CHAPTER 3

RESULTS AND DISSCUSSION

3.1 Optimization of parameters used for FIAS 100-AAnalyst 800

3.1.1 Effect of carrier gas flow rate (Ar)

The flow rate of Argon was examined at 40, 45, 50 and 75 ml min^{-1}. The peak area decreased when higher flow rate was used as seen in Figure 3-1 and Table C-1 in Appendix C. The higher flow rate of carrier gas resulted in decreased signal and decreased sensitivity. The flow rate of 40 ml min^{-1} was hence chosen to carry arsine gas to quartz cell for the IAS 100 coupled with AAnalyst 800 system.

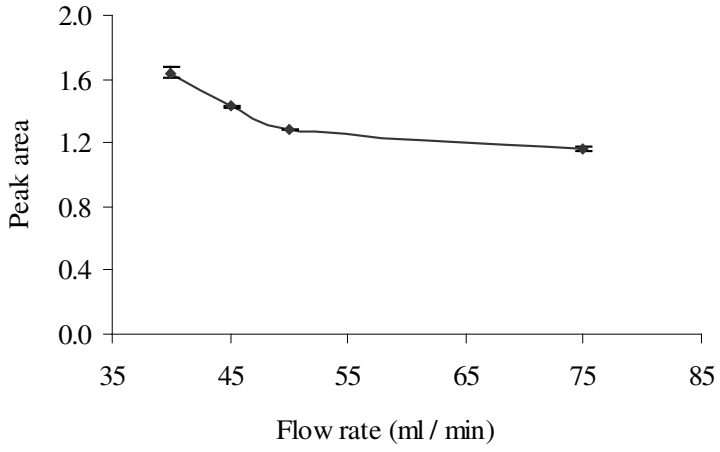


Figure 3- 1 The effect of carrier gas (argon) flow rate on the peak area of arsine generated from FIAS 100- AAnalyst 800 system

3.1.2 Effect of $NaBH_4$ concentration

The effect of $NaBH_4$ concentration, at 0.1, 0.3, 0.5 and 0.7 % (w/v) on the generation of arsine gas was examined. The results are shown in Figure 3-2 and Table C-2 in Appendix C. The maximum peak areas were produced when using the concentration of $NaBH_4$ between 0.3 and 0.5% (w/v). Above 0.5% $NaBH_4$ and below

0.3% $NaBH_4$, the signals were decreased. Thus, $NaBH_4$ concentration of 0.3% (w/v) was selected.

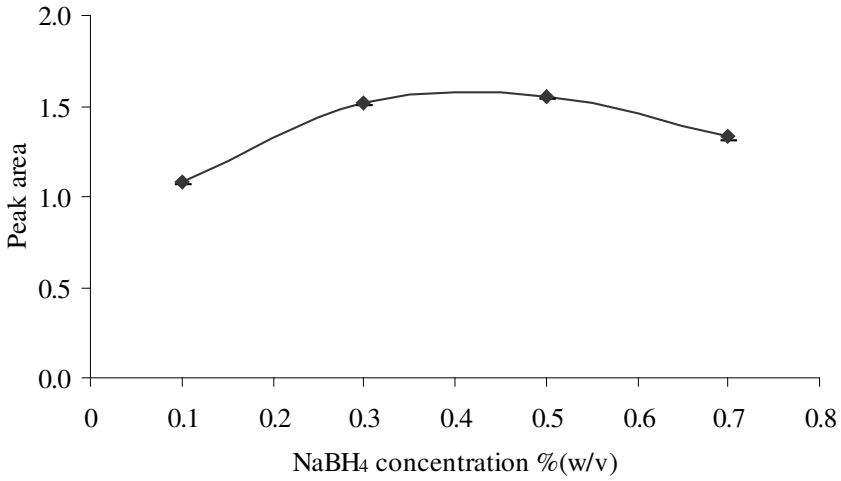


Figure 3- 2 The effect of $NaBH_4$ concentration on the peak area of arsine generated from FIAS 100- AAnalyst 800 system

3.1.3 Effect of HCl concentration

The effect of HCl concentration was examined on both peak area and peak height. The results, shown in Figure 3-3 and Table C-3 in Appendix C, suggested that no significant differences in sensitivity at the different HCl concentrations were observed. This indicated that there was sufficient acid left over from the previous pre-reduction step to allow the arsine generation reaction. However, the concentration of 10% (v/v) HCl was selected to ensure that there is always.

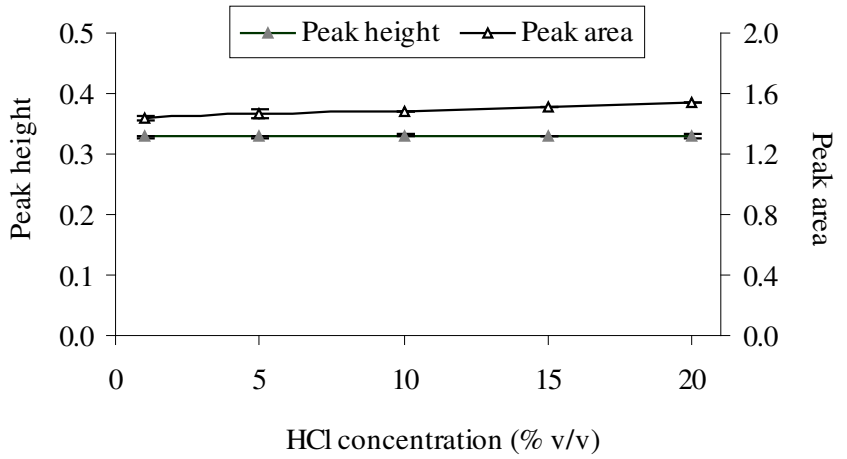


Figure 3- 3 The effect of HCl concentration (%v/v) on the peak height and peak area of arsine generated from FIA S100-AAnalyst 800 system

3.1.4 Effect of Potassium iodide / Ascorbic concentration

The effects of using Potassium iodide /Ascorbic acid as a reducing agent are given in Figure 3-4 and Table C-4 in Appendix C. The best signal was obtained when using 3 to 5 (%w/v) KI/Ascorbic acid. Therefore, 3 (%w/v) of KI/Ascorbic acid has been chosen to use in this study.

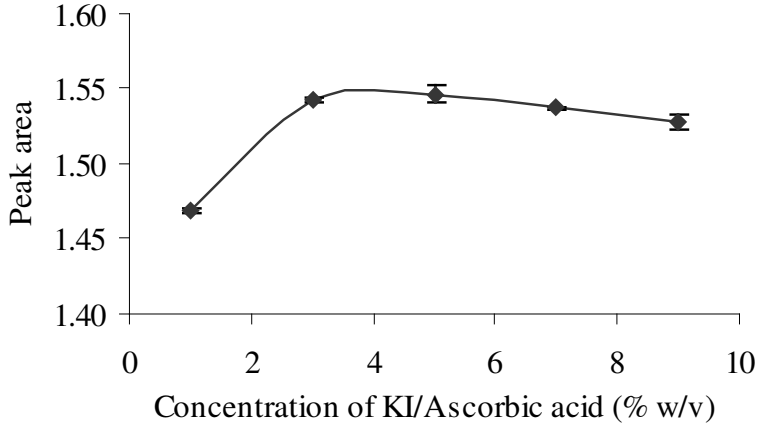


Figure 3- 4 The effect of KI/ Ascorbic acid reagent using as reducing agent on the peak area of arsine generated from FIAS 100-AAnalyst 800 system

3.1.5 Effect of reduction time

To provide short time analysis, the effect of reduction time was tested at 0, 15, 30, 45, 60 and 75 minutes. The results are given in Table C-5 in Appendix C and Figure 3-5. The reaction was found to be completed after 15 minutes. The reduction time of 15 minutes was thus selected in this study.

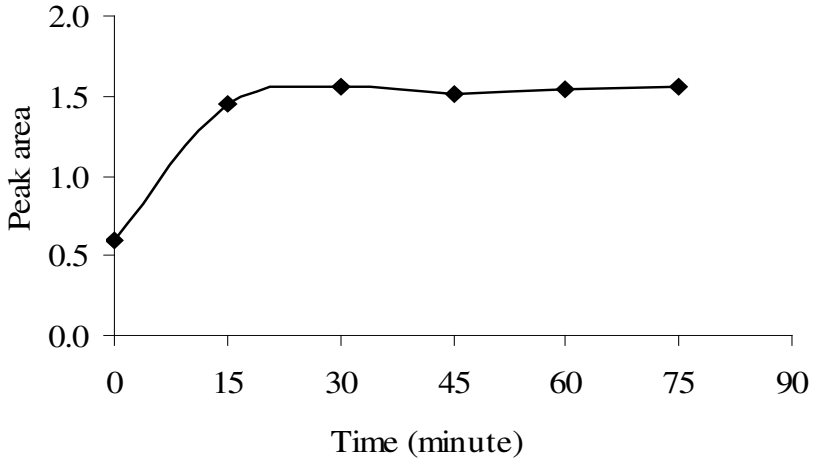


Figure 3- 5 The effect of reduction time on the peak area of arsine generated from FIAS 100-AAnalyst 800 system

3.1.6 Effect of atomization temperature

To complete the arsine atomization, the temperature should be high enough. Effect of atomization temperature is shown in Figure 3-6 and Table C-6 in Appendix C.

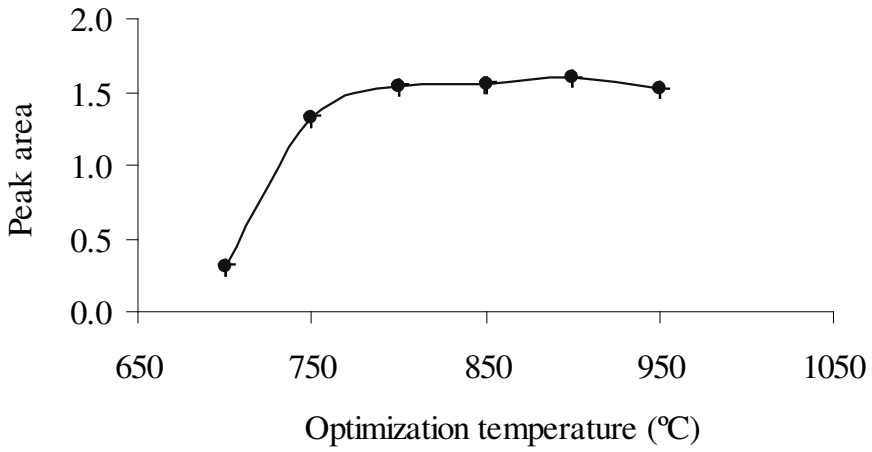


Figure 3- 6 The effect of optimization temperature on the peak area of arsine generated from FIAS 100-AAnalyst 800 system

It is clearly seen that optimization of arsine was not completed at temperature below 800ºC. The maximum sensitivity was obtained at 800-900ºC. However, the most perfect peak shape was only obtained at 900ºC. Hence the 900ºC was selected for further study in order to complete optimization and prevent a memory effect.

3.2 Comparison of the method used for extraction

The results of using autoclave and hot plate to extract soil samples are shown in Figure 3-7 and Table C-7 in Appendix C.

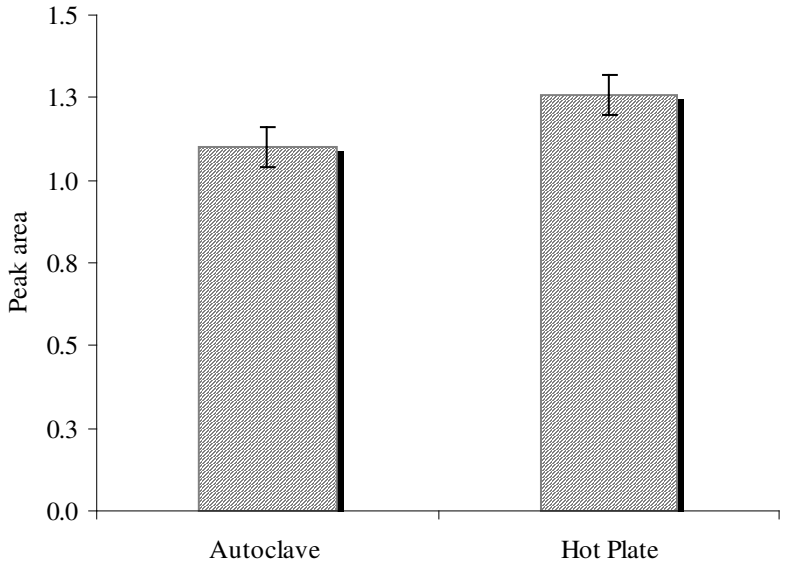


Figure 3- 7 Peak are generated from extractants of soil samples for extraction using autoclave and hot plate

Although both methods produced good reproducible value, the hot plate extraction method was giving significantly higher value than autoclave method when using statically analysis (t-Test, $p<0.05$). Slightly better precision, as shown by the lower %RSD (Table C-7 in Appendix C), was found for hot plate method. Therefore, the hot plate method was selected to extract all soil and plant samples in this study.

3.3 Standard addition

To test the effect of sample matrix when used hydride generation technique, the slope of standard curves prepared using standard addition method was compared to the one prepared in DDW. The effect of matrix was tested both in soil and plant samples. The results in Figure 3-8 and Figure 3-9 (Table C-8 and C-9 in Appendix C) show that no significant difference of the slope value between standard curve and stand addition from soil and plant samples (t-Test, $p<0.05$). Therefore, it can be concluded that there is no interference from the samples matrix.

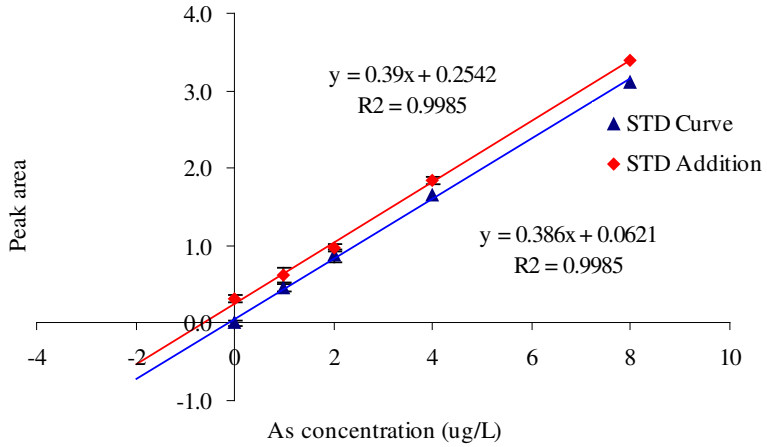


Figure 3- 8 Comparison standard calibration curve and standard addition curve method for soil sample

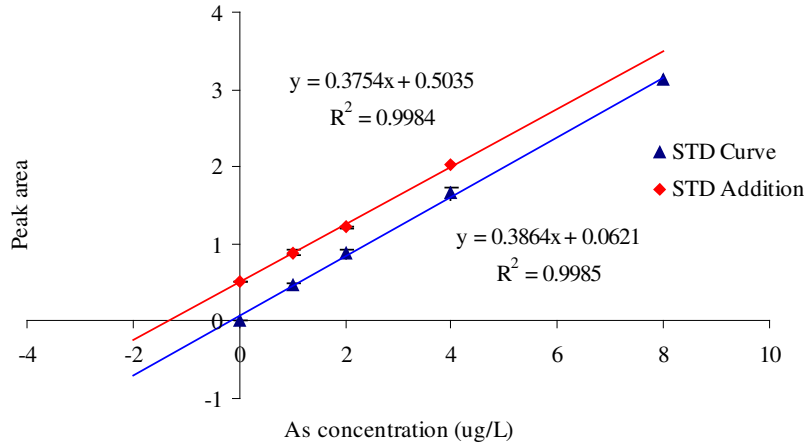


Figure 3- 9 Comparison standard calibration curve and standard addition curve method for plant sample

3.4 Method of validation

3.4.1 Detection limit (DL)

The calculation of DL for both AAS Perkin Model 5000 and FIAS-100 AAnalyst 800 followed Equation 2-5 (details in Chapter 2, section 2.10.1). The DL of AAS Perkin Model 5000 was 3.6 µg L^{-1} (Table C-10 in Appendix C) and DL for AAnalyst 800-FIAS 100 was 0.1 µg L^{-1} (Table C-11 in Appendix C).

3.4.2 Precision

The precision was presented in the term of %RSD of 10 replicated measurements of one soil sample and one plant sample. The percentage of relative deviation value (%RSD) of soil and plant samples were 8.7 and 8.4, respectively (Table C-12 and C-13 in Appendix C).

3.4.3 Accuracy

Certified Reference Material (CRM) PACS-2 obtained from the National Research Council of Canada, was analyzed using the same method as soil sample. The results are given in Table 3-1. The obtained value was found at 27.46 ± 0.43 when certified value is 26.2 ± 1.5 mg/ kg. The percent relative error was found at 6%. Therefore, it can be concluded that there is no significant differences from obtained value and certified values when using t-Test with a certainly of a 90% confidence level.

Table 3- 1 Arsenic concentration in Certified Reference Material (CRM) PACS-2

Repeated	Measured value ($mgkg^{-1}$)	Average ± SD	Certified value	% relative error
1	28.01			
2	26.96	27.46 ± 0.43	26.20 ± 1.50	6
3	27.42			

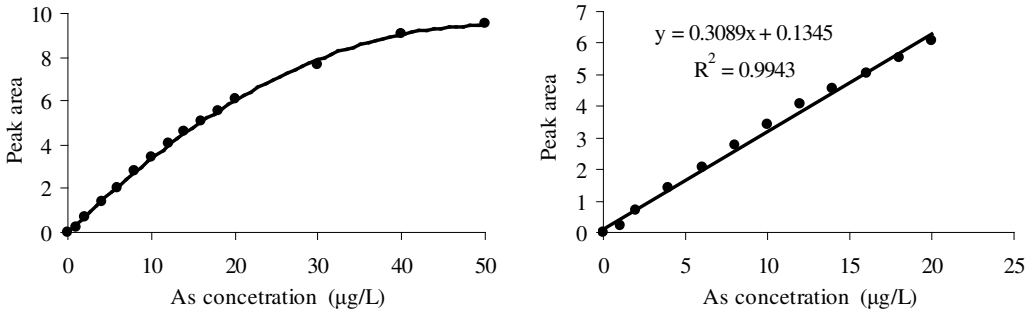
3.4.4 Percent Recovery

Both soil and edible plant samples were spiked with a known concentration of arsenic and were left for one night before analysis. The recoveries

were at 94.6 -106.4 % for soil and 106.2- 111.3 % for plant samples (Table C-14 in Appendix C).

3.4.5 Linear dynamic range

The linear dynamic range for the FIAS 100 -AAnalyst 800 system was in the range of 0.1 - 20 µg L^{-1} as shown in Figure 3-10 (Table C-15 in Appendix C). At the concentration above 20 µg L^{-1} the curve deviated from the linear line.



A B

Figure 3- 10 Peak are generated from FIAS100-AAnalyst 800 system

3.5 Total amount of arsenic in soil and edible plant samples

3.5.1 Arsenic level in soil

Forty soil samples from 8 Villages number 1, 2, 8, 9, 11, 12, 13 and 14 in the Ronphibun Sub-District in March 2004. Thirty-Five samples, excluding 5 samples of Village number 12 were extracted and analyzed using the Perkin Elmer AAS model 5000at DTU. Five samples from Village No. 12 were extracted and determined using the Perkin Elmer FIAS 100 coupled with AAnalyst 800 at PSU.

The arsenic concentrations in soil ranged from 0.6 to 491 mg kg^{-1}. The highest concentration was found in soil collected from Village 13, M_{13} $B_{394/1}$, which was considered as a high risk area. The concentration range of arsenic in the high risk area varied from 3.8 to 491 mg kg^{-1}, while in the low risk area it varied from 0.6 to 26.8 mg kg^{-1}. The results are given in Table C-16 in Appendix C.

Average in soil samples taken from Villages No. 1, 2, 12 and 13 (High risk area) were 12.7 ± 8.40, 107 ± 61.5, 66.9 ± 27.0 and 186 ± 161, respectively. Average concentration in soil samples taken from Villages No. 8, 9,11 and 14 were 5.65 ± 1.40, 1.83 ± 1.39, 1.83 ± 1.39 and 8.34 ± 3.37, respectively.

When comparing the result of the arsenic concentrations in this study with the data in Table 1-2 (Chapter 1 section 1.2.1), it was found that all house of Villages No. 2, 13 and 12 had 4 out of 5 houses arsenic mg kg^{-1} levels in soil > 40 which are considered higher than the average high risk to health level (Sheppard, 1992; Lioa *et al.,* 2005). However, the average arsenic concentration in Village No.1 which had been previously classified in the high risk area group, had similar values to those of low risk area group, and none of the samples were found to reach toxic levels. This might due to an insufficient number of samples in this Village. It would be interested to carry out more samples in different sites in Village No. 1 before classifying it as a risk area if soil arsenic concentration is used as an indicator.

Distribution of arsenic concentrations in Ronphibun Sub-district is shown in Figure 3-11. High arsenic concentration (>40 mg kg^{-1}) was found in Village of 2, 12 and 13.

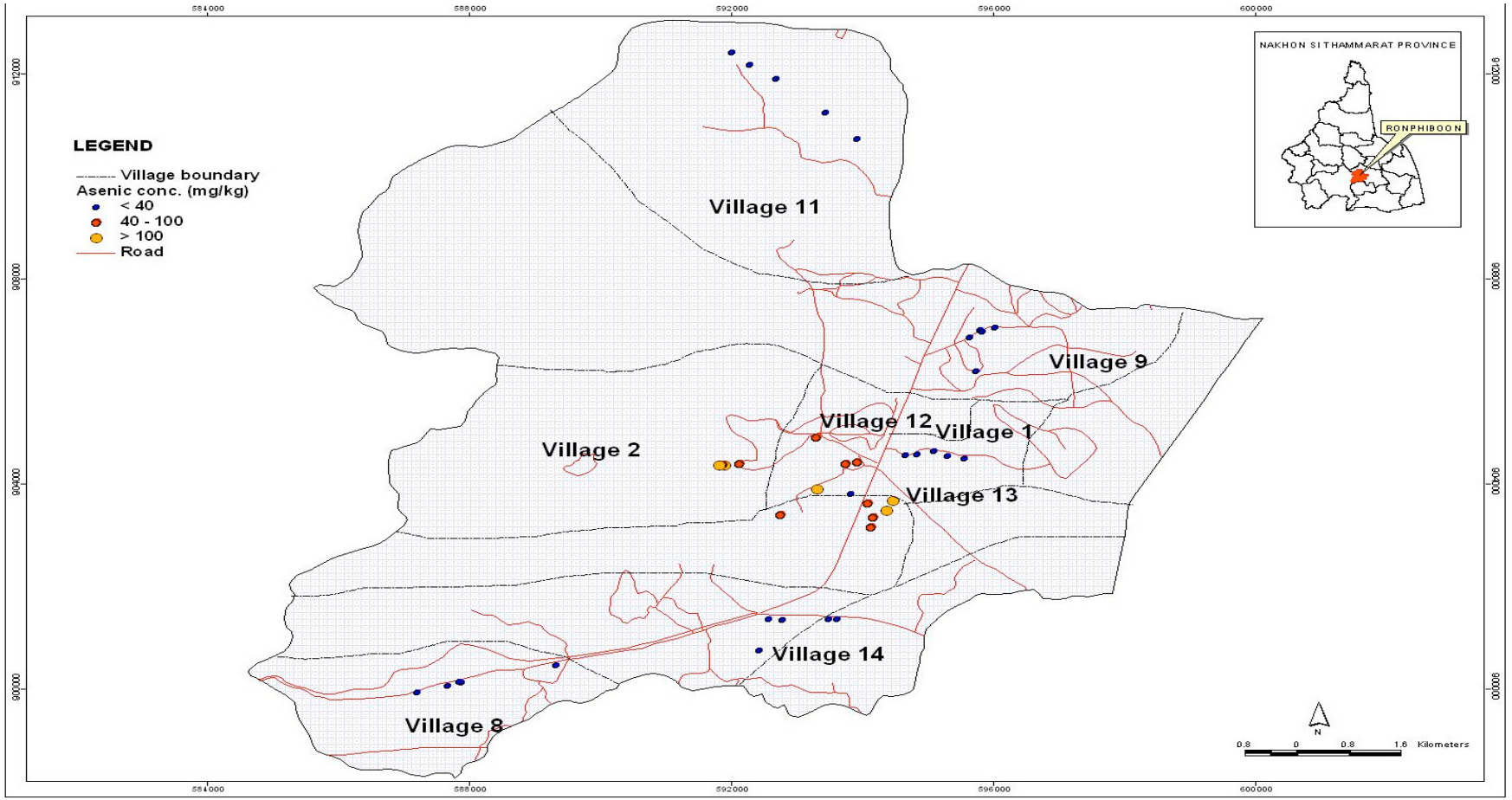


Figure 3- 11 Distribution of arsenic concentration in soil samples collected from Villages No. 1, 2, 8, 9, 11, 12, 13 and 14 in Ronphibun Sub-district**,** Nakhon Si Thammarat

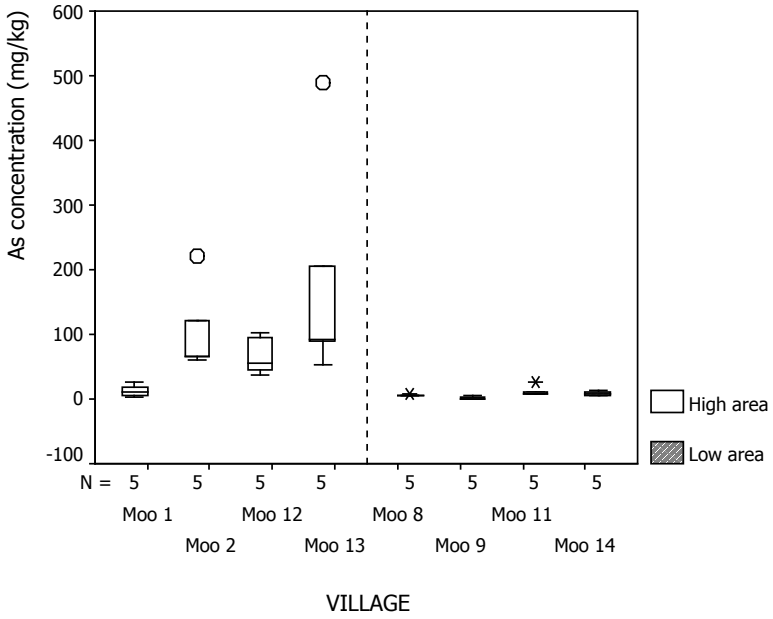


Figure 3- 12 Box and outlier plot presents Q_1, Q_2 and Q_3 of arsenic level in soil of each village (Moo) in Ronphibun Sub-district Nakhon Si Thammarat

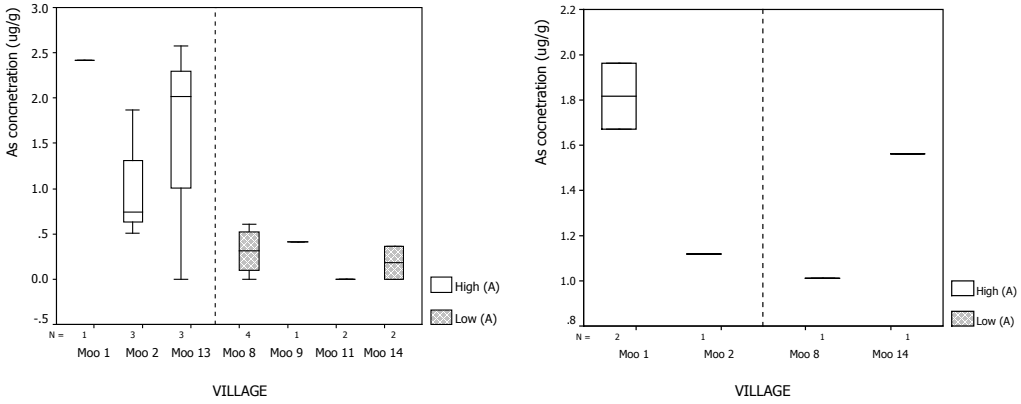
Moo1, Moo2, Moo 12, Moo13 = Previously considered as High risk areas
Moo8, Moo 9, Moo11, Moo14 = Previously considered as Low risk areas
○ indicate out side value (outlier value)
* indicate extreme out side vale (Extreme value)
Q_1 = Quartile 1 (25%), Q_2= Quartile 2 (50%) and Q_3 = Quartile 3 (75%)

Figure 3-12 is a Box plot of arsenic contaminated in soil samples taken from high and low risk areas. It is clearly seen that the arsenic level in soil in the high risk area is generally much higher than those in the low risk area. The median of arsenic contamination in Villages No. 1, 2, 12 and 13 were 11.2, 60.0, 36.0 and 88.2. The low risk area had median concentration range from 1.06 to 9.09. Village No.1 which previously considered as a high risk area, had a slightly higher arsenic value in soil than the low risk areas. The only three Villages which had high arsenic contamination in soils are Village No. 2, 12, and 13.

3.5.2 Arsenic level in plants

Thirteen species of edible plants grow in the contaminated area were studied in this work. The arsenic concentrations range in all plant samples varied from non detected (ND) to 7.4 μg g^{-1} dry weight (Table C-18 in Appendix C).

Arsenic concentrations in edible roots were measured in sixteen *Alpinia* sp. (Galanga) and five *Curcuma longa* (Curcuma) samples taken from high and low risk area. The distribution of arsenic in Galunga and Curcuma are shown in Figure 3-13. The ranges of arsenic concentrations were from ND to 2.6 μg g^{-1} for *Alpinia* sp. and from 1.1 to 2.0 μg g^{-1} for *Curcuma longa*.



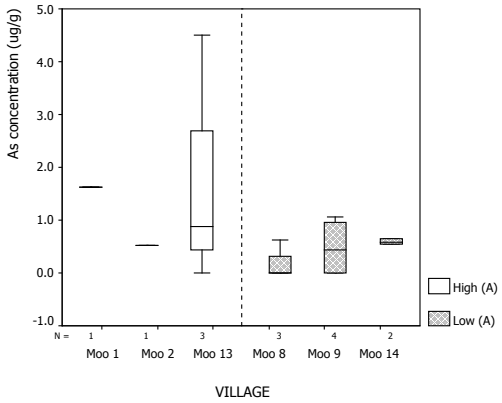
Alpinia sp. (Galanga -ข่า) *Curcuma longa* (Curcuma- ขมิ้น)

Figure 3- 13 Box plot of arsenic concentration presented in plants that have edible root

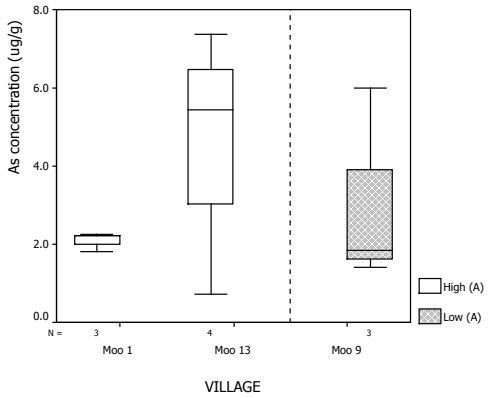
High risk area : Village No. 1, 2, 12 and 13

Low risk area : Village No. 8, 9, 11 and 14

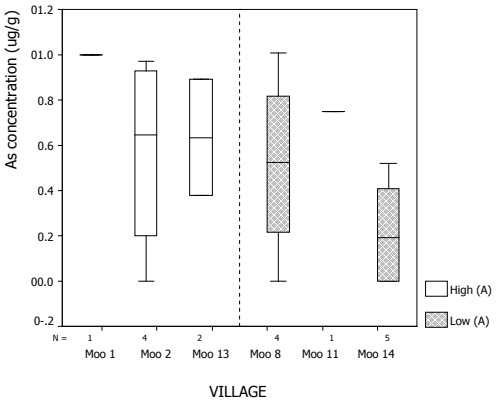
The arsenic concentrations in leaves (Figure 3-14) were studied in *Ocimum sanctum* Linn (Holy basil), *Ocimum* sp. (Sweet basil), *Polyscias* sp. (Polyscias leaves), *Cymbopogon* sp. (Lemon grass), *Ipomoea* sp. (Water morning glory) and *Citrus* sp. (Citrus leaves). Values varied from ND-4.5, 1.8-7.4, ND-1.0, ND-1.0, 1.0-2.3 and 0.2-0.3 μg g^{-1}, respectively. The arsenic concentrations were found in the same range as previous works of Na Chiengmai, (1991) and Rakwong, (1999). The highest arsenic concentration was found in *Ocimum* sp. from $M_{13}B_{381}$ at 7.4 μg g^{-1}.



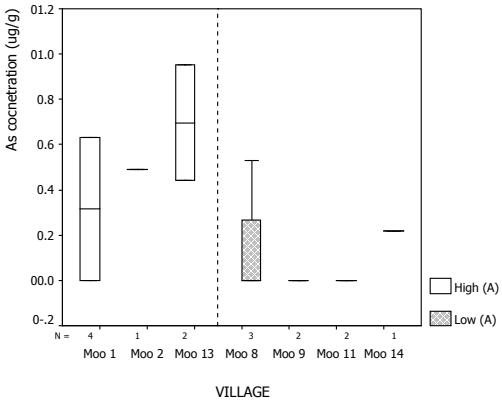
Ocimum sanctum Linn
(Holy basil - กระเพรา)



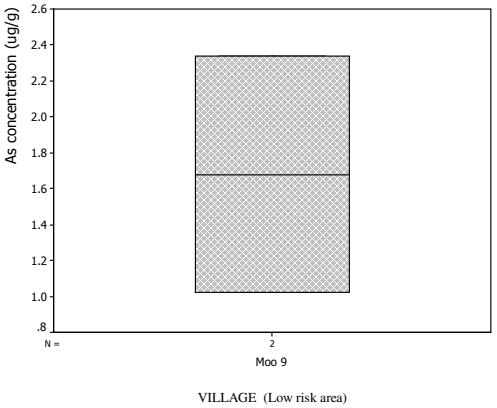
Ocimum sp.
(Sweet basil – โหระพา)



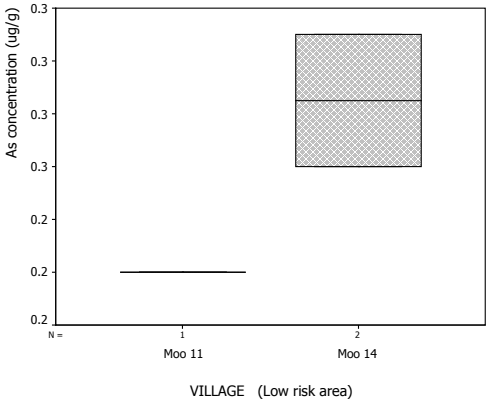
Polyscias sp.
(Polyscias leaves –ใบเล็บครุฑ)



Cymbopogon sp.
(Lemon grass -ตระไคร้)

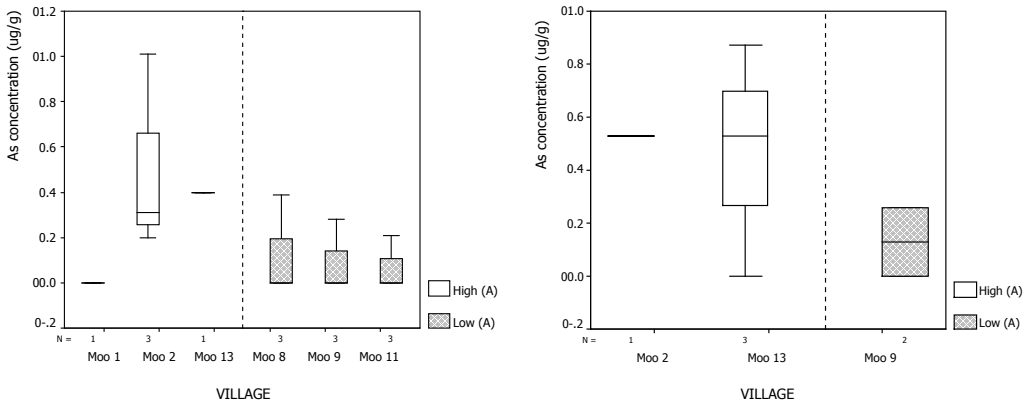


Ipomoea sp.
(water morning glory -ผักบุ้ง)



Citrus sp.
(Citrus leaves – ใบมะกรูด)

Figure 3- 14 Box plot of arsenic concentration presented in the plants that have edible leaves
High risk area : Village No. 1, 2, 12 and 13
Low risk area : Village No. 8, 9, 11 and 14



Psidium guajava (Guava-ฝรั่ง) *Carica* sp. (Papaya -มะละกอ)

Figure 3- 15 Box plot of arsenic concentration presented in the plants that have edible fruit
High risk area : Village No. 1, 2, 12 and 13
Low risk area : Village No. 8, 9, 11 and 14

Arsenic levels in some fruits growing in the area are presented in Figure 3-15. The concentration ranges of arsenic were from ND-1.0 μg g^{-1} for the *Carica* sp. (Papaya) and ND-0.5 μg g^{-1} for *Psidium guajava* (Guava). However, arsenic in *Arece* sp. (Betel nut), *Musa* sp. (Banana) and *Capcicum* sp. (Chilli) were low and less than 0.001 and 0.036 μg g^{-1} for samples were anlysed with FIAS100-AAnalyst 800 and HG-Perkin Elmer Model 5000, respectively. Although it had been previously reported that there is no relationship between arsenic levels in soils and in plants growing in the area (O' Neill, 1995; Huang, 1994), it can be seen in Figure 3-13, 3-14 and 3-15 that arsenic concentration in edible plans growing in the high risk area (high arsenic contamination in soil) is generally higher than the low risk area.

According to Thai dietary regulations the maximum allowed value of arsenic in food is < 2μg g^{-1} (FDA, Ministry of Public Health, 2004). It was found in 11 plant samples, from 3 out of 16 samples of *Alpinia* sp., 6 out of 10 samples of *Ocimum* sp, 1 out of 14 samples of *Ocimum sanctum* Linn. and 1 out of 2 samples of *Ipomoea* sp. contained arsenic concentration > 2μg g^{-1}. Although, these kinds of plants are often used for Thai dishes, but only small amounts are needed in each dish. To ensure the degree of risk, more detailed studies may be required.

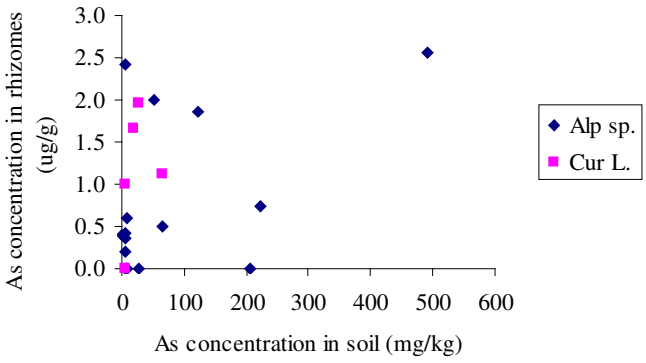
3.6 Relationship between arsenic contents of soil and plant

Plants can uptake arsenate from soil solution through the phosphate uptake system (Asher and Reay, 1979). The relationship between soils arsenic and plants arsenic are shown in Figure 3-16. There is no clear relationship which can be seen, although slightly higher accumulation of arsenic in edible parts of plant growing in the high risk area was found (see section 3.5.2). This may due to various factors including: surface area of the root, root cationic exchange capacity, different uptake system, life cycle of the plant, and selectivity of individual kind of plants.

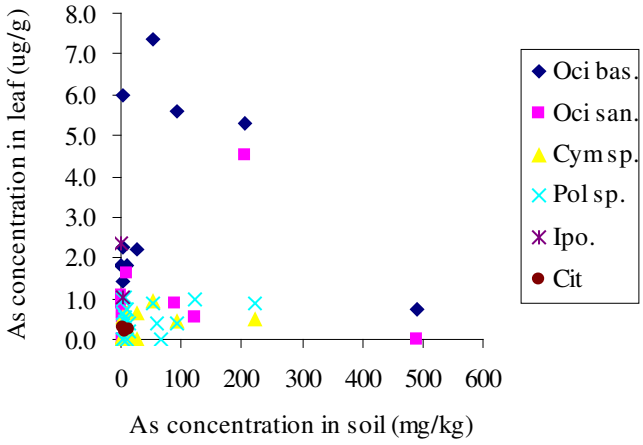
A bioconcentration factor (BCF) is defined as a proportion constant relating a chemical concentration in the plant samples to the concentration of such chemical in soil under the equilibrium condition (Hoffman *et al*., 1995), as shown in Equation 3-1.

$$BCF = \frac{arsenic\ concentration\ in\ plant\ tissue}{arseinc\ concentration\ in\ soil} \qquad 3\text{-}1$$

High BCF value (≥ 0.10) are found in some plants, *Ipomoea* sp. > *Ocimum* sp.> *Ocimum sanctum* Linn > *Curcuma longa.*> *Alpinia* sp. (Table 3-2 and Table C-19 in Appendix C). The BCF of *Ipomoea* sp. in this study is (1.29 ± 0.92) much higher than BCF value of 0.0004 in the one that were grown in a mined tailing spill in China (Liu, *et al.,* 2005). Moderate accumulation is found in *Ocimum* sp. and *Ocimum santum* Linn, with BCF value 0.48 ± 0.6 and 0.27 ± 0.55, respectively. In the other plant species of this study, BCF range is from ~0-0.14. The BCF of arsenic uptakes by plants typically varied from 0.01 to 0.1 (Kloke *et al*., 1984). Warren *et al*. (2003) reported the BCF value ranged from 0.01-0.2 in lettuce.



A



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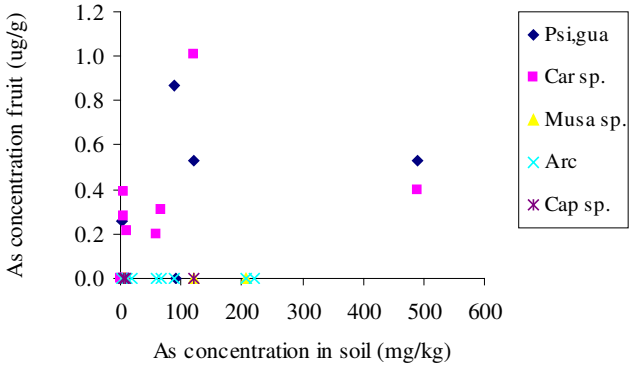


C

Figure 3- 16 The relationship between soil arsenic and accumulated in different part of plants growing in Ronphibun Sub-district Nakhon Si Thammarat

(A : in Rhizome; B : in Leaves; and C: in Fruits)

Alp sp. = *Alpinia* sp., Ipo sp. = *Ipomoea* sp., Car sp. = *Carica* sp.,
Cit sp. = *Citrus sp.*, Cap sp. = *Capcicum* sp. Cur. = *Curcuma longa.*
Oci sp. = *Ocimum* sp., Musa. = *Musa* sp. Cym sp.=*Cymbopogon* sp.
Pol sp. = *Polyscias* sp. Arc sp. = *Arece* sp Oci san.=*Ocimum sanctum* Linn
Psi gua =*Psidium guajava*

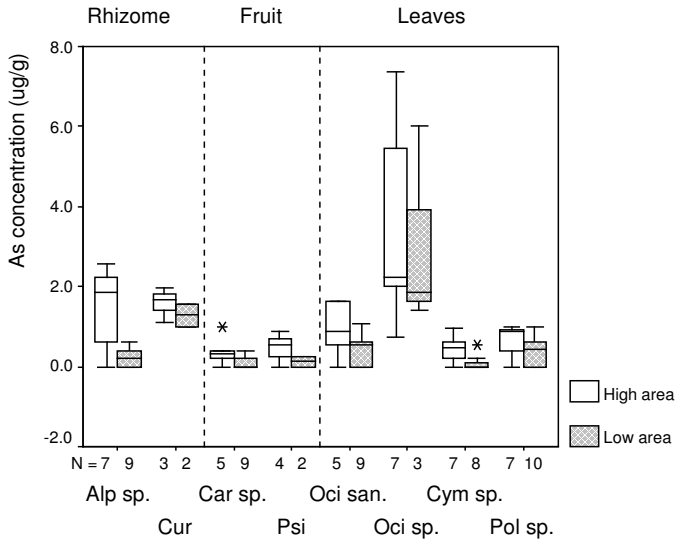
Table 3- 2 Bioconcentration factor value (BCF) of each plant growing on Ronphibun Sub-district Nakhon Si Thammarat

Type of plant **BCF range** **Average** **Number of samples**
Root
Alpinia sp. ~0-0.66 0.1 ± 0.19 16
Curcuma longa 0.02-0.32 0.14 ± 0.11 5
Leaves
Ocimum sp. ~0-1.46 0.48 ± 0.60 10
Ocimum sanctum Linn. ~0-1.81 0.27 ± 0.55 14
Cymbopogon sp. ~0-0.12 0.02 ± 0.03 15
Polyscias sp. ~0-0.22 0.05 ± 0.07 17
Ipomoea sp. 0.37-2.21 1.29 ± 0.92 2
Citrus sp. 0.02-0.07 0.04 ± 0.02

	3
Capsicum sp.	
	~0
	~0
	3
Fruit	
Carica sp.	
	~0-0.07
	0.01 ± 0.02
	14
Musa sp.	
	~0
	~0
	6
Arece sp.	
	~0
	~0
	10
Psidium guavava	
	~0-0.09
	0.02 ± 0.03
	6

Figure 3-17 is a Box plot of a arsenic contamination in plant samples growing in the high and low risk area. It is clearly seen that arsenic concentration in plants grown in a high contaminated area (Village No. 1, 2 and 13) were higher than the same plants collected from low contaminated areas (Village No. 8, 9, 11 and 14).

There are at least two works (O' Neill, 1995; Huang, 1994) reporting that the level of arsenic in plants has no relationship with the level of arsenic in soil where the plants are growing. However, the result from this study differs with the conclusion of those two works.



remark: no samples from Village 12

Figure 3-17 Box plot of arsenic concentrations of arsenic accumulated in edible part of plants which are grown in both high and low contaminated areas

Moo1, Moo2, Moo 12, Moo13 = Previously considered as High risk areas
Moo8, Moo 9, Moo11, Moo14 = Previously considered as Low risk areas
○ indicate out side value (outlier value)
* indicate extreme out side vale (Extreme value)
Q_1 = Quartile 1 (25%), Q_2= Quartile 2 (50%) and Q_3 = Quartile 3 (75%)

Alp sp. = *Alpinia* sp., Car sp. = *Carica* sp. Cur. = *Curcuma longa.*
Oci bas. = *Ocimum sp.* Cym sp. = *Cymbopogon* sp. Oci san. = *Ocimum sanctum Linn.*
Pol sp. = Polyscias sp. Psi = Psidium guajava

3.7 Risk assessment study

The purpose of this part was to evaluate a risk magnitude of arsenic Ronphibun Sub-district Nakhon Si Thammarat, province in order to estimate the risk of local people who consumes edible plants that are grown in the area. The calculation of risk followed the equation in Risk Assessment Guidelines (U.S. EPA, 1992) as shown in equations 3-2 and 3-3.

CDI (Chronic daily intake) = [As concentration x Daily intake] (3-2)

Risk = CDI x oral slope factor (3-3)

Average arsenic concentration of all plant samples in each village was used to calculate the risk of each Village consuming edible plant grown in this area. Daily intake is the average plants consumption. The Department of Health purposed the daily intake for Thai people is 0.003 kg /kg $_{\text{body weight}}$ /day (Ministry of Public

Health, 1995). The oral slope factor is the slope of the relationship between oral intake of inorganic arsenic and skin cancer risk. The slope factor, 1.5 which is estimated from the data provided in Tseng *et al*. (1968) and Tseng (1977) on about 40,000 persons exposed to inorganic arsenic (IRIS, 1998). In addition, the number of cancer prospected can be calculated with Equation 3-4:

Lifetime cancer prospected = Risk x number of risked people (3-4)

Number of risked people is the population in each village multiple by percentages of people consuming plant that are grown in the area using the information provided by Rakwong (1999); 76 % in high risk area and 80% in low risk area.

All edible plants in this study (13 species) are commonly used in Thai-food consumption in daily life. In this study, the total amount of each plant consumption was estimated from popular thirteen recipes of southern Thai dishes. The result is shown in Table 3-3.