

# Influence of Lherzolite on the Growth of Chinese Spinach and Soil Respiration in Cadmium Contaminated Soil

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## Abstract

A glasshouse pot experiment was conducted to investigate cadmium (Cd) tolerance on the growth of Chinese spinach (*Spinacia Oleracea* L.) grown in sandy loam soil and microbial respiration after application of lherzolite (0%, 2.5% and 5%) with added Cd (0, 2.5 and 5 mg·kg<sup>-1</sup>). Soil pH tended to increase with increasing application rate of lherzolite about 1.5 units after plant harvest. Plant growth and microbial respiration in one hand decreased with the rates of Cd application in soil but on the other hand lherzolite application in soil reduced Cd toxicity and enhanced plant growth and microbial respiration. Microbial respiration of soil showed significant positive relationship with shoot and root dry weight of spinach but negative relationship of Cd concentration in plant tissue. This result indicated that application of lherzolite detoxified Cd toxicity in plant resulted in an increase plant growth and microbial respiration in Cd contaminated soil.

## Keywords

Lherzolite, Cd, Chinese Spinach, Soil Respiration, Contamination

## 1. Introduction

Various industrial activities, manufacturing, and intensive agriculture have greatly increased the heavy metal contamination of soils over the past century. Cadmium contamination in soils has become a major environmental issue worldwide because Cd is ubiquitous in the human environment and has been identified as one of the most deleterious heavy metal pollutant [1]. Cadmium

may easily transfer from soil to food plants through root systems and accumulate in the plant tissues. In this pathway, Cd may enter the food chain and affect human health [2]. Among the heavy metals in polluted soil, Cd is potentially harmful not only on humans and animals, but also on microbial activity which plays important role in maintaining soil fertility [3] [4].

Various techniques have been used for the restoration of soils contaminated with heavy metals. Current technologies for soil remediation are time consuming or too expensive. Therefore, it is important to develop techniques that can treat and stabilize heavy metals in situ in an effective and cost-effective manner [5]. It is therefore, important to find out suitable additives to immobilize Cd in soil. Several additives have been screened for their potential to immobilize heavy metals in soils [6]. Each additive has a different effect on the bioavailability of metals, micronutrient availability, soil pH, and soil microstructure [7]. Liming can lead to significant decrease in soil solution concentration and DTPA or EDTA extractable Cd in soils amended with calcium carbonate [8].

Alkaline additives reduce heavy metal solubility in the soil by increasing soil pH and concomitantly increasing metal sorption to soil particles [9] [10]. Soil pH is one of the main parameters controlling the solubility and mobility of heavy metals in soils [11]. Agricultural liming materials, such as  $\text{CaCO}_3$ , increase soil pH and thereby affect the activity and composition of microbial populations [12]. Microbial and chemical responses to lime vary with soil type and management. In acid soils, liming can create better environmental conditions for the development of acid-intolerant microorganisms resulting in increased microbial biomass and soil respiration [13]. Shah *et al.* [14] showed a 20-fold increase in bacterial numbers after the application of  $7.5 \text{ Mg}\cdot\text{ha}^{-1}$  of ground limestone to an acidic podzolic soil.

Lherzolite [15] in powder form, is commercially available and is mined in Toono, Iwate Prefecture, Japan by the Miyamori Saiseki Co. Ltd., Japan. This material is mostly used as a raw material for concrete for making road in Japan, but in addition, this product is also used as a raw material of Mg fertilizer. Lherzolite is a mixture of  $\text{SiO}_2$  (38.5%),  $\text{CaO}$  (2.6%),  $\text{MgO}$  (36%),  $\text{Fe}_2\text{O}_3$  (5.9%),  $\text{Al}_2\text{O}_3$  (1.9%), and Ni (0.17%) and has a pH 9.0. We hypothesized that this material may be useful to reduce the bioavailability and enhance plant growth and soil respiration. Therefore, the aim of this study was to assess the effect of lherzolite on the bioavailability of Cd to Chinese spinach and soil respiration.

## 2. Materials and Methods

### 2.1. Pot Experiment

A pot experiment was carried out in the roof top of a building of Asian University for women in Chittagong, Bangladesh. A sandy loam surface soil (0 - 15 cm) was used for this experiment. Soil sample was air dried and passed through a 4-mm sieve for using in the pots for plant growth. A sub sample was air dried and passed through a 2-mm sieve and stored for laboratory analysis. Physical and chemical properties of the soil are presented in **Table 1**. Soil pH was meas-

ured at 1:2.5 soil to water ratio, soil organic carbon was measured by Walkley and Black [16] and cation exchange capacity (CEC) was measured with 1 M  $\text{NH}_4\text{OAc}$  extraction [17]. The hydrometer method [18] was used for the particle size distribution. The available Fe, Mn, Zn, Cu and Cd contents of soil were extracted by 0.005M DTPA [19] and measured by atomic absorption spectrophotometer 240 AA (Agilent Technologies, Australia) (Table 1).

Moist soil equivalent to 2 kg dry mass was placed in plastic pot (20 cm height and 15 cm diameter) after mixing with Cd at levels of 0, 2.5, 5  $\text{mg}\cdot\text{kg}^{-1}$  and lherzolite at levels of 0%, 2.5% and 5% on dry weight basis and their combinations. The source of Cd was  $\text{CdSO}_4$  (ACS grade, Sigma-Aldrich Co.) The lherzolite used in this experiment was collected from the Laboratory of Plant Physiology and Nutrition, Faculty of Agriculture, Iwate University, Japan. Each pot received a uniform basal dose of NPK fertilizer (N-P-K = 137-32-70  $\text{kg}\cdot\text{ha}^{-1}$  as recommended by Soil Resources Development Institute, Bangladesh [20]. The pots were arranged in a completely randomized design (CRD) with three replications.

Six seeds of Chinese spinach (*Spinacia Oleracea* L.) were sown in each pot and water was applied to field capacity. After 12 days of emergence, 3 healthy seedlings were kept in each pot. The plants were harvested at 45 days of growth and separated into shoot and roots. The plant parts were dried at room temperature to remove excess water prior to oven dry at  $65^\circ\text{C}$  for 72 h and dry mass was recorded. Plant samples were digested with nitric-perchloric acid mixtures (3:1) [21] and the Cd concentration in the digested solutions was analyzed using atomic absorption spectrophotometer 240 AA (Agilent Technologies, Australia). Reagent blanks were also processed. All results are presented on dry weight (DW) basis.

**Table 1.** Physical and chemical properties of the soil used in the experiment.

Physical properties		Values
Moisture content (%)		7.81
Water holding capacity (%)		44.5
Particle size distribution (%)	Sand	74
	Silt	12
	Clay	14
Textural class		Sandy Loam
Chemical properties		
Organic carbon (%)		0.78
Organic matter (%)		1.17
Cation Exchange Capacity ( $\text{cmol}\cdot\text{kg}^{-1}$ soil)		4.39
pH		5.32
Available Olsen P ( $\text{mg}\cdot\text{kg}^{-1}$ )		3.21
Fe ( $\text{mg}\cdot\text{kg}^{-1}$ )		92
Mn ( $\text{mg}\cdot\text{kg}^{-1}$ )		25
Zn ( $\text{mg}\cdot\text{kg}^{-1}$ )		1.6
Cu ( $\text{mg}\cdot\text{kg}^{-1}$ )		1.8
Cd ( $\text{mg}\cdot\text{kg}^{-1}$ )		Below the detection limit (<0.003)

After the harvest, soil samples were collected from each pot, air-dried, and ground to pass a 2-mm sieve. Soil pH was measured the same way as mentioned above. The same soil was used to measure microbial activity or Basal respiration. The most widely used technique to quantify microbial activity is the evaluation of soil basal respiration. Soil respiration was measured by trapping the CO<sub>2</sub> evolved from the soil during incubation period (11 days) in a closed system [22]. For this purpose, 100 g (oven dry basis) soil at 50% of water holding capacity (optimum moisture content for microbial activity) was placed in 1 litre capacity incubation jars and then 20 ml of 0.5 M NaOH in 100 ml capacity beakers were placed in each jar to trap the evolved CO<sub>2</sub>. Three jars with 0.5 M NaOH without soil were used as control. Incubation of all jars was kept at room temperature (25°C). Carbon dioxide absorbed in 0.5 M NaOH after 11 was analysed. The trapped CO<sub>2</sub> was determined by measuring electrical conductivity (EC) of the NaOH traps were measured and CO<sub>2</sub> absorbance was analysed by calibration curve.

## 2.2. Statistical Analysis

Microsoft Excel and Minitab program [23] were used for analysis of variance (ANOVA).

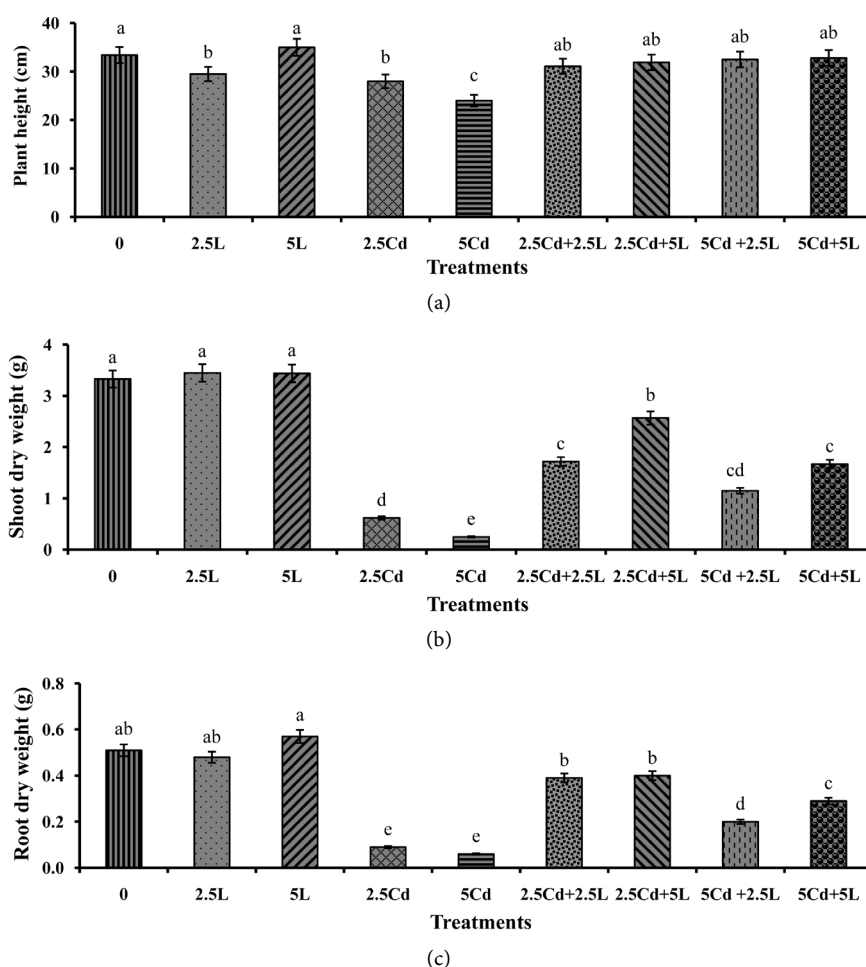
## 3. Results

### 3.1. Growth of Spinach

The growth of spinach was significantly affected by application of lherzolite and cadmium. The height of the plant after 45 days of growth in the control pot (without Cadmium and without lherzolite application) was 29.5 cm. The height of plant increased significantly ( $p < 0.05$ ) with the application of lherzolite at both 2.5% and 5% levels compared to the application of Cd at 5 mg·kg<sup>-1</sup> level (Figure 1(a)).

The shoot dry weight was the highest in the control pot (without Cd and lherzolite) and was the lowest in 5 mg·kg<sup>-1</sup> Cd treated pot (without lherzolite). Dry weight of shoot increased gradually with the increase of the doses of lherzolite with and without Cd application in soil. Dry weight of shoots of spinach decreased from 3.33 g·pot<sup>-1</sup> in control pot (without Cd) to 0.25 g·pot<sup>-1</sup> with the application of 5 mg·kg<sup>-1</sup> Cd. Shoot dry weight in the pot of 5 mg·kg<sup>-1</sup> Cd (0.25 g) increased (1.67 g) by 7 folds with the application of 5% lherzolite in the same 5 mg·kg<sup>-1</sup> Cd treated pot. Application of 5 mg·kg<sup>-1</sup> Cd decreased dry weight of shoot by 13 folds but 55% of its reduction was recovered by the application of 5% lherzolite compared to control indicating further cultivation of plants will increase the efficiency of growth enhancement and decrease Cd tolerance to plants (Figure 1(b)). Dry weight of shoots of Chinese spinach was negatively correlated ( $-0.874$ ,  $p < 0.001$ ) with the concentration of Cd of the shoots.

Like shoot dry weight, root dry weight was influenced by lherzolite and Cd application. Lherzolite enhanced root growth both in Cd treated and untreated



**Figure 1.** Growth parameters of Chinese spinach; (a) Height of the plants; (b) Dry weight of shoots and (c) Dry weight of roots. Vertical bar represent standard deviation of the three replicates. Bars with the same letters among the treatments are not significantly different from each other at  $p < 0.05$ . 2.5 L and 5 L denote 2.5 and 5% lherzolite; 2.5 Cd and 5 Cd denote 2.5 and 5  $\text{mg}\cdot\text{kg}^{-1}$  Cd.

soil as did for shoot growth. Root dry weight decreased from 0.51 g in control pot (without Cd and lherzolite) to 0.06 g in 5  $\text{mg}\cdot\text{kg}^{-1}$  Cd added pot. In one hand, Cd application decreased root growth. On the other hand, it increased gradually with the application of lherzolite. Application of 5% lherzolite increased root dry weight (0.06 g to 0.29 g) by 4 folds in 5  $\text{mg}\cdot\text{kg}^{-1}$  Cd treated pot which indicates reduction of Cd toxic effect and enhanced root growth (Figure 1(c)).

### 3.2. Cadmium Concentration in Shoots of Chinese Spinach

Cadmium concentration was measured only in the shoots of Chinese spinach because less amount of dry matter of roots to digest. Plants grown in the control (0%) and lherzolite (2.5% and 5%) amended soil without Cd addition, concentration of Cd was below the detection limit indicating no Cd was added from the amendments of lherzolite as contaminates. Shoot Cd concentration was the

highest of  $301 \text{ mg}\cdot\text{kg}^{-1}$  in the plants grown in  $5 \text{ mg}\cdot\text{kg}^{-1}$  Cd treated soil pot and of  $101 \text{ mg}\cdot\text{kg}^{-1}$  in the plants grown in  $5 \text{ mg}\cdot\text{kg}^{-1}$  Cd treated soil amended with 5% lherzolite that resulted 3 folds (70%) reduction of Cd uptake in shoots of Chinese spinach due to lherzolite addition. Concentration of Cd decreased from  $191 \text{ mg}\cdot\text{kg}^{-1}$  to  $73 \text{ mg}\cdot\text{kg}^{-1}$  when 5% lherzolite was added in the  $2.5 \text{ mg}\cdot\text{kg}^{-1}$  Cd treated pot indicates higher reduction of Cd uptake due to lherzolite application (**Figure 2**).

### 3.3. Soil pH

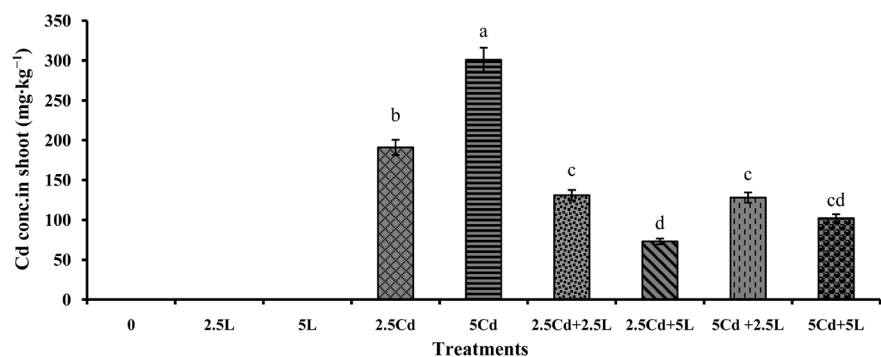
Soil pH measured after the plant harvest, pH in the control soils was 4.4. Soil pH increased with the rates of lherzolite up to 5.92. In the 5% lherzolite application pot, soil pH was about 1.5 units higher than the control pot. Soil pH showed significant negative relationship ( $p < 0.05$ ) with shoot Cd concentration and positive relationships with  $\text{CO}_2$  respiration and shoot dry weight (**Figure 3**).

### 3.4. $\text{CO}_2$ -C Respiration

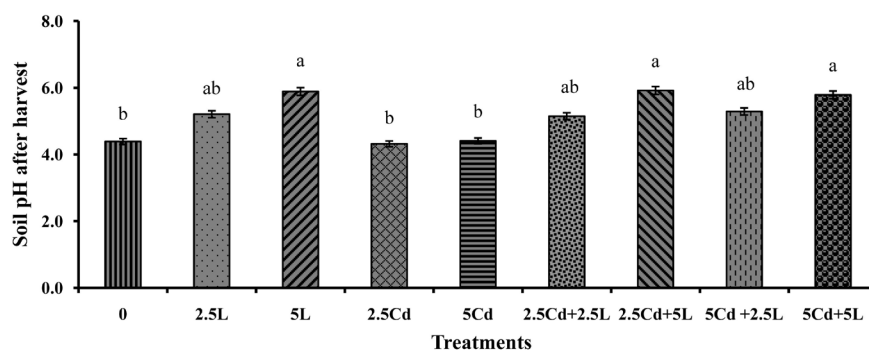
Similar to shoot and root dry weights of spinach,  $\text{CO}_2$ -C respiration was influenced negatively by doses of Cd and positively by the application of lherzolite. During the first day of incubation,  $\text{CO}_2$ -C respiration increased with the rate of lherzolite in Cd contaminated soil. The rate effect of lherzolite was negligible in the Cd non contaminated soil. The  $\text{CO}_2$ -C respiration decreased from  $60 \text{ mg}\cdot\text{kg}^{-1}$  (in the control) to  $20 \text{ mg}\cdot\text{kg}^{-1}$  ( $5 \text{ mg}\cdot\text{kg}^{-1}$  Cd treated pot) soil per day. Highest dose (5%) of lherzolite in  $5 \text{ mg}\cdot\text{kg}^{-1}$  Cd ( $49 \text{ mg}\cdot\text{kg}^{-1}$  soil per day) treated soil increased  $\text{CO}_2$ -C respiration by 2.5 folds compared to  $5 \text{ mg}\cdot\text{kg}^{-1}$  Cd treated soil without lherzolite application (**Figure 4**).

## 4. Discussion

In the lherzolite amended soils, pH of the soils increased. This increase of soil pH in lherzolite amended soil will lead to reduction of Cd bioavailable in soil. The chemical composition of lherzolite may cause to either fixation of Cd by



**Figure 2.** Cadmium concentration in the shoots of Chinese spinach. Vertical bar represent standard deviation of the three replicates. Bars with the same letters among the treatments are not significantly different from each other at  $p < 0.05$ . 2.5 L and 5 L denote 2.5% and 5% lherzolite; 2.5 Cd and 5 Cd denote 2.5 and 5  $\text{mg}\cdot\text{kg}^{-1}$  Cd.



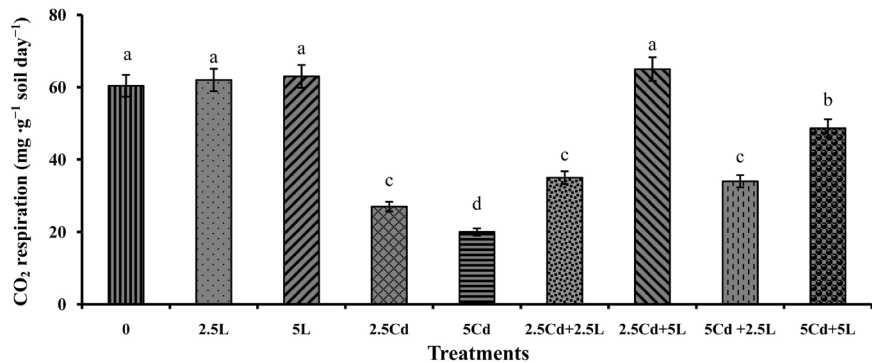
**Figure 3.** Soil pH after harvest of Chinese spinach. Vertical bar represent standard deviation of the three replicates. Bars with the same letters among the treatments are not significantly different from each other at  $p < 0.05$ . 2.5 L and 5 L denote 2.5% and 5% lherzolite; 2.5 Cd and 5 Cd denote 2.5 and 5  $\text{mg}\cdot\text{kg}^{-1}$  Cd.

providing large surface areas or to precipitation of this metal. The use of Ca and Mg carbonates and Ca oxides [24] [25], and zeolites, bringite, and hydrous oxides of Fe, Al, and Mn and hydroxyapatite-like compounds derived from oyster shell [26] [27] [28] can reduce metal mobility significantly in soils and their uptake by plants.

Increasing soil pH was a common mechanism of action for all the amendments. Our results of pH increase are in accordance with those of other researchers [26] [29]. The increase of pH value of soil lowered the solubility of heavy metals in the soil [8] [30]. Lee *et al.* [8] found that soil pH was increased after addition of Ca carbonate and decreased significantly DTPA extractable Cd in a sandy soil. In addition, the rising of soil pH increased the pH-dependent charges and also increased the metals adsorbed on soil particles [31]. In this study, lherzolite behaved like those of red mud as well as lime, bringite and hydroxyapatite-like compounds. We can speculate that the fixation mechanism (reduction of bioavailable Cd) of Cd in the case of lherzolite could be mostly due to pH increase and also ion exchange and precipitation or coprecipitation of metals [32].

It was clearly shown in this experiment that growth of Chinese spinach was greatly enhanced by lherzolite and Cd concentration in plant tissues was largely reduced. The reduced bioavailability of Cd by amendment with lherzolite may be chiefly a consequence of pH rise. This increase of soil pH in the soil amended with lherzolite reduced the phytotoxicity of Cd, resulting in significantly lower Cd concentrations of shoots Chinese spinach (Figure 2 and Figure 3). The improvement Chinese spinach growth may be related with the addition of lherzolite and that could be partially explained by its detoxifying effect of Cd in the soil by other nutrients (Si, Ca, and Mg) derived from lherzolite. Besides Ca and Mg, essential nutrients, Si has also been shown to have a beneficial role in plant growth [33]. Liang *et al.* [33] observed that addition of Si at 400  $\text{mg Si kg}^{-1}$  (as  $\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O}$ ) increased soil pH, decreased Cd availability in soil, and thus reduced Cd concentration in plant. Kashem and Kawai [34] found that Cd





**Figure 4.** CO<sub>2</sub>-C respiration of soils after harvest of Chinese spinach. Vertical bar represent standard deviation of the three replicates. Bars with the same letters among the treatments are not significantly different from each other at  $p < 0.05$ . 2.5 L and 5 L denote 2.5% and 5% lherzolite; 2.5 Cd and 5 Cd denote 2.5 and 5 mg·kg<sup>-1</sup> Cd.

concentration in the shoots of Japanese Mustard spinach was reduced by 40% when this crop was grown under 10-fold concentration of Mg in the hydroponics, indicating detoxifying effect of Mg on plant Cd.

This study also showed that soil respiration (CO<sub>2</sub> evolution) decreased with increasing Cd concentrations in soil which indicates that Cd has a toxic effect on microbial respiration. Similar of our result was observed by other investigators [35] [36]. Verma *et al.* [36] found soil respiration (CO<sub>2</sub> evolution) significantly decreased in Cd contaminated soils compared to uncontaminated soils. The addition of lherzolite to the soil diminished the inhibitory effects of Cd on biological parameters and hence enhanced soil respiration. The addition of this kind of material can help in the remediation of Cd polluted soils.

## 5. Conclusion

Cadmium is toxic to plant and on soil microbial activity. Addition of Cd to the soil caused to decrease plant growth and microbial respiration that can be detoxified or diminished by the application lherzolite in soil. This result indicates that lherzolite can be used to remediate Cd contaminated soil.

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