Research article

Effect of Adding Agricultural and Organic Lime on Soil Properties and Survival Rate of Pathogenic Bacteria (Coliform and *E. coli*) in Farmland Soils of Kampong Cham Province, Cambodia

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Abstract Cow manure and compost has been applied as fertilizer for many years by farmers in Kampong Cham province. Improperly processed of cow manure and compost may contained pathogenic bacteria such coliform and *E. coli* that are harmful to humans. Pathogenic bacteria coliform and *E. coli* are both recognized as hazardous microorganism in the environment and for public health. It is an important pathogen associated with several foodborne and waterborne outbreaks of gastrointestinal illness, which has been widely reported in Cambodia. Also, coliform and *E. coli* was detected in farmlands soils of Samraong and Baray Communes, Kampong Cham Province. The contamination of agricultural land, surface water, irrigation water, and fresh vegetables, can become a reservoir of infections. Therefore, it is necessary to eliminate the pathogenic bacteria from the contaminated areas. Many studies have shown that inactivation of pathogens in biosolids depends on several factors, from that the soil pH is one of the dominant factors affecting the inactivation of pathogenic bacteria. Increasing of pH can be achieved by adding agricultural limestone calcium carbonate ($CaCO_3$) or quick lime (CaO). The objectives of this study are to investigate and evaluate the effects of adding different agricultural and organic lime materials on soil chemical properties and the survival rates of pathogenic bacteria coliform and *E. coli*. Pot experiments was conducted and different $CaCO_3$ and CaO material such $CaCO_3$ powder, eggshell, clamshell, and wood ash are used for the soil amendment. The $CaCO_3$ and CaO added to soil at the ratio of 0 g, 5 g, and 15 g to 750 g of soil to each pot. The CaO obtained by burning the $CaCO_3$ powder, eggshell, clamshell, and wood ash at 800˚C. Soil chemical and biological parameters such as pH, EC, K^+, Na^+, Ca^{2+}, coliform and *E. coli* were determined. The results showed that increase in soil pH due to lime addition were observed. However, there's no significant difference of soil pH responding to amounts of lime added 5 g, 10 g and 15 g. Similar trends were also observed between the unburned and burned lime material. The survival rate of coliform and *E. coli* were decreased with unburned and burned lime added, the pathogenic bacteria can be eliminated at 7 days after addition of lime. Since the performance of unburned and burned lime on the survival rates of pathogenic bacteria no different. So, it is not necessary to burn the lime material of $CaCO_3$, eggshell, clamshell, and wood ash. As burning material need the temperature at 800 °C, farmers need a burner machine that can reach to that temperature. Farmers in Cambodia usually burning wood or charco to get the heat. Therefore, it is recommended that farmers can use the unburned lime instead of burned materials in Kampong Cham Province.

Keywords: agricultural lime, calcium carbonate, fertilizers, shells, soil pH, microorganisms

INTRODUCTION

Organic amendments or fertilizers including livestock manure and compost are valuable sources of nutrients for plant's growth. The organic matter also contributing to improve the soil quality and fertility. Application of organic waste products to agricultural land without control of their hygienic safety is one of the potential risks by which pathogens may enter the human food chain (Nicholson et al., 2005). Pathogenic bacteria in raw vegetables and their consumption affects human health, resulting in diarrhea or serious disease (Beuchat, 1996; Summer and Peters, 1997). Muyleang and Mihara (2019) reported that the coliform and *E. coli* was found in farmland soils in Samraong and Baray Commune, Prey Chhor District, Kampong Cham Province. The occurrence of coliform and *E. coli* maybe a potential of food-borne pathogens in the farmlands soils as well as vegetable. Also, the application of fresh cow manure or immature compost that contained pathogenic bacteria can spread the pathogens to the farmland's soils. The contamination of agricultural lands, surface water, irrigation water, and fresh vegetable can become a reservoir of infection. To produce safety agricultural production, it is necessary to eliminate pathogenic bacteria such as coliform and *E. coli* in farmlands. The inactivation of pathogens in biosolids depends on several factors such as temperature, moisture content, competition from indigenous microflora, soil types and texture (Martin et al., 1990; Ward et al., 1981; Russ and Yanko, 1981). However, the survival rate of *E. coli* became remarkably low with pH higher than 9.0. Increasing of pH can be achieved by adding of agricultural lime ($CaCO_3$) and quick lime (CaO). Lime is commonly used to farmlands soil for neutralizing soil acidity for centuries and has been demonstrated to improve crop yield (Acosta et al., 2000). Calcium carbonate ($CaCO_3$) is one of the basic inorganic and inexpensive materials that were applied in agriculture industries to stabilize the soil pH. About 95% of $CaCO_3$ can be obtained from shellfish and the rest is organic matter and other compounds (Hamester et al., 2012). An increase in soil pH, with liming application help to increase in microbial activity, resulting in increased decomposition of soil organic matter. Therefore, the study was proposed different lime material application and its effect on soil properties and the microbial survival rates.

OBJECTIVE

The objectives of this study are to investigate and evaluate the effects of adding different agricultural and organic lime materials on soil chemical properties and the survival rates of pathogenic bacteria coliform and *E. coli*.

METHODOLOGY

Experimental Designed and Materials Used

Pot experiment was conducted in the experimental room at Tokyo University of Agriculture from August to September 2021 during summer season. Fresh cow dungs were collected from Onoji Farm and added to soil samples to get pathogenic bacteria contamination in Fig. 2.

$CaCO_3$ Eggshell Clamshell Wood ash

Fig. 1 Agricultural and organic lime materials

Unburned and burned lime materials were used for the experiment. The unburned lime materials such Agricultural lime $CaCO_3$ powder, organic lime such eggshell, clamshell, and wood ash (Fig. 1), and the burned lime materials were obtained from burned of $CaCO_3$, eggshell, clamshell, and wood ash at 800℃.

Fig. 2 Cow manure collection in Onoji Farm, Tokyo, Japan (left) and pot experiment design (right)

The pH of the soil samples used for the experiment was around 6.8 to 6.9. The amounts of lime materials were added from 5 g, 10 g, and 15 g to 750 g of soil, this amount added in the experiments (Fig. 3) was decided based on practical use in Cambodia (Chakraborty, 2014). For the actual field of lime added was from 33 kg a^{-1}, 66 kg a^{-1}, and 99 kg a^{-1}.

Chemical Properties of Lime Materials Used

The lime materials of $CaCO_3$, eggshell, clamshell, and wood ash that used for the experiment has the calcium carbonate content (CCC%) of 99.9%, 97.8%, 98.4% and 40% respectively. The pH of each material ranged from 9.5 to 9.9 (Table 1).

Table 1 Chemical properties of lime materials used in the experiments

Lime materials	Calcium carbonate content (%)	pH	EC (mS/cm)	Ca^{2+} (mg/L)
$CaCO_3$	98.9	9.5	0.45	23
Eggshell	97.8	9.5	0.26	20
Clamshell	98.4	9.5	1.03	14
Wood ash	40	9.9	14.6	170

Samples Preparation

- Soil samples from Samraong and Baray Communes were used for the experimental. The samples were mixed and sieved with 2 mm sieve to remove the leaves, root and stone, and air dried at room temperature before used for the experiment. The initial soil physical, chemical, and biological properties were analyzed.
- Eggshell and clamshell were collected from daily consumption in Cambodia, all the eggshell and clamshell were washed and air dried before crushed to powder. For $CaCO_3$ and wood ash were obtained from the commercial products. Also, for the CaO that was used for the experiment was obtained from burned of the raw materials of $CaCO_3$, eggshell, clamshell, and wood ash at 800℃.

Treatments	Unburned	Burned	Mass of lime (g)
T0 (Control)	600 g (soil) + 150 g (cow manure)	600 g (soil) + 150 g (cow manure)	0
T1-1	Soil + cow manure + $CaCO_3$ powder	Soil + cow manure + CaO powder	5
T1-2			10
T1-3			15
T2-1	Soil + cow manure+ Eggshell	Soil + cow manure+ Eggshell	5
T2-2			10
T2-3			15
T3-1	Soil + cow manure+ Clamshell	Soil + cow manure+ Clamshell	5
T3-2			10
T3-3			15
T4-1	Soil + cow manure+ Wood ash	Soil + cow manure+ Wood ash	5
T4-2			10
T4-3			15

Fig. 3 The experimental design of lime materials added treatments

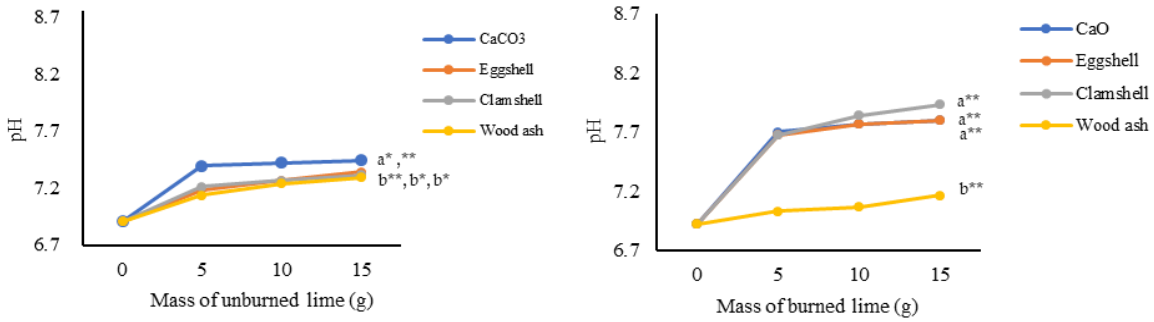
Data Collection and Analysis

The soil samples were collection from the first day before and after added of different lime materials and the following collection were done in every two days. The samples were analyzed on the soil water contents, soil pH, EC, K^+, Na^+, Ca^{2+}, coliform and *E. coli*. T-test was used to see the significant difference between the variables.

RESULTS AND DISCUSSION

Effect of Unburned and Burned Lime Materials on Soil Chemical Properties

Increases in soil pH due to lime addition were observed in this study. Similar tends of soil pH increased were observed between the unburned lime ($CaCO_3$) and burned lime (CaO) materials in (Fig. 4).

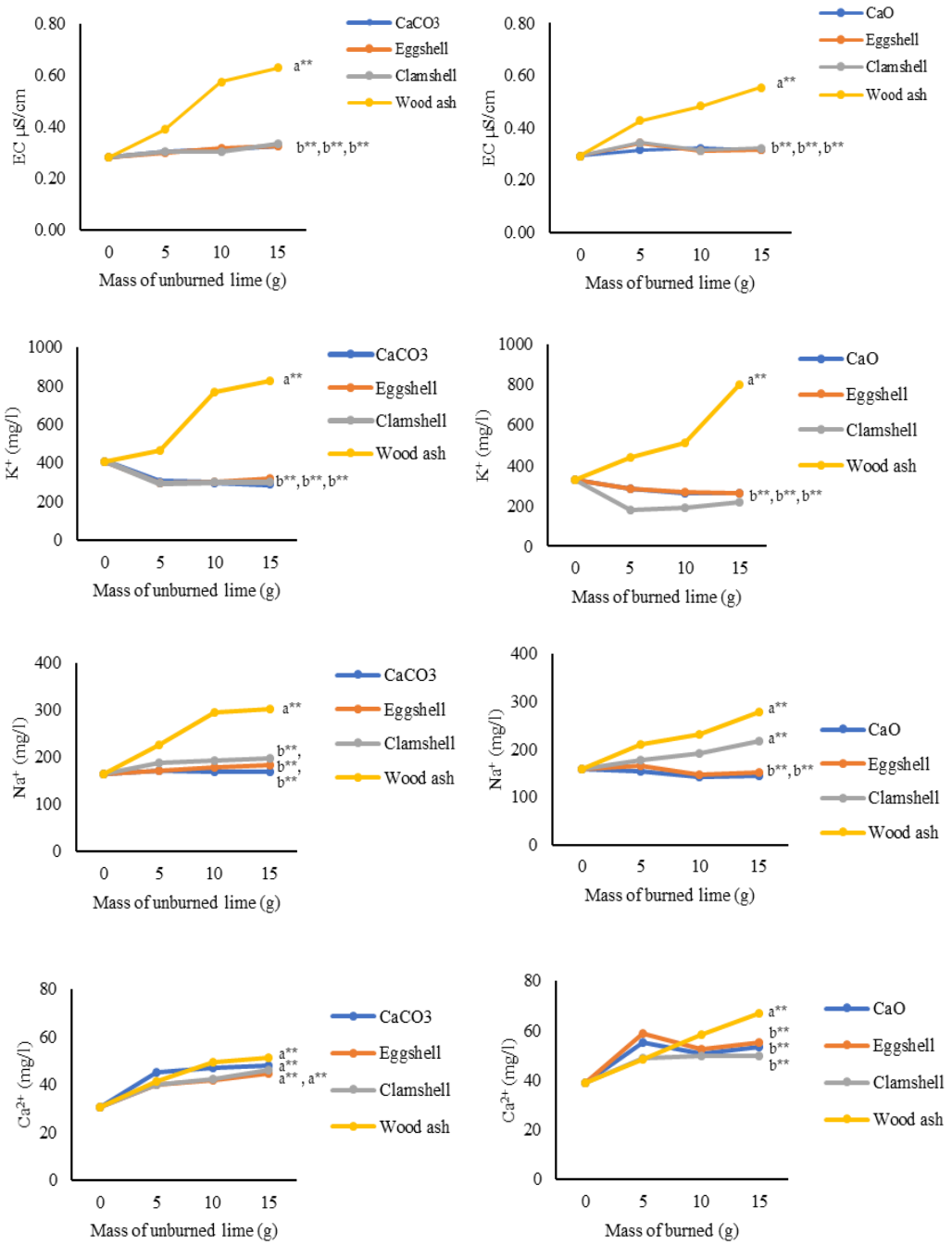


**: Significance difference at 95%, p<0.05, **: p<0.01*

Fig. 4 The effect of unburned and burned lime materials on soil pH

Addition of $CaCO_3$, the calcium ions (Ca^{2+}) from the lime adsorb on soil particle surface and then the carbonate (CO_3) take place and reacts with the acidity to form water and carbon dioxide. However, the effect of wood ash on soil pH became lower due to losses of K_2O after burning at 800℃. Moreover, the CaO materials provided more effectiveness to increase soil pH than $CaCO_3$ materials, except the burned wood ash which losses of K_2O. Burned lime (CaO) reacts with water to form calcium hydroxide ($Ca(OH)_2$) and the solubility is higher than of $CaCO_3$. The wood ash

without extra burning should be applied to the farmlands directly. There was no significant difference of soil pH responding to the amounts of lime added in this study. Therefore, the amounts of lime added less than 5 g should be considered.



**: Significance difference at 99%, $p<0.01$

Fig. 5 The effect of unburned and burned lime materials on soil EC, K^+, Na^+, and Ca^{2+}

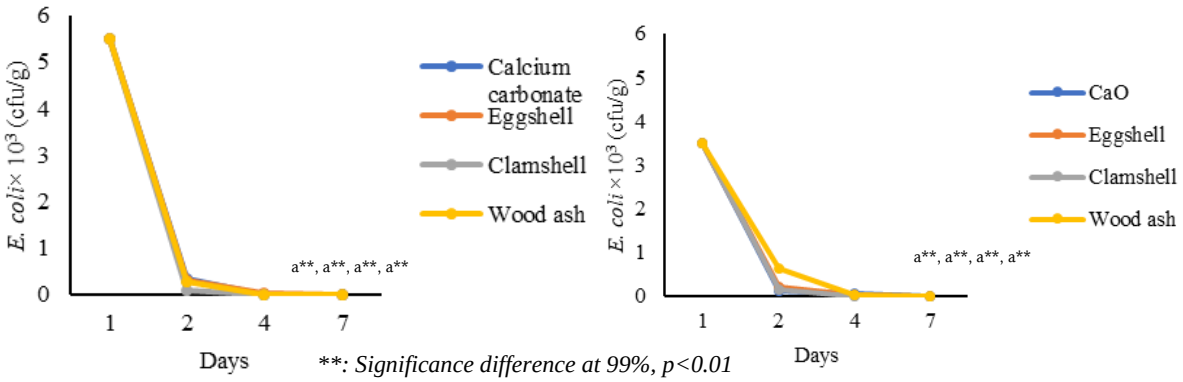
All the treatment with unburned and burned lime material application generally increased the soil EC, K^+, Na^+, and Ca^{2+} when compared with control. However, the application of wood ash increasing the soil EC higher than other unburned and burned lime treatments (Fig. 5). Increased in EC was associated with increased concentration of K^+, Na^+ and Ca^{2+} in $CaCO_3$, eggshell, clamshell, and wood ash treatment. Similar trend was also found in the burned lime treatments, the soil EC

was also slightly increase with burned lime application (Fig. 5). Additionally, increased of wood ash rates significantly effect to increase of soil EC compared to other lime treatment.

Wood ash and burned wood ash shown significantly increased higher of soil cation such as K^+, Na^+ and Ca^{2+} with increasing rates of wood ash application while other treatment the change of application rate with the increasing of nutrients was not significantly different. Increase in K^+, Na^+ and Ca^{2+} reflected to the supply of these elements by wood ash and the high solubility of wood ash one applied to the soil. It was observed that the unburned lime such $CaCO_3$, eggshell, clamshell, wood ash and burned CaO, eggshell, clamshell, wood ash effects the soil solution chemistry in two ways, all the material ack as liming agent and as a supplier of nutrients. There is evidence that the limes application and wood ash could represent increased in availability of nutrients for plants. However, large concentration of basic cations obtained with higher application rates could be a concern because of potential solute transport to surface water and ground water. For maximum effectiveness, limes should be uniformly spread and incorporated into the soil. However, farmers in Cambodia tend to spread the lime by hands is therefore high laborious is need. Therefore, lime application from $CaCO_3$, eggshell, clamshell, and wood ash must be applied at the reasonable rates to avoid any risk for the environment, soil quality and farmers laborious.

Effect of Unburned and Burned Lime Materials on Survival Rate of Coliform and *E. coli*

The survival rate of coliform decreased due to lime materials. Lime increased the soil pH, reducing the survival rate of coliform, even the pH of soil didn't reach to 9.0 (less than 8.0). Pathogenic bacteria were eliminated at 7 days after the addition of lime materials (Fig. 6). However, the burned wood ash had low potential in eliminating coliform compared with other materials as its low increases in soi pH compared to other lime materials. Also, there is no significant difference of survival rate of coliform to the amounts of lime added. So, the amounts of less than 5 g or 33 kg a^{-1} should be considered. Similar trends of CaO used were also observed compared with $CaCO_3$ materials. Beside that the CaO from the burned material of $CaCO_3$ working well on eliminating the pathogenic bacteria compared to other burned materials. Increase in soil pH, EC, and concentrations of elements such K, Ca and Na have been shown to be main driver in shaping the bacterial communities in various soils. Both burned and unburned lime materials could eliminate *E. coli* in 7 days. Therefore, farmers can apply lime materials directly without burning.



**: *Significance difference at 99%, p<0.01*

Fig. 6 The effect of unburned and burned lime materials on the survival rates of *E. coli*

CONCLUSION

Different lime materials application increased soil pH, EC, K^+, Na^+, and Ca^{2+}. Similar tends were observed on burned lime material. CaO materials has more effective to increase soil pH than $CaCO_3$ materials, however, the effect of burned wood ash on soil pH became lower due to losses of K_2O after burning at 800℃. The wood ash without extra burning should be applied to the farmlands directly. There was no significant difference of soil pH responding to the amounts of lime added in this study. However, Wood ash and burned wood ash shown significantly increased higher of soil

cation such as K^+, Na^+ and Ca^{2+} than other lime treatment, increasing of wood ash application rates lead to increase of soil cation while other treatment the change of application rates has not significantly different to the increased of soil cation. It was observed that the unburned lime such $CaCO_3$, eggshell, clamshell, wood ash and burned CaO, eggshell, clamshell, wood ash effects the soil solution chemistry in two ways, as liming agent and as a supplier of nutrients. There is evidence that the limes application and wood ash could represent increased in availability of nutrients for plants. The survival rate of coliform decreased due to lime materials addition. Pathogenic bacteria were eliminated at 7 days after the addition of lime materials. Both burned and unburned lime materials could eliminate *E. coli* in 7 days. Lime increased the soil pH, reducing the survival rate of coliform, even the pH of soil didn't reach to 9.0 (less than 8.0). Since there is no significant difference of soil pH, and the survival rates of coliform and *E. coli* to the amount of lime added, so the amounts of less 5 g of lime adding should be considered. Moreover, lime application also effects to increase the soil cation large concentration of basic cations obtained with higher application rates could be a concern because of potential solute transport to surface water and ground water. For maximum effectiveness, limes should be uniformly spread and incorporated into the soil.

However, farmers in Cambodia spread the lime by hands is therefore needed high laborious. Therefore, lime application from $CaCO_3$, eggshell, clamshell, and wood ash must be applied at the reasonable rates to avoid any risk for the environment as well as to avoid the labor shortage. Also, farmers in Cambodia usually burning wood or charco to get the heat. To avoid extra laborious, it is recommended that farmers can use the unburned lime instead of burned materials in Kampong Cham Province. Inconclusion increases in soil pH, EC, K^+, Na^{2+}, and Ca^{2+} by adding of $CaCO_3$, eggshell, clamshell and wood ash can eliminate pathogenic bacteria effectively within 7 days, therefore, farmers can directly use eggshell, clamshell, and wood ash without burning for eliminating pathogenic bacteria in the farmland's soils. Additionally, local farmers should be trained on production and application of these lime materials.

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