Research article

Effect of Fertilization on Soil Microorganisms in Kampong Cham Province, Cambodia

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Abstract Fertilizers are one of the most important nutrient inputs into soil for supplying nutrients that were absorbed by plants. Application of organic and inorganic fertilizers has given an effect to soil properties directly or indirectly. Recently, organic fertilizers application such as compost, cow manure or green manure were introduced to local farmers in Kampong Cham Province, Cambodia. However, improper agricultural practices incorporating with raw materials or immature compost have a direct impact on plant health and crop productivity with resulting in contamination of soils with pathogenic microbes. Therefore, the objective of this study was to investigate the survival of pathogenic microbes with organic and inorganic fertilization in Kampong Cham Province, Cambodia. Twenty samples of soils where collected from farmlands where organic and inorganic fertilizers were applied in Samraong and Baray Communes. Also, 5 samples of compost and cow manure were collected in the same areas. From these samples of soil, compost and cow manure, the biological properties such as pathogenic bacteria *Escherichia coli* and fungi were analyzed. The experiment results indicated that there was a certain contamination of *E. coli* in these samples of soil, compost and cow manure. Also, the degrees of contamination were divided into 4 categories as very low, low, medium and high, respectively. In addition, the results of pathogenic bacteria *E. coli* in samples of soil were summarized in hazard map. However, the correlation between microbes such as pathogenic bacteria *E. coli* or fungi and fertilization was not observed statistically, while organic and inorganic fertilizers has been applied to the soil. It was considered that the sources of *E. coli* are not only from organic fertilizer applied in this area, but also transported from upstream, as there are many range lands for breeding cows in the upstream of both Samraong and Baray Communes.

Keywords fertilization, pathogenic bacteria, *E. coli*, contamination, hazard map

INTRODUCTION

Although soil is an excellent culture media for growth and develop of various microorganisms (Balasubramanian, 2017), intensive agriculture depending on agricultural chemicals resulted in negative effects on soil environment over the past decades. Sustainable crop cultivation needs the use of appropriate fertilizers that rich in nutrients, free from pathogenicity and contributes to increase in soil fertility. Hartemik (2006) defined soil fertility as the capacity of soil that supplies nutrients in adequate amounts and in proper balance for sustainable biological productivity, maintains environmental quality and promotes plant and animal health. Hence, fertilizers are one of the most important nutrient input into soil for supplying nutrients that were absorbed by plants. Organic and inorganic fertilizers supplied to plants provided the necessary nutrient for plant growth and maximum in yields (Alimi et al., 2007). Application of organic and inorganic fertilizers gave an effect to soil properties directly or indirectly. Recently, organic fertilizers application was introduced to local farmers in Kampong Cham Province, Cambodia, as it contributes to increase in

nutrient contents and improve on soil physical, chemical and biological properties (Pinamonti, 1998; Brown et al., 2004; ERECON, 2009). However, improper agricultural practices incorporating with raw materials or immature compost result in contamination of soil with pathogenic microbes. Therefore, the attentions have been given to safety use of raw materials or compost for supplying nutrients to crop and soil as well as eliminating pathogenic microbes under the different types of fertilization in Kampong Cham of Cambodia.

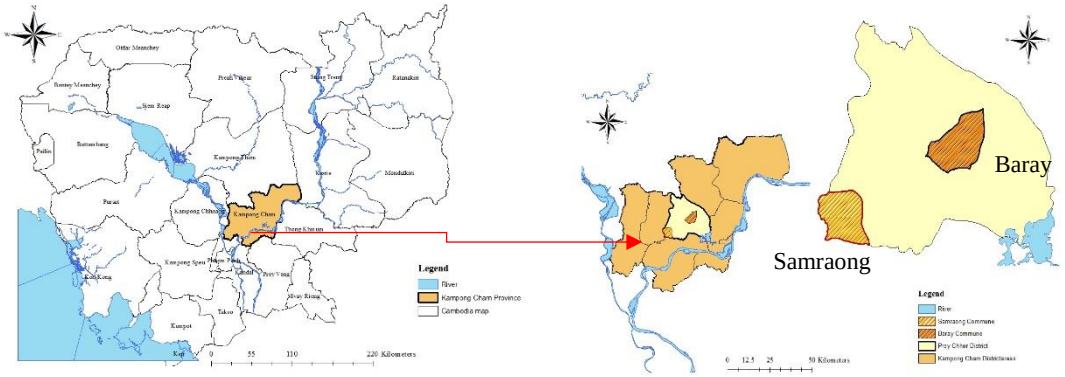
OBJECTIVE

The objective of this study was to investigate the survival of pathogenic microbes with organic and inorganic fertilization in Kampong Cham Province, Cambodia.

METHODOLOGY

Study Site

Samraong and Baray Communes, Prey Chhor District, Kampong Cham Province, Cambodia (Fig. 1) were chosen to be the study site. Samraong Commune consists of 11 villages and 1,714 households. Also, Baray Commune consists of 13 villages and 2,446 households. Ninety percent of the population are dependent on agriculture with the produce of rice and some amount of vegetables (CDB, 2010). Recently, agriculture practices in these villages have changed from traditional to market oriented with integrated used of organic and inorganic fertilizers. However, the experience of organic fertilizers application in Samraong Commune has been applied for 3 years and 10 years in Baray Communes (ERECON, 2017).



Cambodia Map

Kampong Cham Province

Fig. 1 Map of the study areas

Data Collection and Analysis

Secondary data collection: Relevant documents were collected from the research institutions, journals and reports of the project implement and the experts who had carried out studies in study areas in order to better understanding the issues involved.

Primary data collection: Interviewed farmers with the design questionnaires in Samraong and Baray Communes and 20 famers households were selected by using simple random method. The information of questionnaires survey based on general information, economical and agricultural condition of farmers in the study areas.

Field data collection: Twenty samples of soils were collected from Samraong and Baray Communes. Also, 5 samples of compost and cow manure were collected from the same farmlands.

Pathogenicity Test for *E. coli* in the Field

Instant-check was used to investigate the survival of pathogenic microbe directly in the farmland. The instant-check 25 ESCM from EIKEN Chemical for analyzing the presence of *E. coli* in soil samples (Fig. 2). Two replicated of each soil samples were tested. The plates were put in outside temperature from 30-35 ºC for 48 hours. Presence of pink and blue colonies showed the present of *E. coli* in the samples. The colonies were counted and categorized into different degree of contamination according to the number of colonies detected.

Laboratory Test

Samples of soil, compost and cow manure which from the farmlands were analyzed on its chemical, physical and biological properties. For the biological properties, pathogenic bacteria *E. coli* and fungi were analyzed by using distill dilution and plate counts method, the medium for growing the *E. coli* is XM-G Agar and Difco™ Cook Rose Bengal Agar for fungi.

Interpolation Map of *E. coli* Contamination by Inverse Distance Weight

Interpolation is use for predicts values for cells in a raster from a limited number of sample data points and can be used to predict unknown values for any geographic point data, such as elevation, rainfall, chemical concentration and noise levels. The Inverse Distance Weight (IDW) interpolation was applied for estimating unknown values with specifying search distance closet points. To do this we first started with known values and estimate the unknown points through interpolation. For creating the interpolated map, the ArcGIS software was used as a tool to record the data of *E. coli* from the laboratory and created the IDW interpolated *E. coli* hazard map.

Table 1 Community database

	Samraong Commune	Baray Commune
1. Population	8,123	10,637
2. Total number of families	1,714	2,446
3. Villages	11	13
4. Cultivated area/household	Less than 1 ha	Less than 1 ha
5. Main crops	Rice and vegetables	Rice and vegetables
6. Soil type	Brown hydromorphics, regurs, and cultural hydromorphics	Brown hydromorphics, regurs, and cultural hydromorphics
7. Number of families using chemical fertilizer	1,587	1,479
8. Project implemented	Project on Promoting Sustainable Agriculture in Kampong Cham Province, Cambodia supported by JICA (2011-2016)	Project on promotion of organic farming through composting and liquid fertilizer making in Wat Chas and Roung Kor Villages, Baray Commune supported by MAFF, Japan (2006-2009)

RESULTS AND DISCUSSION

Information of Community Database

The community database Table 1 showed that there were 8,123 and 10,637 population in Samraong and Baray Communes, respectively. The total number of families are 1,714 in Samraong and 2,446

in Baray Communes. Farmers in Samraong and Baray Communes has owned cultivation land less than 1 ha. Rice and vegetables are cultivated for both sale and self-consumption. In Samraong and Baray Communes, the projects on promoting the sustainable use of organic fertilizer were implemented, however the project periods were different. In Samraong Commune, the project were implemented from 2011 to 2016 and in Baray Commune from 2006 to 2009. After the project implemented till now, each farmers have accumulated the experiences on applying organic fertilizers around 3 years in Samraong and 10 years in Baray Communes.

Pathogenicity of *E. coli*

The results of instant-check (Fig. 2) showed that there was presence of pathogenic bacteria *E. coli* in both soils. The presence of *E. coli* can be categorized into different degree of contamination according to number of *E. coli* detected (Kaneko, 1999). Therefore, the *E. coli* contamination were divided in to 4 categories very low, low, medium and high, respectively.

Fig. 2 Instant check of *E. coli* contamination

Table 2 Degree of contamination of *E. Coli* in farmland soils

No.	Communes	OFD (%)*	Instant Check of *E. coli* in farmland	Determine	Degree of pollution	*E. coli* $\times 10^4$ (cfu/g)*	Fungi $\times 10^4$
1	Samraong	26.1	>30	++++	High	37.47	48.48
2	Samraong	62.3	0-10	+	Very low	33.23	50.59
3	Samraong	33.5	>30	++++	High	36.08	52.73
4	Samraong	27.4	>30	++++	High	34.17	54.95
5	Samraong	18.3	>30	++++	High	45.23	48.23
6	Samraong	0.1	21-30	+++	Medium	35.55	57.92
7	Samraong	1.2	11-20	++	Low	1.45	59.11
8	Samraong	0.9	0-10	+	Very low	9.92	54.47
9	Samraong	0.0	20-30	+++	Medium	38.69	4.97
10	Samraong	0.0	11-20	++	Low	56.45	55.86
11	Baray	11.3	21-30	+++	Medium	4.19	47.74
12	Baray	17.8	>30	++++	High	1.39	47.77
13	Baray	7.1	0-10	+	Very low	12.84	46.20
14	Baray	58.7	0-10	+	Very low	6.99	38.93
15	Baray	75.0	20-30	+++	Medium	10.25	45.99
16	Baray	0.0	>30	++++	High	35.53	44.41
17	Baray	0.0	>30	++++	High	42.58	42.38
18	Baray	0.0	21-30	+++	Medium	69.42	48.59
19	Baray	0.0	21-30	+++	Medium	25.42	36.85
20	Baray	0.0	21-30	+++	Medium	24.98	41.87

**E. coli colonies in soil samples which counted in laboratory, OFD (%) is the Organic Fertilizer Dependences*

Laboratory Test of *E. coli*

According to laboratory test for *E. coli* analysis, the samples of soils, compost and cow manure (Tables 2 and 3) showed that there were certain degrees of *E. coli* contamination observed. The

presence of *E. coli* in the samples of soils from the laboratory analysis were related with the degree of contamination by the instant-check done in the farmland. Additionaly, fungi were also presented in the samples of soils which were summarized in Table 2. In addition to soil samples, the samples of compost and cow manure also showed the *E. coli* contamination through the laboratory test. So, it was discussed that immature raw materials or compost and cow manure were improperly applied into farmlands, the pathogenic microbes were easily transferred to soils, as Stuart (2006) stated that largely exposure of crops to untreated livestock waste or plant residues with improperly composted manure are likely sources of *E. coli* in farmlands.

Table 3 Number of *E. coli* in organic fertilizers

No. *	Communes	Types of Organic fertilizer	*E. coli* $\times 10^4$ (cfu/g) in organic fertilizer	OFD (%) of corresponding farmland	*E. coli* $\times 10^4$ (cfu/g) in applied farmland
1	Samraong	Compost 1	9.99	26.1	37.47
2	Samraong	Compost 2	20.91	62.3	33.23
3	Samraong	Compost 3	40.81	33.5	36.08
4	Samraong	Compost 4	56.98	27.4	34.17
5	Samraong	Compost 5	30.92	18.3	45.23
11	Baray	Cow manure 1	50.68	11.3	4.19
12	Baray	Cow manure 2	34.35	17.8	1.39
13	Baray	Cow manure 3	46.95	7.1	12.84
14	Baray	Cow manure 4	41.90	58.7	6.99
15	Baray	Cow manure 5	9.98	75.0	10.25

** These site numbers in Table 3 are corresponding to that in Table 2.*

Correlation Coefficient Anaylsis

The relationships between *E. coli* or fungi with the percentage of organic fertilizers dependence (OFD) were not observed siganificantly (Fig. 3). Since it was discussed that the presence of *E. coli* in farmland soils were not only from the fertilization, although the samples of compost and cow manure showed certain contamination of *E. coli* as shown in Table 3. Also, the suvival conditions of *E. coli* in compost and cow manure gave clear tendency that those materials were immature and not ready to apply into farmlands.

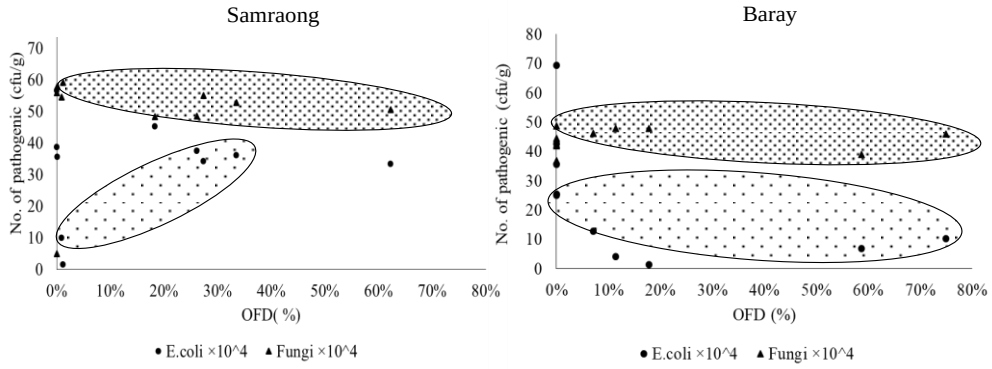


Fig. 3 Correlation between *E. coli* or fungi with OFD in Samraong and Baray Communes

So, precautions should be taken before applying those organic materials into farmlands. The survival of *E. coli* depends on many factors such as temperature, pH and water content, and according to these conditions, it can survive extended periods in water as well as soils. In addition, *E. coli* can be transfered from compost and cow manure to soils and crops. Once *E. coli* is

innoculated in soils or crops, it may survive for more than six months in soils or for up to 150 days in plants. Additionally, presence of fungi in soils were observed in the study areas, but the species were not identified. Fungi has various functions to decompose detritus and recycling nutrient back to soils and certain fungi in soils has a positive relation with plant roots to absorb nutrient and water (Einallah et al., 2014).

It was considered that the sources of *E. coli* in farmlands are not only from organic fertilizer applied in this area, but also transported from upstream, as there are many range lands for breeding cows in the upstream of both Samraong and Baray Communes.

Interpolated Maps of *E. coli* Contamination by Inverse Distance Weight

Based on the degree of *E. coli* contamination in farmlands of Samraong and Baray Communes as shown in Table 2, the interpolated maps of *E. coli* contamination were summarized in Fig. 4. This is a method to estimate unknown value point areas from known value points. These interpolated maps showed the spread of *E. coli* contamination in the study areas. The dark areas in the maps showed higher possibility of contamination of *E. coli* compared to the areas of lighter color.

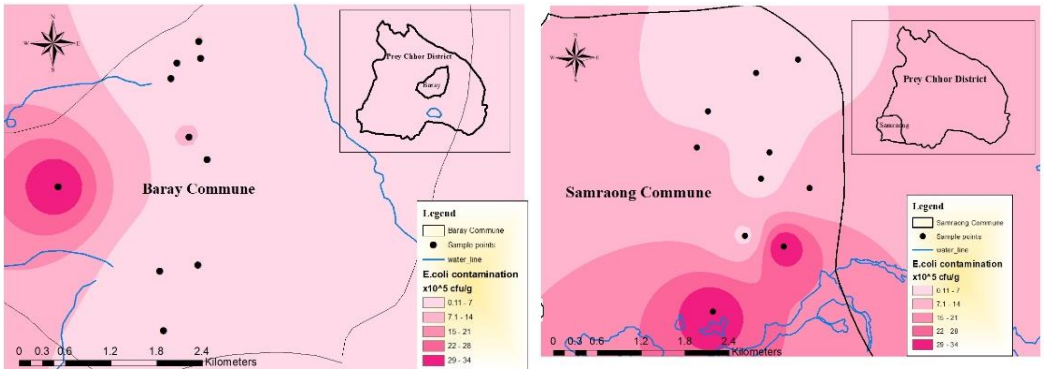


Fig. 4 Interpolated maps of *E. coli* contamination in Samraong and Baray Communes

CONCLUSION

In generally, good combination of organic and inorganic fertilizers is recommended for soil fertility management. However, maturity or well decomposition of organic materials such as compost and cow manures should be observed well before applying organic fertilizers into farmlands for eliminating survival of *E. coli* in soils. Since the relationship between *E. coli* or fungi with the percentage of organic fertilizers dependence (OFD) were not observed significantly, it was considered that the sources of *E. coli* in farmlands are not only from organic fertilizer applied in this area, but also transported from upstream, as there are many range lands for breeding cows in the upstream of both Samraong and Baray Communes. Also, it is suggested that developed *E. coli* interpolation map may be available as bio-hazard map for local farmers for producing safe agro-production and controlling the spread of *E. coli* to other areas.

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