

Effectiveness of chelating agent-assisted Fenton-like processes on remediation of glucocorticoid contaminated soil using chemical and biological assessment: performance comparison of CaO_2 and H_2O_2

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Abstract

Glucocorticoids (GCs) have drawn great concern due to widespread contamination in the environment and application in treating COVID-19. Most studies on GC removal mainly focused on aquatic environment, while GC behaviors in soil were less mentioned. In this study, degradation of three selected GCs in soil have been investigated using citric acid (CA)-modified Fenton-like processes ($\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ and $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatments). The results showed that GCs in soil can be removed by modified Fenton-like processes (removal efficiency > 70% for 24 h). $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ was more efficient than $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ at low oxidant dosage (< 0.28–0.69 mmol/g) for long treatment time (> 4 h). Besides the chemical assessment with GC removal, effects of Fenton-like processes were also evaluated by biological assessments with bacteria and plants. $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ was less harmful to the richness and diversity of microorganisms in soil compared to $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$. Weaker phytotoxic effects were observed on GC-contaminated soil treated by $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ than $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$. This study therefore, recommends CaO_2 based treatments to remediate GC-contaminated soils.

Highlights

- H_2O_2 and CaO_2 based oxidation can effectively remove GCs from soils.
- CaO_2 based oxidation was more efficient than H_2O_2 at low oxidant dosage.
- CaO_2 based oxidation was less harmful to soil microorganisms than H_2O_2 .
- H_2O_2 based oxidation showed stronger phytotoxic effect than CaO_2 .

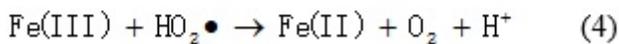
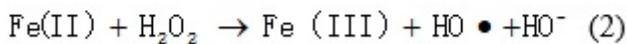
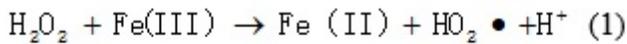
1 Introduction

Pharmaceuticals and pharmaceutical byproducts have since been identified as one of the major groups of the emerging environmental pollutants and are present in different segments of the environment, including soils (Wu and Bi 2019). Synthetic glucocorticoids (GCs) are a class of steroid hormones which could potentially disturb the corticosteroid signaling pathways both in wildlife and humans (Wu et al. 2019), and are among the most prescribed drugs in the world against allergies, autoimmune, and inflammatory disorders (Banaschik et al. 2015). Moreover, GCs are urgently used to treat patients infected by the 2019 novel coronavirus (COVID-19) (Russell et al. 2020). Due to the vast usage, GCs have been identified as a group of emerging environmental pollutants which are present in different segments of the environment (Ai et al. 2016; Schriks et al. 2015; Suzuki et al. 2015). Exposure to exogenous GCs has been associated with health complications such as obesity, osteoporosis, cardiovascular disease, impaired development, inflammations, type-2-diabetes, and autoimmune diseases (Schriks et al. 2015). Therefore, it is imperative and urgent to control GC pollution in the environment.

Fluocinolone acetonide (FA), triamcinolone acetonide (TA), and clobetasol propionate (CP) are amongst the principal contributors to activities of GCs in the environment (Ai et al. 2016). However, they have been reported to be relatively stable and difficult to remove completely by traditional wastewater treatment

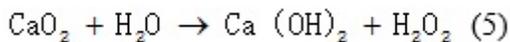
plants (WWTPs) (Weizel et al. 2018). Piram et al. (2008) even found higher concentrations of FA in effluents compared to influents of a WWTP attributing to GC conjugate hydrolysis. Therefore, these GCs can be released into the soil through untreated wastewater, WWTP effluents, waste activated sludge, pharmaceutical residues, and animal manures (Abdellah et al. 2020), causing soils to become significant reservoirs for GCs (Shargil et al. 2016). To make matters worse, the GCs accumulated in soil may enter the food chain (Shargil et al. 2016). Shargil et al. (2016) studied GCs occurrence in lettuce plants and found that corticosterone was present in the biosolids (11–92 ng/g) and lettuce plants (1-900 ng/g), indicating a cumulative tendency of GCs in plants. It is therefore, imperative to reduce these pollutants from the soil so as to guarantee safety both for humans and ecological systems.

As a group of non-biodegradable organic pollutants (Weizel et al. 2018), FA, TA, and CP can be degraded by $\cdot\text{OH}$ radicals during advanced oxidation processes (AOPs) (Zhang et al. 2018a; Zhang et al. 2018b). As a typical representative of AOPs, Fenton processes are widely used in the remediation of contaminated soils due to their cost-effectiveness and practical viabilities (Hwang and Kim 2021; Zhou et al. 2017). However, conventional Fenton process is only limited for narrow pH (pH = 2–4) because iron ions will be precipitated at higher pH values (Li and Zhang 2014). To overcome this limitation, researchers have modified the conventional Fenton process by using Fe(III) and chelating agents such as citric acid (CA) to replace Fe(II) as shown in Eqs. 1–4 (Hwang and Kim 2021; Miao et al. 2015; Zhang et al. 2017). In this modified Fenton system, the use of Fe(III) and chelating agents enable the generation of $\cdot\text{OH}$ radicals occurred under neutral conditions (Miao et al. 2015), which could maintain soil functions and decrease the chemical costs of soil remediation.



Besides the narrow pH limitation, another limitation for the application of conventional Fenton process is the storing and transport of hydrogen peroxide (H_2O_2). Since H_2O_2 is unstable and has short lifespan in soil, alternative oxidants have been considered for soil remediation (Zhang et al. 2018a). As a potential replacement for liquid H_2O_2 , calcium peroxide (CaO_2) is widely used in water treatment, sediment and soil remediation (Ndjou'ou and Cassidy 2006). CaO_2 dissolves in water forming H_2O_2 in a wide pH range and over a relatively longer period of time in soil (weeks to months) (Eq. 5), achieving higher pollutant removal rate than liquid H_2O_2 without introducing additional toxic chemicals (Bogan and Trbovic 2003; Ndjou'ou and Cassidy 2006; Northup and Cassidy 2008; Zhang et al. 2015). Our previous research found that CaO_2 is effective in GC degradation in aqueous solutions due to its synergistic effects in oxidation, adsorption, and base catalysis (Zhang et al. 2018a). Moreover, CaO_2 is a slow oxygen-releasing reagent which can

promote the micro-biological mineralization of pollutants in soil in long term (Lu et al. 2017). Therefore, CA-modified Fe(III) catalyzed CaO₂ remediation has significant potential.



Though chemical remediation by modified Fenton oxidation may be effective for GC removal in soil, the associated effects on terrestrial organisms and plant growth after GC removal are largely unknown. After soil remediation by modified Fenton oxidation, the residual chemicals may cause ecological toxicity because they change the soil properties and microorganisms (Long et al. 2016; Ping et al. 2018). Additionally, some studies indicated that free radicals and oxidizing species produced during AOPs may be harmful to plant species (Abdul et al. 2012). To our knowledge, most studies evaluated the efficiency of oxidation process in soil remediation only by chemical analysis of pollutant removal; however, studies describing the biological toxicity remain incomplete.

We therefore determined whether the modified Fenton-like oxidations (H₂O₂/Fe(III)/CA treatment and CaO₂/Fe(III)/CA treatment) are effective in GC removal in soil and evaluated their influences on the treated soils using both chemical and biological assessments. Effects of H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments on bacteria in soil and subsequent plant growth were investigated and comparisons drawn. This study therefore, provides reference for the development of modified Fenton-like remediation technologies for soils contaminated with pharmaceuticals, particularly the GCs.

2 Materials And Methods

2.1 Chemicals, soils, and seeds

The three GC standards (FA, CAS: 67-73-2; TA, CAS: 76-25-5; CP, CAS: 25122-46-7) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Their chemical structures were as shown in Fig. 1. CaO₂ reagent (75%, 80–150 mesh) purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Methanol, acetonitrile and methyl tert-butyl ether (MTBE) were of chromatographic grade and obtained from Sigma-Aldrich. Hydrogen peroxide (H₂O₂, 30% (w/v)), citric acid (2-Hydroxy-1,2,3-propanetricarboxylic acid, CA, purity > 99.5%) and iron (III) chloride hexahydrate (Fe(III)) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPPO) was obtained from Sigma-Aldrich (St. Louis, USA). Other chemicals were of analytical grade and purchased from Macklin Inc. (Shanghai, China). Ultrapure water was collected from a Barnstead Ultra-Pure instrument (EMD Millipore, Billerica, MA, USA).

A silty clay soil was collected from Donghua University Songjiang campus (N 31°3'30"; E 121°12'18") and air-dried and sieved through a 2-mm mesh before use. The main characteristics (average data) of the soil were as follows: sand grains (> 20 μm) 14.0%, silt grains (2–20 μm) 57.2%, clay grains (< 2 μm) 28.8%,

soil pH 7.54, moisture content 1.44%, organic content 3.22%. Pea seeds were purchased from a local agricultural market and stored at 4°C before use. Each 300 seeds weighed between 150 – 180 g.

2.2 Experimental procedure

The performance of $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ and $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ on FA, TA, and CP removal from soils were separately evaluated. The GC polluted soil slurry was prepared by mixing 10 g of soil with 50 mL of GC solution (prepared by spiking concentrated GC solution into deionized water) on a mechanical shaker overnight. The initial GC concentration in soil solution was 5 μmol GC per gram dry weight (dw) of soil. Appropriate CA and Fe(III) solutions were spiked into the GC polluted soil slurry and then preset amount of oxidant (H_2O_2 or CaO_2) was added into the GC polluted soil slurry to start the experiment while magnetically stirred. Effects of oxidant (H_2O_2 or CaO_2) type and treatment time (0–24 h) on GC removal were evaluated by adding 0.69 mmol/g dw H_2O_2 or CaO_2 into GC polluted soil slurries with molar ratio of oxidant (H_2O_2 or CaO_2):Fe(III):CA fixed at 4:1:2. Effects of oxidant dosage (0–1.39 mmol/g dw) were evaluated with molar ratio of oxidant:Fe(III):CA fixed at 4:1:2 for 4 h. Effects of CA and Fe(III) dosages on GC removal during $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ oxidation were investigated at various molar ratios of CaO_2 :Fe(III):CA (20:1:2, 20:2:4, 20:5:10, 20:7.5:15, and 20:10:20) at fixed CaO_2 dosage (1.11 mmol/g dw) for 4 h (The oxidant dosages and ratio-values were chosen based on our pre-experiments with data not shown). Detailed experimental design was as shown in Table S1 in supporting information (SI). For the blank, same volume of deionized water was spiked into soil slurry without GC addition and with no CA, Fe(III), and oxidant added. For the control, same volume of deionized water was spiked into the GC polluted soil slurry with no CA and Fe(III) solution and oxidant added. All the experiments were conducted under dark conditions. To study the effects of $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ and $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ on soil indigenous microorganisms and phytotoxicity, experiments were performed at oxidant dosage (H_2O_2 or CaO_2) of 1.11 mmol/g dw and molar ratio of oxidant (H_2O_2 or CaO_2):Fe(III):CA at 4:1:2 with pH unadjusted. For the blank, same volume of deionized water was spiked into soil slurry without GC addition and with no CA and Fe(III) solution and oxidant added. 10 mL of soil slurry samples were taken at the preset sampling times with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) as the terminator and analyzed immediately.

2.3 Analysis

2.3.1 GCs extraction and analysis

The soil sample preparation procedure for GCs followed the method of our previous research (Zhang and Li 2014). Briefly, soil samples were freeze-dried and extracted with methanol and acetate buffer solution (pH 5.0) mixture (90:10, v/v) by ultrasonic. The extracts were evaporated in a rotatory evaporator and extracted with solid phase extraction (SPE) according to Jia et al. (2016). Detailed analysis method can be found in Text S1 in SI. The extracts of SPE were then concentrated with nitrogen to a final volume of 5 mL for Ultra High Performance Liquid Chromatography (UHPLC, Dionex UltiMate 3000, Thermo Fisher Scientific Inc, USA) analysis. Detailed methods for UHPLC were as reported in our previous researches (Zhang et al. 2018a; Zhang et al. 2018b) and shown in Text S2 in SI. Under these experimental conditions,

the quantification limits of FA, TA, and CP were 1.82, 1.63, and 1.47 nmol/g dw, respectively. The recoveries for FA, TA, and CP were in the range of 85–91%, 83–89% and 94–102%, respectively.

2.3.2 Molecular analysis of microbial communities in soil

To evaluate the individual influence of H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA on microorganisms, the bacterial communities in soil samples after H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments were analyzed. The procedure was performed according to a previous report (Ping et al. 2018). Detailed information was as described in Text S3 in SI. The raw sequences data have been deposited with the accession number SRP198736 (https://www.ncbi.nlm.nih.gov/Traces/sra_sub/).

2.3.3 Seed germination analysis

Seed germination experiments were conducted to detect the phytotoxic effects of GC polluted soil before and after H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA separate treatments (Gao et al. 2019; Jia et al. 2018). 50 mL of soil slurry samples were collected before (the “blank”) and after GC polluted (the “control”) and after H₂O₂/Fe(III)/CA treatment or CaO₂/Fe(III)/CA treatment. Then 50 pea seeds were uniformly put into the soil sample in a seedbed (plastic mesh with each hole containing a seed) in a petri dish (Φ 140 mm). The dish was placed inside a germination chamber equipped with day light fluorescent lamps. After six days of germination, germination rate, shoot length, and dry weight were measured as described in Text S4 in SI. The significance test of difference was analyzed with SPSS statistical software (Version 16, SPSS Inc., Chicago, IL, USA).

2.3.4 Other analysis

Electron paramagnetic resonance spectroscopy (EPR) was used for detection of free radicals during CaO₂/Fe(III)/CA treatment as described in Text S5 in SI. Oxidation reduction potential (ORP) and pH was recorded in situ using a multi-probe instrument (HANNA instrument HI9829, Italy). H₂O₂ concentration was analyzed by potassium titanium (IV) oxalate using a spectrophotometer (TU-1810, China) at 387 nm (Gao et al. 2019).

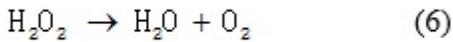
3 Results And Discussion

3.1 GC degradation performance by oxidant/Fe(III)/CA treatments in soil

3.1.1 Effects of oxidant dosage

The results of GC removal at varying treatment times (0–24 h) with oxidant dosage of 0.69 mmol/g dw and molar ratio of oxidant:Fe(III):CA = 4:1:2 were as shown in Fig. 2. GC removal efficiency increased with the increase of treatment time from 0 to 24 h. At treatment time of 24 h, the GC removal efficiencies were 70–77% and 78–89% for H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatment, respectively, indicating that GCs in soil can be removed by modified Fenton-like processes.

The removal efficiency of FA, TA and CP during H₂O₂/Fe(III)/CA treatment reached 74%, 70%, and 77% at 24 h, respectively. In comparison, the removal efficiency of FA, TA and CP during CaO₂/Fe(III)/CA treatment reached 78%, 78%, and 89% at 24 h, respectively (Fig. 2). At treatment time of 12h for FA, 8h for TA, and 4h for CP, reactions with CaO₂/Fe(III)/CA treatment began to show higher GC removal efficiencies than those with H₂O₂/Fe(III)/CA treatment (Fig. 2). CaO₂ can release H₂O₂ continuously and slowly upon dissolution (Eq. 5), thus reducing the loss of H₂O₂ to O₂ (Eq. 6) and volatilization, achieving a greater oxidant efficiency than liquid H₂O₂ (Zhang et al. 2015).



After 4h, the increasing tendency of GC removal efficiency slowed down for both H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments (Fig. 2). At treatment time of 24 h, the removal efficiencies for all target GCs were greater than 50% (Fig. 2). Considering both the removal of GCs and the time efficiency, treatment time of 4h was chosen for the following experiments.

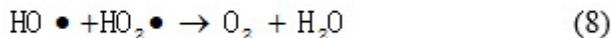
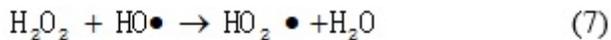
3.1.2 Effects of oxidant dosage

The results of GC removal at varying oxidant concentrations (0–1.39 mmol/g dw) with the molar ratio of oxidant:Fe(III):CA = 4:1:2 at 4 h were as shown in Fig. 3. The GC removal rate increased with the increase of oxidant concentrations from 0 to 1.39 mmol/g except for the case of CP at H₂O₂ dosage of 1.39 mmol/g (Fig. 3). Substantive increments in H₂O₂ and CaO₂ dosages increased the generation of reactive radicals (such as •OH) as shown in Eqs. 1–5 (Hu et al. 2019), thus improving the GC degradation.

At oxidant dosages of 0.28 mmol/g for FA and 0.28–0.69 mmol/g for CP, reactions with CaO₂/Fe(III)/CA treatment showed higher GC removal efficiencies than those with H₂O₂/Fe(III)/CA treatment (Fig. 3). Similar results were also obtained by Bogan and Trbovic (2003) and Ndjou’ou and Cassidy (2006) in treating polycyclic aromatic hydrocarbon (PAH)-contaminated soil and total petroleum hydrocarbons (TPH)-contaminated soil by CaO₂ and H₂O₂, respectively. In conditions with insufficient oxidants, CaO₂ was more effective than H₂O₂ in removing organic pollutants (Zhang et al. 2015). However, at high oxidant concentrations of 1.11–1.39 mmol/g dw, H₂O₂/Fe(III)/CA treatment performed better than CaO₂/Fe(III)/CA in GC removal at 4h (Fig. 3). Northup and Cassidy (2008) reported that complete dissolution of unbuffered CaO₂ required 62 days. In conditions with sufficient oxidants, H₂O₂ can generate more reactive radicals than equimolar quantities of CaO₂ in a short reaction time (Northup and Cassidy 2008), leading to the higher GC removal efficiencies.

It is worth noting that there was a comparative decrease (14%) for CP degradation when H₂O₂ dosage increased from 1.11 to 1.39 mmol/g (Fig. 3c). This could be attributed to the reactive effects of radical scavengers as shown in Eqs. 7–9 (Lemaire et al. 2013). When H₂O₂ was in excess, it became a scavenger for •OH (Eqs. 7–9), thus inhibiting oxidation of CP (Seol and Javandel 2008). Since the O/C ratio of CP is 20% lower than that of TA and FA and the H/C ratio of CP is 7% and 4% lower than that of TA and FA

respectively (Fig. 1), the oxidation state of CP was the lowest among the target GCs (Liu et al. 2019), causing CP the most sensitive one to $\cdot\text{OH}$ concentration among the three target GCs.



As the increasing tendency of GC removal rate slowed down with CaO_2 dosage increase from 1.11 to 1.39 mmol/g, the overall effect on GC removal was limited ($\leq 5\%$). This could be due to the fact that increasing CaO_2 dosage increased the soil pH by releasing $\text{Ca}(\text{OH})_2$ (Eq. 5). In alkaline conditions, the Fe(III) precipitates and H_2O_2 decomposes into oxygen without generating $\cdot\text{OH}$ (Seol and Javandel 2008). Considering the operational cost, CaO_2 dose of 1.11 mmol/g was selected for the follow-up experiments. The removal rate of FA, TA, and CP reached 60%, 54% and 57%, respectively at 4 h with CaO_2 dosage of 1.11 mmol/g. Similar magnitudes of organic pollutant degradation were also observed in Fe(II), Fe(III) and ascorbic acid, CA activated CaO_2 and H_2O_2 systems. Zhang et al. (2017) reported that the removal rate of nitrobenzene and carbon tetrachloride in water was 59% and 15%, respectively with $\text{CaO}_2/\text{Fe}(\text{III})/\text{ascorbic acid}$ treatment for 90 min. Lemaire et al. (2013) reported an oxidation ratio of 45% for PAHs in soil with $\text{H}_2\text{O}_2/\text{Fe}(\text{II})/\text{CA}$ treatment.

3.1.3 Effects of CA and Fe(III) dosages

To investigate the effect of CA on activating $\text{CaO}_2/\text{Fe}(\text{III})/\text{CA}$ for GC contaminated soil remediation, the GC removal efficiencies at various CA dosages were evaluated as shown in Fig. 4. The GC removal rate was below 25% in $\text{CaO}_2/\text{Fe}(\text{III})$ system at various Fe(III) dosages without CA addition (Fig. 4a). The addition of CA to $\text{CaO}_2/\text{Fe}(\text{III})$ system improved GC removal significantly (Fig. 4b). In absence of CA, the soil pH increased with CaO_2 addition. At alkaline conditions, Fe(III) precipitated quickly (Zhang et al. 2017), thus decreasing the generation rate of $\cdot\text{OH}$ radicals. Conversely, with CA addition, the concentration of Fe(III) could be maintained as the soil pH decreased and the Fe(III)-CA complex formed (Zhang et al. 2017), promoting the generation rate of $\cdot\text{OH}$ radicals and thus improved GC removal efficiency. Miao et al. (2015) reported that the chelators could effectively enhance the removal of tetrachloroethene during percarbonate/Fe(III) treatment by chelating with Fe(III). Moreover, the acidic pH caused by adding CA would neutralize the alkalinity from CaO_2 and accelerate H_2O_2 release and $\cdot\text{OH}$ generation during CaO_2 dissolution (Wang et al. 2016). Wolanov et al. (2013) reported that the dissolution of CaO_2 would decrease to zero with pH rising to 11. Arienzo (2000) detected the pH dependency of the H_2O_2 concentration in CaO_2 slurry and found that acidic condition could accelerate the release of H_2O_2 from CaO_2 . To further confirm the effect of acidification and acceleration of H_2O_2 or $\cdot\text{OH}$ generation by CA during $\text{CaO}_2/\text{Fe}(\text{III})/\text{CA}$ treatment, changes in pH and H_2O_2 concentrations during the treatment were investigated and the EPR analysis was used. As shown in Fig. 4c-d, with the increase of CA dosage, the

solution pH decreased and H₂O₂ concentration increased. The EPR spectrum which has four split lines with 1:2:2:1 height ratio and $g = 2.005$ at the symmetry center represented the formation of •OH radicals during CaO₂/Fe(III)/CA treatment (Fig. 4d).

In addition, the GC removal rate increased with the increase of CA and Fe(III) except for CP removal at CaO₂:Fe(III):CA = 20:10:20 (Fig. 4b). Decreasing removal rate of CP with increasing CA concentration was observed at CaO₂:CA < 4:3. Possible explanation for this declining removal efficiency of CP could be the scavenging effects of CA to •OH radicals. CA is a competitor for •OH radical and part of the oxidant may be consumed by CA (Zhou et al. 2017). In the present case, the high content of CA (CaO₂:CA < 4:3) may have consumed a large part of the oxidant and •OH radical, causing the decrease of CP removal efficiency.

3.2 Effects of oxidant/Fe(III)/CA treatment on indigenous microorganisms in soil

Microbial samples were taken from the control, H₂O₂/Fe(III)/CA, and CaO₂/Fe(III)/CA treatments conducted at 4 h. Effective sequences of 890,856 were achieved from 9 samples with the minimum number of 38,745 from each sample. Through clustering, a total of 3,212 Operational Taxonomic Units (OTUs) were recovered at the cutoff level of 3%. These data were sufficient enough to ensure the accuracy of the rarefaction analysis (Fig. S1).

3.2.1 Microbial richness and diversity

To illustrate the community diversity and sample coverage, the alpha-diversity indices were calculated and listed in Table 1. All Good's coverages from these samples were beyond 99%, reflecting a credible presentation (Shi et al. 2018). According to Table 1, the samples without oxidation (control) showed higher microbial richness and more microorganism species than the samples with either H₂O₂/Fe(III)/CA or CaO₂/Fe(III)/CA treatments. This could be largely associated with the sensitivity of microorganisms to oxidants (Ping et al. 2018). As an oxidant, the •OH radical released by H₂O₂ and CaO₂ directly attacks the polyunsaturated fatty acids in microbial membranes (Ping et al. 2018). This initiates lipid peroxidation, which kills or inactivates some microorganisms in soil (Ping et al. 2018), thus lowering the richness and diversity of microorganisms in the soil. Moreover, the richness and diversity of microorganism species after H₂O₂/Fe(III)/CA treatment were much lower than those after CaO₂/Fe(III)/CA treatment (Table 1). Contrary to H₂O₂ which releases high levels of •OH radicals in a short time, the solid CaO₂ could release H₂O₂ and •OH radical upon dissolution (Eq. 5) and maintain lower levels of H₂O₂, •OH radical, and O₂ (Eq. 6) over a relatively longer period as noted earlier (Sect. 3.1.1), thus minimizing the sudden shock to microorganisms. Additionally, CaO₂/Fe(III)/CA treatment is more compatible with microbial reactions than H₂O₂/Fe(III)/CA treatment as the operating pH is near neutral (pH = 7.9 at CaO₂/Fe(III)/CA = 20:5:10). Based on the above results, CaO₂/Fe(III)/CA treatment is therefore, a preferred approach for

contaminated soil remediation as it is favorable to the microorganisms compared to H₂O₂/Fe(III)/CA treatment.

Table 1

Richness and diversity indexes of soil samples' microbial communities in Control, H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments

Samples	Ace ^a	Chao ^a	Sobs ^a	Shannon ^b	Simpson ^b	Good's coverage ^c
Control	1411	1467	1255	6.15	0.00425	99.00%
H ₂ O ₂ /Fe(III)/CA	806	812	801	5.73	0.0150	99.95%
CaO ₂ /Fe(III)/CA	1246	1257	1209	5.87	0.0140	99.65%
^a ACE, Chao and Sobs richness indicators: the total number of OTUs estimated by infinite sampling. A higher number indicates higher richness.						
^b Shannon and Simpson diversity indicators: the indexes to estimate species diversity. A higher Shannon value or lower Simpson value represents higher diversity.						
^c Good's coverage index: the index to estimate the microbial evenness. A higher percent indicates higher evenness.						

3.2.2 Microbial composition

According to the Venn diagram (Fig. S2), OTUs implied that only 193 OTUs from a total of 3,212 were shared by the three treatment approaches, which implied that microbial communities in control, H₂O₂/Fe(III)/CA, and CaO₂/Fe(III)/CA treated samples were different.

The dominant distributions of phylum and genus level were depicted in Fig. 5 and Fig. S3, respectively. In phylum level, *Proteobacteria* (control 41%, H₂O₂/Fe(III)/CA 25%, CaO₂/Fe(III)/CA 22%), *Firmicutes* (control 0.5%, H₂O₂/Fe(III)/CA 30%, CaO₂/Fe(III)/CA 29%), *Bacteroidetes* (control 9%, H₂O₂/Fe(III)/CA 15%, CaO₂/Fe(III)/CA 16%), and *Actinobacteria* (control 24%, H₂O₂/Fe(III)/CA 6%, CaO₂/Fe(III)/CA 10%) were predominant (Fig. 5). In genus level, the dominant distributions were *Nitrosomonadaceae* (5%) for the control and *Peptostreptococcaceae* (H₂O₂/Fe(III)/CA 9%, CaO₂/Fe(III)/CA 9%) for oxidized samples (Fig. S3).

After H₂O₂/Fe(III)/CA or CaO₂/Fe(III)/CA treatment, the proportion of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* decreased while the percent of *Firmicutes* and *Bacteroidetes* increased. The lipid and humic-like substances in soil greatly affected the distribution of phylum *Proteobacteria* while the aromatic and humic-like substances affected the phylum *Actinobacteria* (Liu et al. 2019). The percent decrease of *Proteobacteria* and *Actinobacteria* after H₂O₂/Fe(III)/CA or CaO₂/Fe(III)/CA treatment indicated the content decrease of the lipid, aromatic and humic-like substances in soil after oxidation.

Bacteroidetes was important in organic matter degradation and the carbon cycle (Newton et al. 2011) and *Firmicutes* was important in the synthesis and humification of fulvic-like substances (Liu et al. 2019). Besides, *Firmicutes* and *Bacteroidetes* can release extracellular enzymes to promote the degradation and hydrolysis of semi-cellulose, cellulose, and proteins (Kong et al. 2018). This implies that the proportionate increase in *Firmicutes* and *Bacteroidetes* enhanced hydrolysis, degradation of organic matter, and humification after $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ or $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment.

3.3 Phytotoxic effects of oxidant/Fe(III)/CA treatment

The phytotoxicity of $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ and $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatments on GC contaminated soil was evaluated by monitoring germination rate, shoot length, and plant dry weight of pea (Gao et al. 2019). As depicted in Fig. 6, the pea seeds in the control (non-treated GC-contaminated soil) had lower germination rate (7% reduction), shoot length (41% reduction), and plant dry weight (41% reduction) compared to those in the blank (uncontaminated soil), demonstrating the strong toxicity effect of GCs to plants. After the application of $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ treatment on the GC-contaminated soil, a greater negative impact on the germination (33% reduction), shoot length (31% reduction), and plant dry weight (64% reduction) of pea seeds was observed compared to those in control (Fig. 6). This meant that $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ treatment increased the phytotoxic effects of the GC-contaminated soil. In contrast, $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment showed much weaker phytotoxic effects to pea seeds than with similar germination rate and plant dry weight before and after treatment (Fig. 6). A greater shoot length (7.08 cm) was found after $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment of the GC-contaminated soil (5.67cm in control, Fig. 6). As shown in Table S2, the ORP for GC-contaminated soil increased from 116 to 559 mV after $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ treatment. Comparatively, the ORP changed slightly (from 116 to 121 mV) after $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment, indicating that the oxidative stress caused by CaO_2 was much weaker than that caused by H_2O_2 . Furthermore, the release of Ca^{2+} from CaO_2 during $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment may also contribute to the germination and growth of pea seeds. It has been well established that Ca^{2+} and calmodulin are key regulatory elements in many cellular processes in animals and plants (Corpas and Barroso 2018). The presence of Ca^{2+} also closely correlates with that of Ca^{2+} -dependent protein kinase predominantly involved in lipid metabolism, oxidative stress, mediating pathogen resistance, and pollen tube growth (Corpas and Barroso 2018). Since $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ treatment was more phytotoxic on terrestrial plants, $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatment was recommended to remove GCs from the contaminated soil and simultaneously reduce the phytotoxicity to terrestrial plants.

4 Conclusions

$\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ and $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ treatments were effective for GC removal in soils. Both H_2O_2 and CaO_2 systems achieved over 53% of GC removal efficiencies at oxidant dosage of 1.11 mmol/g dw with the molar ratio of oxidant:Fe(III):CA = 4:1:2 at 4 h. $\text{CaO}_2/\text{Fe(III)}/\text{CA}$ could be more efficient than $\text{H}_2\text{O}_2/\text{Fe(III)}/\text{CA}$ for GC removal at low oxidant dosages (< 0.28 - 0.69 mmol/g) for long treatment times (> 4 h).

H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments had significant influences on bacteria in treated soils and subsequent plant growth. The richness and number of microorganism species decreased and the microbial communities changed after H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments. Compared to H₂O₂/Fe(III)/CA treatment, CaO₂/Fe(III)/CA treatment was less harmful to the richness and diversity of microorganisms in soil. Weaker phytotoxic effects were observed on GC-contaminated soil treated by CaO₂/Fe(III)/CA than H₂O₂/Fe(III)/CA.

Considering both GC removal and subsequent utilization of soils, CaO₂/Fe(III)/CA treatments are recommended for remediation of soils contaminated with GCs or other organic pollutants.

Declarations

Ethical Approval and Consent to participate: Not applicable.

Consent for publication: Not applicable.

Data availability: The authors declare that all relevant data supporting the findings of this study are included in this article and its supplementary information files.

Conflicts of interest: There are no conflicts to declare.

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Figures

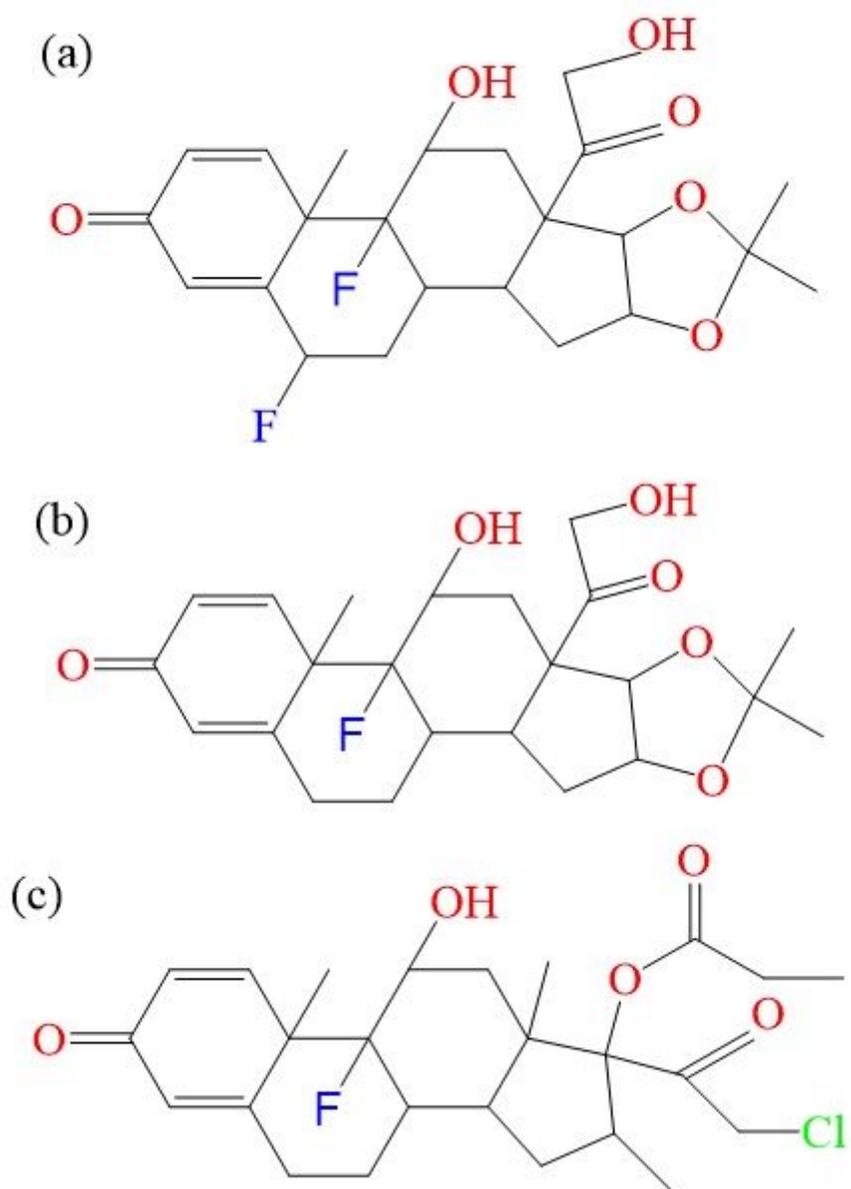


Figure 1

Chemical structure of (a) flucinolone acetonide (FA), (b) triamcinolone acetonide (TA), and (c) clobetasol propionate (CP)

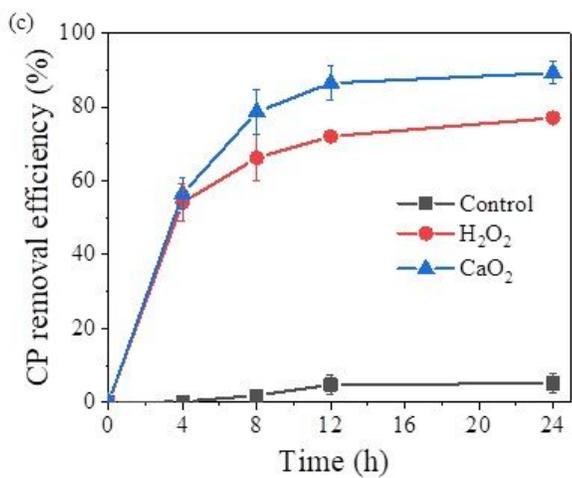
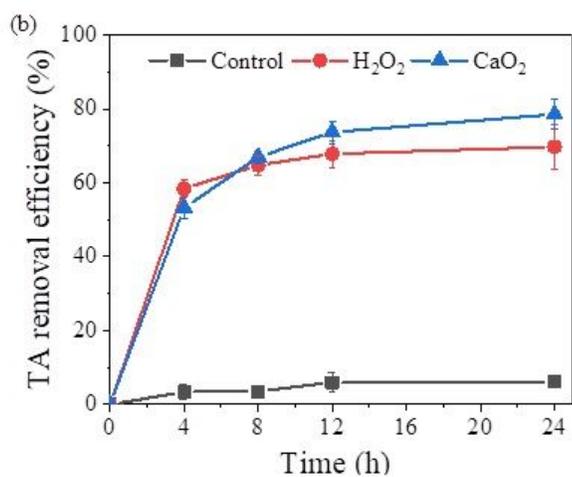
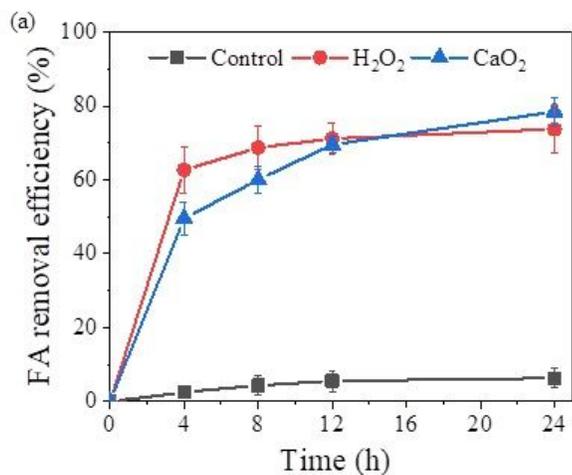


Figure 2

The removal efficiencies of (a) FA, (b) TA and (c) CP during H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments (oxidant concentration = 0.69 mmol/g dw, molar ratio of oxidant:Fe(III):CA = 4:1:2)

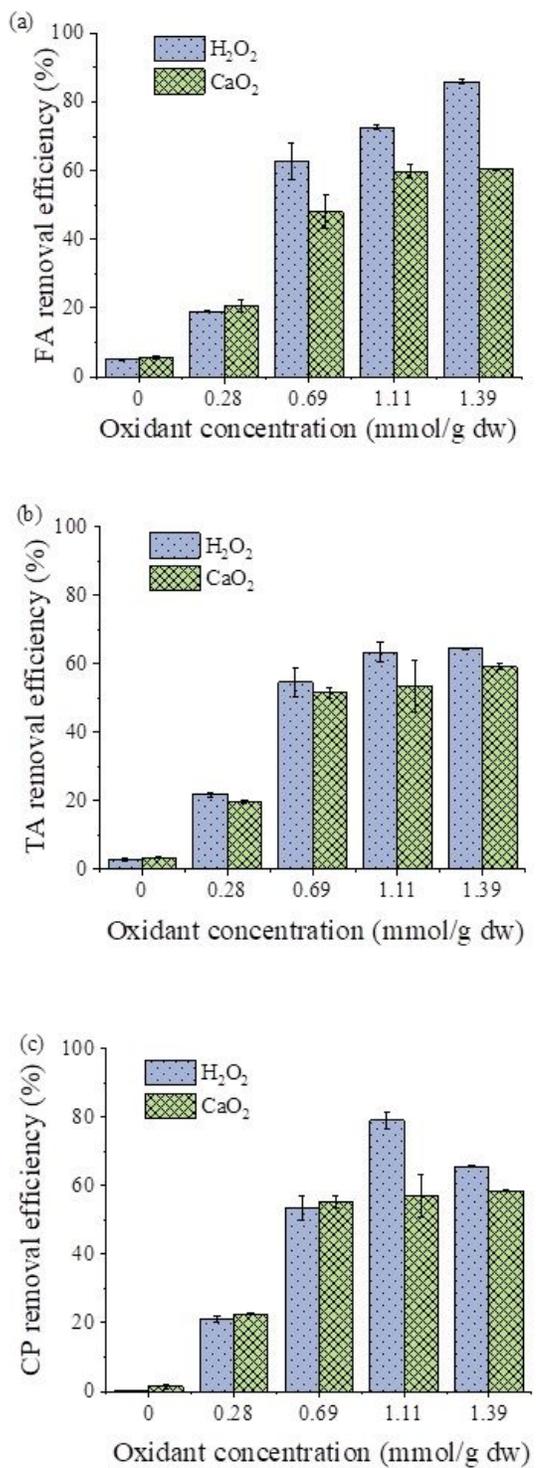


Figure 3

The removal efficiencies of (a) FA, (b) TA and (c) CP by H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments at varying oxidant concentrations (0 - 1.39 mmol/g dw) with the molar ratio of oxidant:Fe(III):CA = 4:1:2

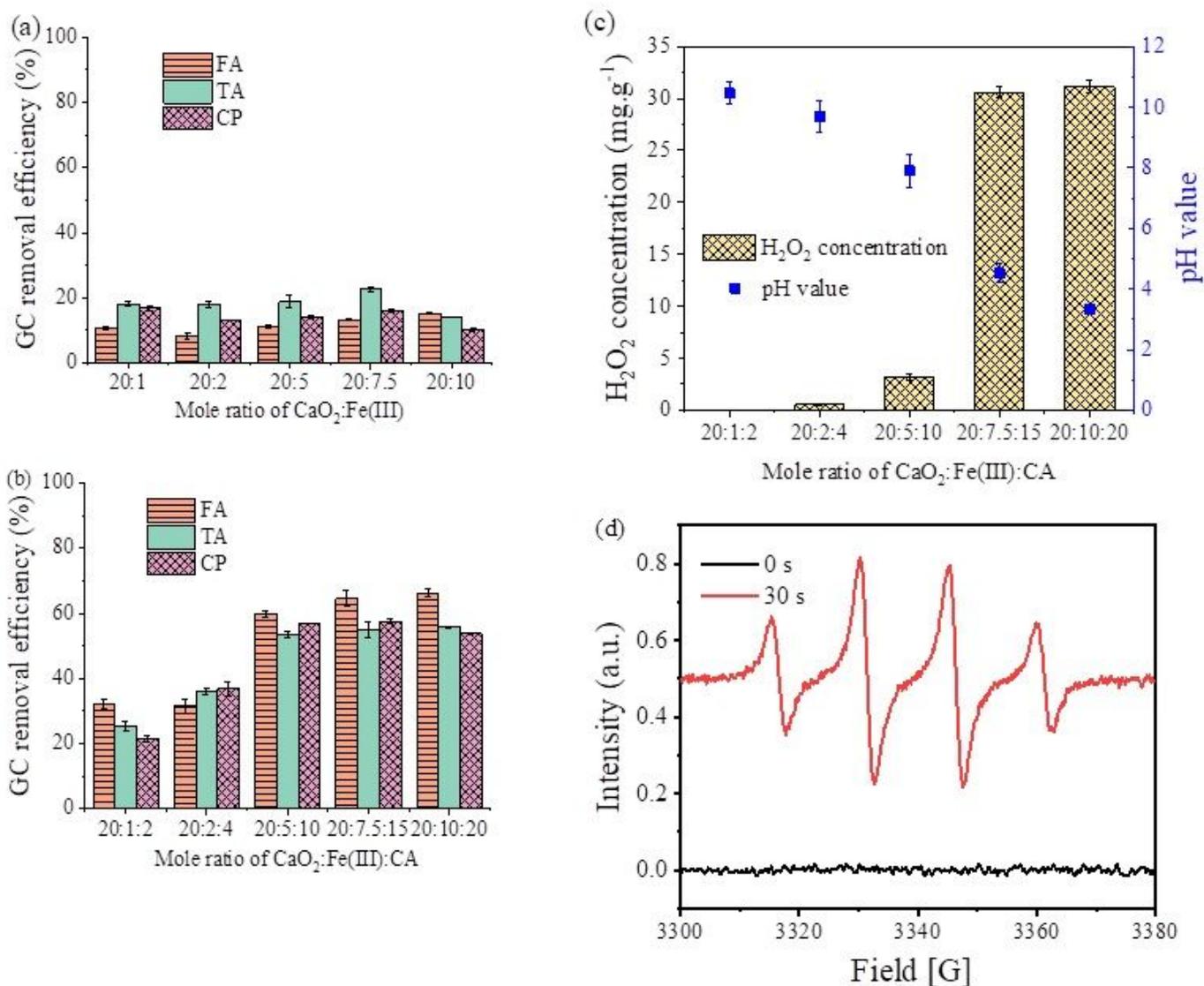


Figure 4

The removal efficiencies of GCs (a) by CaO₂/Fe(III) treatment at varying Fe(III) concentrations and (b) by CaO₂/Fe(III)/CA treatment at varying Fe(III) and CA concentrations; (c) H₂O₂ concentration and pH value at 30 min at various mole ratios of CaO₂:Fe(III):CA; (d) EPR spectrum of radicals released during CaO₂/Fe(III)/CA treatment (CaO₂ dosage = 1.11 mmol/g dw).

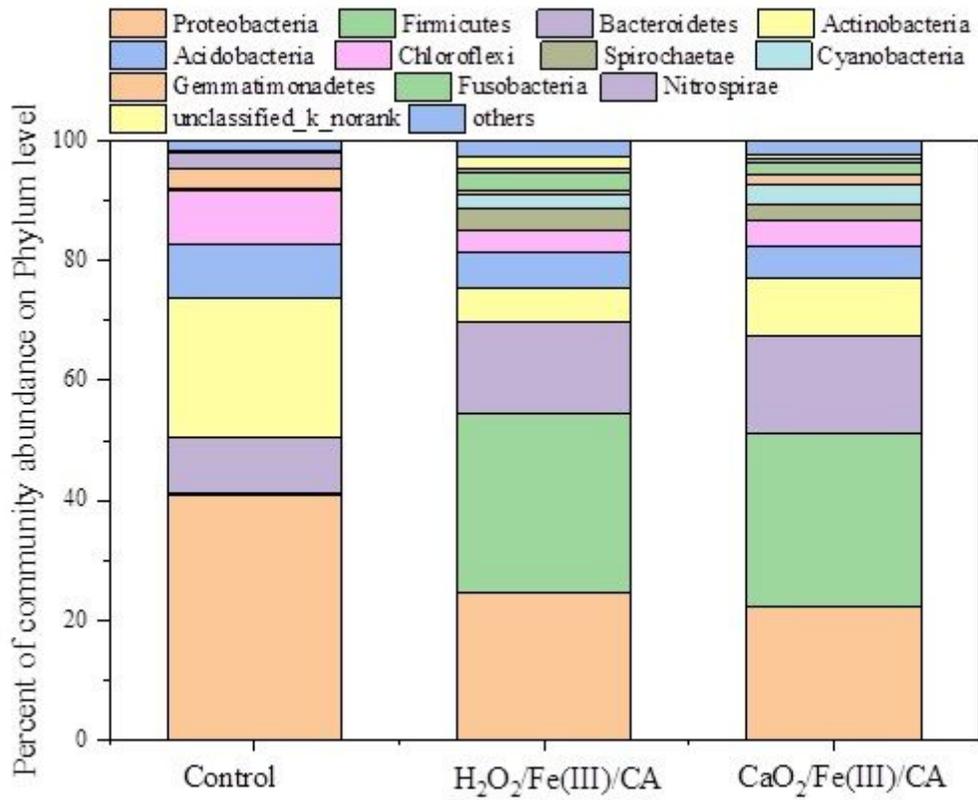


Figure 5

The composition of microbial communities on phylum level in soil samples of control, H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments (oxidant concentration = 1.11 mmol/g dw, Fe(III) = 0.28 mmol/g dw, CA = 0.56 mmol/g dw).

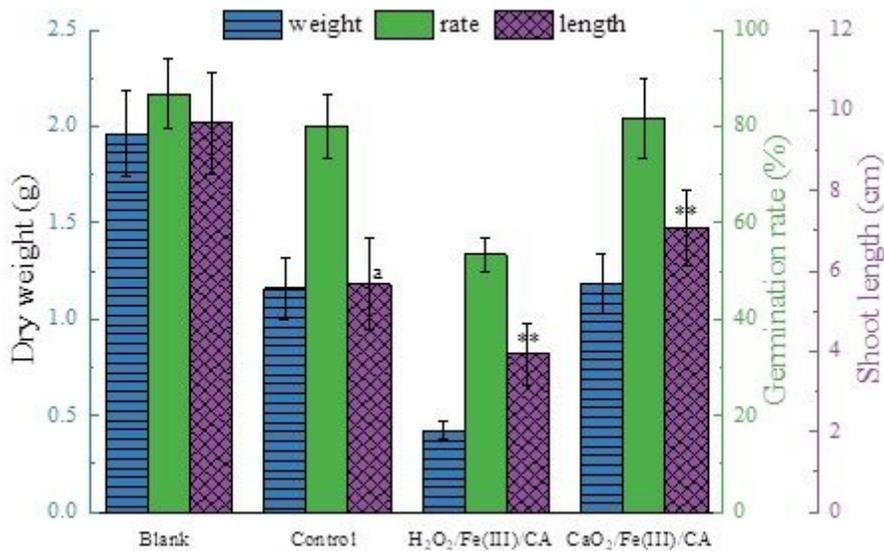


Figure 6

Phytotoxicity of soil samples on seeding germination, shoot length, and plant dry weight of pea of the blank, control, H₂O₂/Fe(III)/CA and CaO₂/Fe(III)/CA treatments. The letter above the bar indicates significant differences on shoot length among the blank and the control at $p < 0.01$. The double asterisk (**) indicates $p < 0.01$ for a Student's t-test carried out on shoot length between the control and oxidant/Fe(III)/CA treatments (oxidant concentration = 1.11 mmol/g dw, Fe(III) = 0.28 mmol/g dw, CA = 0.56 mmol/g dw).

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