-	Nitrate ion

pH	Potential of hydrogen
rpm	Revolutions per minute
S	Second
*XR*D	X-ray diffraction

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