Effect of Composting on the Cu Speciation in Cow-Based Compost Using BCR Sequential Extraction

Natdhera Sanmanee and Sirichai Nubdee

***Abstract*—Compost derived from cow-based manure mixed with agricultural waste was studied for four Cu fractions: exchangeable fraction (Cu-Ex), oxide-bound fraction (Cu-Ox), organically-bound fraction (Cu-Org), and residual fraction (Cu-Res). Cu-Res was found the most for entire process of composting within the range of 68.6-97.1% following by Cu-Org, Cu-Ex, and Cu-Ox indicating the high preference of more stable forms. As the organic matters were decomposed, the mobility of Cu increased corresponding with time ($p<0.01$) especially after reaching mature stage at day 35. Thus, composting process helped to increase the amounts of Cu. Nevertheless, using the compost to the field still needed to consider other characteristics, such as organic matter and other nutrients which should not be depleted by microorganism under too long composting.**

***Index Terms*—Compost, BCR, Sequential extraction, Cu.**

I. Introduction

Organic fertilizers become more popular recently in Thailand as the cost of chemical fertilizers is gradually increasing. Cow manure is used widespread because it is easily to access with lower price. Moreover, its organic based material offers important nutrients, such as nitrogen, phosphorus, potassium, and other micronutrients like copper (Cu) [1]. Nevertheless, fresh cow manure usually has a high ammonia content including harmful pathogens like E.coli. Thus, utilizing cow manure after composting would reduce these risks. Some studies about swine manure and flower waste composts showed increasing concentrations of total Cu during the composting period [1]-[3]. Composting process also reduces phytotoxic effect of heavy metals from sewage sludge but increasing the availability of Cu [4]. However, the amounts of each species and its availability differ depending on types of compost materials and fermenting conditions. For tropical climate where the fermenting process is rather fast, little is known about the change of Cu species and how much of each species that would release from cow manure compost.

The objectives of this research are to quantify the amounts of Cu concentrations in 4 fractions: exchangeable fraction (Cu-Ex), oxide-bound fraction (Cu-Ox), organically- bound fraction (Cu-Org), and residual fraction (Cu-Res) and to study the factors that could influence the releasing process including the relationship among them. The result would enhance the agronomic value where the result led to the idea of selecting the duration to gain the profitable compost and the length of keeping it before its nutrients are used up by microorganisms.

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II. Material and Methods

A. Composting

Cow compost 1,000 Kg derived from 76.6% of com manure was mixed with agricultural wastes—1.5% cow-bone powder, 0.9% bran of rice, 0.6% bat manure, 5.7% dried seaweed from salt farming field, 0.3% dolomite and 14.4% fish ferment juice. The compost was put in ten air-flow bags 15 Kg each and kept under shade with good air ventilation. The composite samples were collect during the fermenting period for 250 g of each bag and combined into one sample. Before sampling, the temperature of each bag was measured. All 14 samples were collected during the fermenting period at day 0, 7, 14, 21, 28, 35, 42, 49, 63, 77, 91, 105, and 119. The samples were passed through 2 mm-sieved and analyzed for the following parameters--moisture, pH, electrical conductivity (EC), organic matter (OM), nitrogen (N), phosphorus (P), and potassium (K).

Organic carbon (OC) was measured using total organic carbon analyzer (TOC) and organic matter was calculate using Van Bemmelen Factor which multiplied OC by 1.724 [5]. Total nitrogen was analyzed using Kjeldahl Method of AOAC official method 955.04 [6]. Total P and total K were analyzed after the Methods of Soil Analysis Part 3 –Chemical Methods [5].

B. Sequential Extraction

The method of sequential extraction used in this study was modified from BCR procedure [3]. The first step is exchangeable fraction which samples were extracted with 40 ml of 0.11 M aCH_3COOH shaking for 16 h at $22\pm5\circ C$. The second step is oxide-bound fraction. The samples were extracted with 40 ml of 0.5 M $NH_2OH{\cdot}HCl$ shaking for 16 h at $22\pm5\circ C$. The third step is organically-bound fraction. The samples were extracted with 10 ml of 8.8 M H_2O_2 after digested for 1 h at $85\pm5\circ C$, the volume reached 2 to 3 ml. Then, the 50 ml of 1 M NH_4OAc was added and adjusted to pH 2 with HNO_3 shaking of 16 h at $22\pm5\circ C$. The last step called residual fraction was added after Wada and Wada [7]. The residues were added with 6 ml of concentrated HNO_3 and 4 ml of 30% HCl (v/v) sporadically stir at $95\pm5\circ C$ for 1 h. All fractions were centrifuged at 5,000 rpm for 20 min. Supernatants of four steps were decanted and analyzed for Cu with Atomic Absorption Spectrophotometer (Perkin Elmer Model 2380).

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C. Statistical Analysis

Pearson correlation was employed for the relationship among parameters. The difference among means was done using Anova and Duncan. All were run by SPSS program.

III. Results and Discussion

A. Compost Characteristics

As shown in Table I, the compost was divided after the temperature into two stages: thermophillic stage (day 0-34) and mature stage (since day 35). The thermophillic stage appeared very fast with in a day of mixing shooting the temperature at day 0 to 37.0 ◦C. At the beginning of this stage, the compost still showed their original textures with the strong scent of ammonia. As, the fermenting process progressed the original texture gradually disappeared along with the diminishing of ammonia scent. This period usually end close to one month for tropical areas in which the ambient temperature rather high while composting in the colder areas would take longer [1]-[3]. The mature stage started from day 35 in which the temperature equal to the ambient temperature. The dark grey-brown color of compost gradually grew darker. The texture was soft and its smell was earthy. No ammonia scent was detected at this stage.

TABLE I: Compost Stage

Compost Stage	Duration (days)	Temperature (◦C)	Ambient Temperature (◦C)
Thermophilic stage	0-34	30.0-38.3	27.0-28.0
Mature Stage	≥ 35	27.3-30.0	27.3-30.0

Other physic-chemical characteristics of day 0, 35 and 119 were shown in Table II. At the day of mixing (day 0), the organic matter was rather high 49.8% and C/N ratio equaled to 21.7. Other parameters such as pH and moisture content were 70.4% and 6.7 which were suitable for microorganisms. When the compost process progressed, the amounts of organic matter continued to decline towards the end of mixing showing the formation change of organic carbon via the following equation:

$$Org + O_2 + H_2O \rightarrow CO_2 + NH_3 + \text{new Org} + \text{heat} + \text{new cells}$$

The reducing amounts of organic matters significantly corresponded with the increasing time (r=-0.551, p<0.01).

TABLE II: Physico-Chemicals Characteristics of Selected Compost Dates

Parameters	Day 0	Day 35	Day 119
Moisture (%)	70.4 ± 2.5	26.9 ± 0.1	11.7 ± 0.2
pH	6.7 ± 0.1	6.7 ± 0.1	5.8 ± 0.7
EC ($dS \cdot m^{-1}$)	11.4 ± 0.1	13.5 ± 0.3	13.5 ± 0.3
Organic Matter (%)	49.8 ± 9.2	39.9 ± 2.5	30.8 ± 6.1
Total N ($g \cdot kg^{-1}$)	13.3 ± 0.7	13.4 ± 0.4	16.1 ± 0.1
Total P ($g \cdot kg^{-1}$)	12.8 ± 2.8	20.6 ± 0.6	17.9 ± 0.3
Total K ($g \cdot kg^{-1}$)	2.7 ± 0.3	3.4 ± 0.1	3.6 ± 0.2
C/N ratio	21.7 ± 1.5	17.2 ± 0.5	11.1 ± 0.2

Moisture content also declined both by evaporation and consumption by microorganisms. The slow reducing of moisture content was controlled by small pore of fermenting bags and there was no adding water during the composting. When the compost age close to one month (on day 28), the moisture had just dropped below 30% reaching the standard of mature fertilizer for sale in Thailand.

The variation of pH in this experiment was not much. The pH was in the range 5.8-6.9. It was still in the optimum rage for microorganism activities, 5.5-8.5 which was the same range of standard of mature fertilizer for sale in Thailand as well.

The electrical conductivity (EC), an indirect indicator of soluble salts gradually increased during thermophillic stage (days 0-7) as shown in Fig. 1. This implied that the organic matters were transformed into nutrients via decomposition process [1]. Slightly decrease of EC was observed during late thermophillic stage (days 14-28). This might be because some released ions were in more stable forms called condensation process. However, as the compost reached the mature stage, the EC seemed to be more stable around 13 $dS \cdot m^{-1}$ which was less than 40 $dS \cdot m^{-1}$, a minimum of toxic level to plant [2].

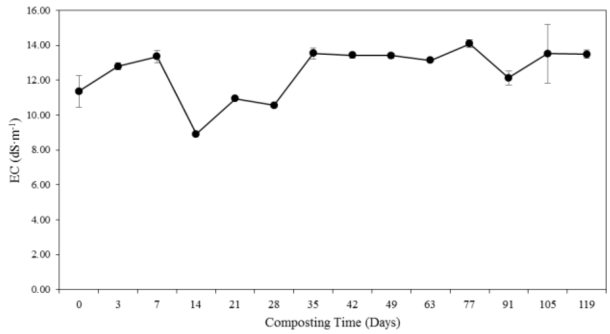

Fig. 1. Electrical conductivity of the compost.

The C/N ratio, a characteristic of the indigenous organic matter was the highest at the day of mixing, 21.7. As the organic materials were decomposed, the C/N ratio was decreasing corresponding with time (r=-0.839, p< 0.01) as shown in Fig. 2. This indicated that both thermophillic and mesophilic microorganisms processed for entire process of mixing. The evolution of C/N ratio appeared to correlate well with organic matters (p<0.01) while it was not with total nitrogen. Therefore, the consumption of carbon by microorganisms would be in the different rate when compare to the nitrogen. So, any effect on organic carbon seemed to respond the change of the C/N ratio in this study.

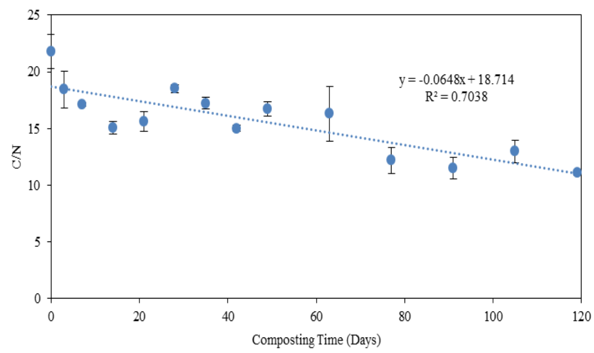

Fig. 2. C/N ratio of compost corresponding with time (r = -0.839, p<0.01).

Macronutrients, nitrogen (N), phosphorus (P) and potassium (K) showed their higher concentrations at days 35 and 119 than the day of mixing (day 0). This implied that composting also helped releasing macronutrients as well. The results were similar to composting with organic based materials such as flower waste and pig manures [1], [8]. However, other compost from sewage sludge where the mineral nutrients and toxic metals were high might be different [9]. More investigation would be needed to elaborate it.

B. Copper Speciation

As shown in Fig. 3, copper found the most in residual form for entire process of composting with the average concentrations in the following order:

Cu-Res >> Cu-Org > Cu-Ex > Cu-Ox
66.9 >> 5.7 > 4.4 > 2.3 $mg\cdot kg^{-1}$

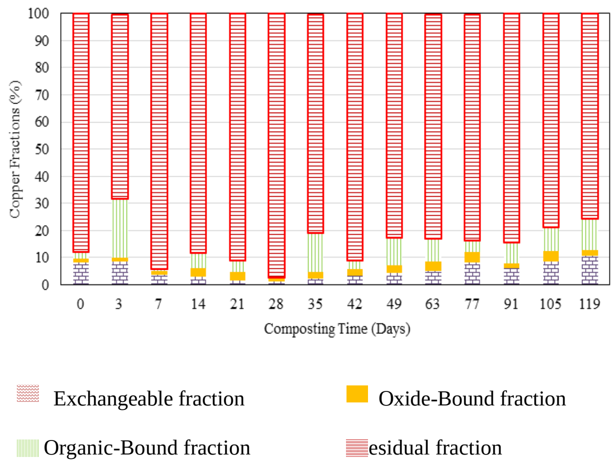

Fig. 3. Sequential extraction of Cu during composting of com manure mixed with agricultural waste.

The proportions of high Cu complexes supported the sequence of complex stability, the Irving-Williams order in which the Cu^{2+} showed the highest stability [10]. In the soil/water environment where the pH above 5.5, Cu^{2+} is not removed easily by cation exchange process with excessive amounts of Ca^{2+}or other exchangeable ions [11]. The rank of Cu found in three major components of soil/sediment is Organics > Fe/Mn oxides >>> Clay minerals [11]. This explained a bit higher amounts of Cu-Org than Cu-Ox. Other researches on swine manures mixed with other agricultural wastes showed high stability complex of Cu with the organic fraction as well even some showed the amounts of organically-bound fraction greater than the residual fraction [8], [10], [12], [13].

As composting process progressed, all fractions significantly increased with time (p<0.01) as shown in Table III leading to the increasing amounts of total Cu concentrations (Fig. 4) while the amounts of organic matter and C/N ratio were reducing (p<0.01). The accumulation of Cu in all fractions came from the change of organic matters through weight loss of the compost during decomposition process [12], [13]. The condensation process appeared rather fast during days 0-77 with the rate of 0.0013 $mg\cdot g^{-1}$ per day. During days 77-105, the total Cu was rather constant. Later it declined towards the end of mixing (day 119) as the initial raw materials were depleted by microorganisms. Others like pig manures mixed with varieties of agricultural wastes such as rice straw, maize straw and sawdust showed the same trends [12]-[13]. The increasing amount of EC through time (Table III) showed significant relationship to the rising of Cu-Ex as well (r=0.443, p<0.01). The amounts of Cu-Ex as shown in Fig. 5 (a) gradually increased with time especially in mature phase (since day 35) showing the continuos releasing of mobility Cu even at nearly end of composting. This fraction might come from releasing of other Cu forms especially when the raw materials like OM continued to deciline (as shown in Table II). This was in contrast with pig manures mixed with other agricultural wastes where the composting process reduced Cu mobility [8], [12] but it was the same as sewage sludge [14]. Thus, varieties of organic materials yield different results. More investigation in this area would be needed to identify the factors.

TABLE III: PEARSON CORRELATIONS BETWEEN CU SPECIES WITH TIME, ORGANIC MATTER, AND C/N RATIO

	Time	Organic Matter	C/N Ratio	EC ($dS\cdot m^{-1}$)
Time	1	-0.551**	-0.839**	0.458**
Cu-Ex	0.921**	-0.408**	-0.730**	0.443**
Cu-Ox	0.442**	-0.300	-0.395**	0.291
Cu-Org	0.758**	-0.188	-0.536**	0.386*
Cu-Res	0.790**	-0.591**	-0.717**	0.328*

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

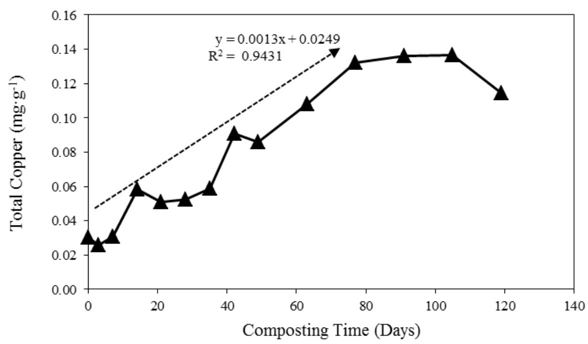

Fig. 4. Total concentration of Cu during composting process: The equation was calculated from the data during days 0-77.

The amounts of other Cu species, Cu-Ox, Cu-Org, and Cu-Res were shown in Fig. 5 (b), (c) and (d). However, after day 77 (Fig. 5 (b) and (d)) both Cu-Ox and Cu-Res were decreasing while Cu-Org was increasing. This implied the higher stability of the organic complex at the very end of fermenting. High stability organic matters that may occur from composting are humic substances [8]. They are known as form strong stability complexes expecially with Cu than other bivalent cations. Nevertheless, all fractions in mature stage still were greater than the thermophillic stage implying the occurance of mineralization. The linear regressiones of all fractions versus times were as followed:

$$Cu\text{-}Ex = 9\cdot 10^{-5}(time) + 5\cdot 10^{-5} \quad R^2 = 0.874$$
$$Cu\text{-}Ox = 3\cdot 10^{-5}(time) + 0.0007 \quad R^2 = 0.5095$$
$$Cu\text{-}Org = 9\cdot 10^{-5}(time) + 0.0014 \quad R^2 = 0.6334$$
$$Cu\text{-}Res = 0.0008(time) + 0.0318 \quad R^2 = 0.7872$$

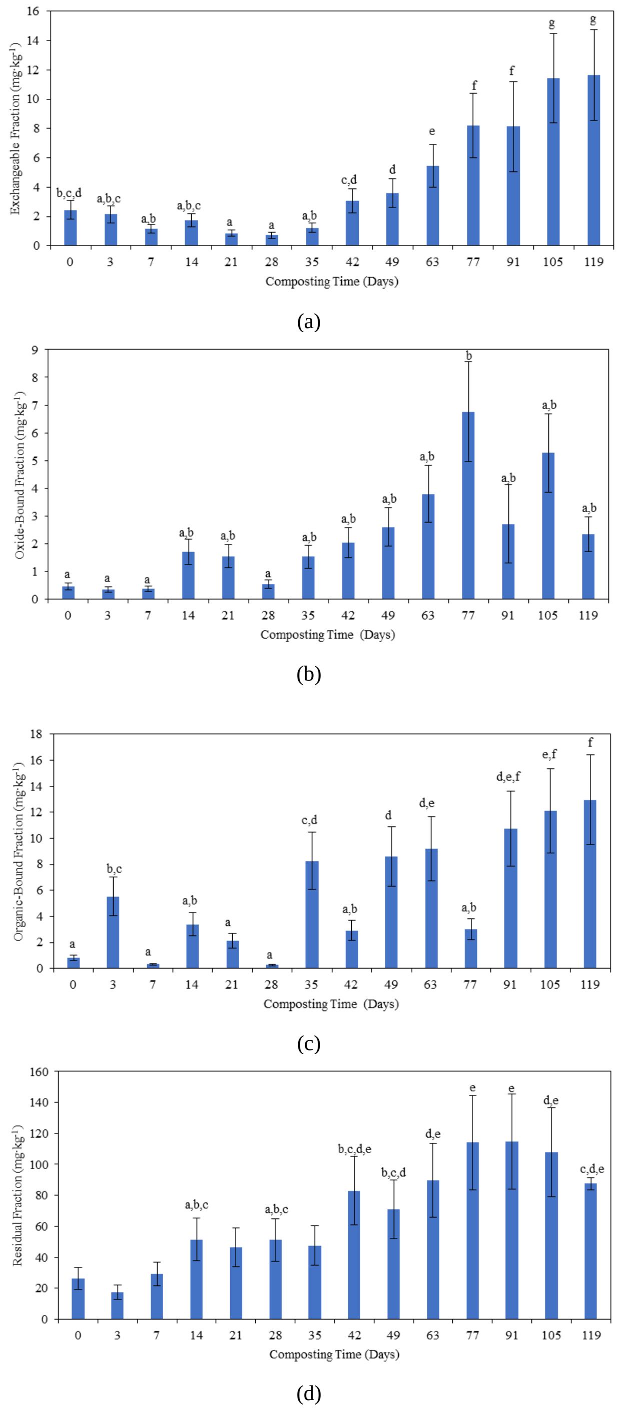

Fig. 5. The different amounts of Cu during composting period of each fraction: (a) Cu-Ex, (b) Cu-Ox, (c) Cu-Org, and (d) Cu-Res. Note: a, b, c, d, e, f, and g are significantly different at $p<0.05$, bar chart = standard error, n =3.

As shown in Table IV, most of Cu species were related to each other supporting the idea of increasing all Cu species in the same direction.

TABLE IV: PEARSON CORRELATIONS AMONG CU SPECIES

Copper	Cu-Ex	Cu-Ox	Cu-Org	Cu-Res
Cu-Ex	1	0.365*	0.772**	0.704**
Cu-Ox	0.365*	1	0.149	0.595**
Cu-Org	0.772**	0.149	1	0.439**
Cu-Res	0.704**	0.595**	0.439**	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Despite its benefit as micronutrients, too much Cu accumulating in the field might lead to phytotoxicity. In this study, the mobility factor (MF), a ratio of Cu-EX to the total content was shown in the Fig. 6. At the beginning, the Cu mobility was high and gradually decreased through time with the lowest value at day 28, 0.013. After that the Cu mobility was increasing again. However, the amounts of Cu-Ex found through entire study were not high enough to threaten the plants. The range of Cu-Ex in this study was 0.7 -11.6 mg·kg^{-1} with an average of 4.4 mg·kg^{-1} while the range of Cu found in most soils was 2-250 mg·kg^{-1} with an average of 50 mg·kg^{-1} [15]. Thus, applying this compost to the field was considered as increasing micronutrients which was more beneficial to plants rather than providing phytotoxic effect. The increasing Cu mobility was the same as the compost from sewage sludge [14]. Some studies about pig manures showed decreasing of Cu mobility towards the end of composting [12], [13]. Thus, different organic materials yield different results. Each type of organic materials should be elaborated to find the attribute factors that influenced on its mobility. The data would help to improve the understanding of composting with different organic materials and how to handle them to gain the most profit to the soil.

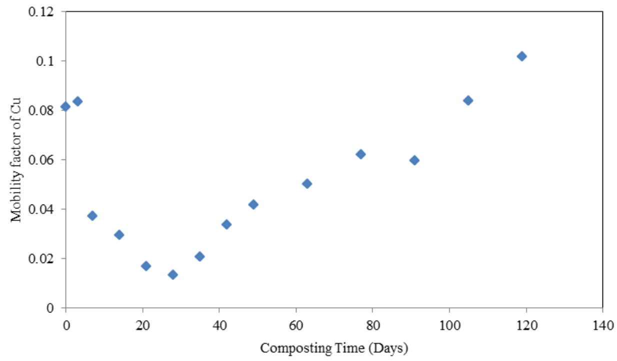

Fig. 6. The mobility factor (MF) during composting period.

In this study, composting process helped increasing Cu concentraition in all fractions. Nevertheless, cow-based compost appeared to have more residual forms than others derived from pig manure and sewage sludge [12], [14]. This might be because cow digestive process was special which could break down tough and course organic materials including storing it till it needed. These breaking organic materials might form more stable complex with Cu which would slowly release into the environment.

IV. CONCLUSION

Cow-based manure compost could be used since day 35 where the mature stage occurred. Composting process affected the Cu speciation by increasing total Cu concentration 4.4-4.5 times especially during days 77-105. All four fractions, Cu-Ex, Cu-Ox, Cu-Org, Cu-Res were increasing corresponding to the time of composting (p<0.01). Nevertheless, the major fraction was found in the residual form, 68.6-97.1% which could slowly release Cu mobility to the environment. To gain high profit of Cu-Ex applying to the field, the date of using this compost should be during days 105-119. Nevertheless, at the very end of mature stage the total Cu started to decline when kept it longer than 105 days.

So, other forms of Cu might act as a source to replenish Cu-Ex where the initial raw materials were used up. Study of how long the compost could be kept would be an interesting next study for utilizing it with high profit.

Other compost characteristics such as organic matter that was reducing through time as decomposing progress might degrade the compost in terms of physico-chemical improvement to soil. Therefore, all compost characteristics should be considered when the date of compost was selected in order to apply to the field. Other types of compost including different fermenting conditions would make the difference as well. More investigations in this area would be needed to clarify it.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Sirichai Nubdee worked on Cu extraction experiment and some statistics. Assoc. Prof. Natdhera Sanmanee who carried the grant of this research worked on other physico-chemical parameters, analyzed the data and wrote this paper.

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References

[1] D. Sharma, K. D. Yadav, and S. Kumar, "Role of sawdust and cow dung on compost maturity during rotary drum," *Bioresource Technology*, vol. 264, pp. 285-289, May 2018.

[2] H. Zhou, H. Meng, L., Zhao, Y. Shen, Y. Hou, H. Cheng, and L. Song, "Effect of biochar and humic acid on the copper, lead, and cadmium passivation during composting," *Bioresource Technology*, vol. 258, pp. 279-286, February 2018.

[3] D. Lu, L. Wang, B. Yan, Y. Ou, J. Guan, Y. Bian, and Y. Zhang, "Speciation of Cu and Zn during composting of pig manure amended with rock phosphate," *Waste Management*, vol. 34, pp. 1529-1536, August 2014.

[4] I. Walter, F. Martínez, and V. Cala, "Heavy metal speciation and phytotoxic effects of three representative sewage sludges for agricultural uses," *Environmental Pollution*, vol. 139, pp. 507-514, February 2006.

[5] D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, and M. E. Sumner, *Methods of Soil Analysis, Part 3. Chemical Methods,* Madison, WI: Soil Science Society American and American Society of Agronomy, 1996, pp. 51-1010.

[6] G. W. Latimer, Jr., *Official Methods of Analysis of AOAC INTERNATIONAL*, 19th ed. Gaithersburg, MD: AOAC INTERNATIONAL, 2012, ch. 2, pp. 13-14.

[7] D. Wada and S. I. Wada, "Kinetics of speciation of copper, lead, and zinc loaded to soils that differ in cation exchanger composition at low moisture content," *Communications in Soil Science and Plant Analysis,* vol. 30, pp. 2363-2375, 1999.

[8] L. Wang, Y. Li, S. O. Prasher, B. Yan, Y. Ou, H. Cui, and Y. Cui, "Organic matter, a critical factor to immobilize phosphorus, copper, and zinc during composting under various initial C/N ratios," *Bioresource Technology*, vol. 289, pp. 1-7, July 2019.

[9] X. Wang, G. Zheng, T. Chen, X. Shi, Y. Wang, and E. Nie, "Effect of phosphate amendments on improving the fertilizer efficiency and reducing the mobility of heavy metals during sewage sludge composting," *Journal of Environmental Management*, vol. 235, pp. 124-132, January 2019

[10] J. W. C. Wong and A. Selvam, "Speciation of heavy metals during co-composting of sewage sludge with lime," *Chemosphere*, vol. 63, pp. 980-986, May 2006.

[11] B. J. Alloway, *Heavy Metals in Soils,* 1st ed. New York: John Wiley and Sons, Inc., 1990.

[12] J. Meng, L. Wang, L. Zhong, X. Liu, P. C. Brookes, J. Xu, and H. Chen. "Contrasting effects of composting and pyrolysis on bioavailability and speciation of Cu and Zn in pig manure." *Chemishpere,* vol. 190, pp. 93-99, April 2017.

[13] S. Wu, Z. Shen, C. Yang, Y. Zhou, X. Li, G. Zeng, S. Ai, and H. He. "Effects of C/N ratio and bulking agent on speciation of Zn and Cu and enzymatic activity during pig manure composting." *International Biodeterioraiton & Biodegradation,* vol. 119, pp. 429-436, September 2016.

[14] H. Miaomiao, L. Wenhong, L. Xinqiang, W. Donglei, and T. Guangming, "Effect of composting process on phytotoxicity and speciation of copper, zinc and lead in sewage sludge and swine manure," *Waste Management,* vol. 29, pp. 590-597, February 2009.

[15] Agency for Toxic Substances and Disease Registry, *Copper: Public Health Statement*, 1600 Clifton Road NE, Mailstop F-32, Atlanta, GA 30333, USA.: Division of Toxicology, 2019, [Online]. ch. 1. pp. 1-10. Available: http://www.atsdr.cdc.gov/toxprofiles/tp132-c1.pdf

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N. Sanmanee, *Trace Metals in the Aquatic Environments (in Thai)*, 2nd ed. Bangkae, Bangkok, Thailand: Charansanitwong Printing Company Limited, 2012, pp. 1-177.

N. Sanmanee, *Environmental Chemistry (in Thai)*, 2nd ed. Nakorn Pathom, Thailand: Silpakorn University Printing, 2018, pp. 1-261

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