CHAPTER 6

ARSENIC TRANSFORMATION IN CONTAMINATED SOILS AND ACCUMULATION IN ROOTS AND SHOOT OF CARROT

6.1 Introduction

Arsenic (As), known as the 'king of poison's', is the most prevalent heavy metals and is found at highest concentration in most contaminated agricultural-soils of northern Thailand. This phenomenon occurs mainly due to various anthropogenic activities including excessive use of chemical fertilizers, arsenical herbicide, pesticides, fresh manures and immature compost (Shutsrirung, 2013). The high and long-term application of these agro-chemicals has ultimately increased the arsenic concentrations of current productive Thailand agricultural-soils. As concentrations in contaminated agricultural-soil of Thailand, Bangladesh, West Bengal (India), Vietnam, Taiwan, Argentina, Chile, and Spain varied widely from 0.1-40 mg/kg (Tu and Ma, 2004). Chemical and biological oxidation-reduction of arsenic species are known to occur in various environments. The redox reactions of arsenic are important since arsenite is about 5 times more toxic to plants and also more water-soluble than arsenate (Woolson,1983). There is structural analogy between arsenate and inorganic phosphate. Therefore, arsenate can enter the cell through the same system as phosphate (Shrestha *et al*., 2008). Arsenic transformation, adsorption and mobility are commonly found on the soil surface and is influenced by various factors such as the contents of iron oxides and organic matter, pH and Eh values, and microbial activity. (O'Neil, 1995; Kumpiene *et al*., 2007). High concentration of arsenic in soils may reduce soil productivity, can be toxic to plants, and enter into the crops and vegetables (De la Fuente, 2010). Various arsenic species both inorganic forms (arsenite: As(III) and arsenate As(V)) and organic forms (dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA)) can be taken up from contaminated soils by various crops such as rice, lettuce, radish and carrot (Smith *et al*., 2008). However, inorganic As species, arsenate and arsenite have been found to be predominated (≥75 %) in various plant species such as carrot lettuce and spinach (Bergqvist. 2011). The intake of As via foods consumption such as rice, vegetables and fruit juice could pose a high risk on

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

human health. When people are exposed to arsenic concentration above permissible limit i.e., 0.05 ppm (Khan *et al*., 2000), it causes various toxic effects on human health. There is no threshold value when As becomes toxic to humans and exposure may give rise to cancer in liver, lung, skin, bladder and kidney even at low concentrations (Smith *et al*.1992). As a result of its toxicity and potential for human exposure, the US Agency for Toxic Substances and Disease Registry has ranked inorganic arsenic as the highest health hazard on their substance priority list.

Carrot (*Daucus carota* L.) is one of the most popular root vegetables consumed by human, because it is rich in valuable phytochemicals such as carotenoids and natural antioxidants having anticancer activity (Sharma *et al*., 2012). In general, plants take up nutrients and toxic metals including As through the roots thus possible accumulation of the As in carrot and a high risk of As intake by root vegetables consumption. Recent literature revealed a certain ability of carrot to accumulate As in its biomass (De Temmerman *et al*., 2012; Mayorga *et al*., 2013; McBride *et al*., 2013). The percentage of inorganic As in the edible tissues of vegetables (carrot, garlic, potato, and beetroot) depends on the species, growth stage, organ, etc. and varies from 28 to 100% of the total As contents (Muñoz *et al*., 2002). Organizations worldwide have sets standards for food to protect public health. The Chinese standards for As are 0.5 mg/kg fresh weight (FW) in rice, beans and vegetables, and 0.2 mg/kg (FW) in potatoes (NFHPC, 2012), whereas the FAO/WHO have set a standard of 0.2 mg/kg in rice (FAO/WHO, 2014). In Thailand a standard of total As for food is 2 mg/kg and inorganic As is 2 mg/kg for aquatic and seafood (Notification of the Ministry of Public Health, BE 2546).

In this experiment, high arsenic resistant isolates BAs11 (*Mycobacterium neoauurum*) and BAs29 (*Bacillus altitudinis*) was selected to evaluate their effects on arsenic transformation in soils and arsenic accumulation in the roots and leaves of carrot. Arsenic sensitive BAs7 (*Bacillus stratosphericus*) was also used for comparison.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

6.2 Materials and methods

6.2.1 Soil sampling and properties analysis

Soil samples were collected from highland areas, Pangda (18°50' 57 N and 98°44'56E), which was contaminated with As on northern Thailand. The soils collected from Pangda was considered as Pangda soils in this experiment. Representative bulk composite soil 0-30 cm was sampled and collected in polythene bags. The bulked soil sample were air-dried, thoroughly mixed and sieved to 2 mm. Some chemical properties and As concentration of the soil samples were evaluated before the experiment.

6.2.2 Bacterial culture preparation and experimental design

High arsenic resistant isolates BAs11 (*Mycobacterium neoauurum*) and BAs29 (*Bacillus altitudinis*) was used in this study. Arsenic sensitive BAs7 (*Bacillus stratosphericus*) was also used for comparison. The three isolates were cultured in nutrient broth until reach maximum growth. The final concentrations of cell suspensions were adjusted to $OD600_{nm}$ = 0.5 that correspond to cell density of approximately 10^8 CFU/ml using UV-visible spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA).

Soils collected from Pangda, Chiang Mai, was applied in this study. Lime requirement of the soil sample was determined (Woodruf, 1948). The three selected isolates were applied in soils with and without liming (Table 6.1). This experiment contained eight treatments with four replications. Nutrient broth without bacteria (control) and the culture broth of each isolate (BAs7, BAs11 or BAs29) were applied to the soils for control and each inoculated treatment, respectively. The broth solution was mixed well into the soils under each treatment to bring the soil moisture content to 60% maximum water holding capacity (MWHC). The mixed soils were incubated under room temperature (30°C) for 15 days. Soil properties and arsenic concentration were determined at 0 and 15 days after incubation. The methods of soil analysis were described under 6.2.4 and 6.2.5.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.1 Experimental treatments using Pangda Soils

Treatment	Soil (g)	$CaMg(CO_3)_2$ (g)	Broth Solution[1/,2/] (ml)	MilliQ Water[1/,2/] (ml)
P1 (Control)	1500	0	15 (nutrient broth)	135
P2 (Control + Lime)	1500	7.5	15 (nutrient broth)	135
P3 (BAs7)	1500	0	15	135
P4 (BAs7 + Lime)	1500	7.5	15	135
P5 (BAs11)	1500	0	15	135
P6 (BAs11 + Lime)	1500	7.5	15	135
P7 (BAs29)	1500	0	15	135
P8 (BAs29 + Lime)	1500	7.5	15	135

[1/] Bacteria were inoculated at a concentration of 10^6 CFU g^{-1}soil, nutrient broth without bacteria was used in the control

[2/] The moisture of the sample was adjusted to about 60% MWHC

6.2.3. Pot experiments

The Pangda soils which had been incubated for 15 days (from 6.2.2) was used for the pot experiment. Completely randomized design (CRD) was used in this experiment. Pot experiment was set up using 200 g of soil sown with carrot (D. *carota*.) seeds (3 seeds per pot), Experimental pots were sufficiently irrigated with MilliQ water for the entire study. All the pots were placed in a conditions room (25˚C) and (12:12 light:dark photoperiod and a light level of approximately 5.8 klux). After ten days of germination, only one plant/pot was selected. Carrots from each pot were harvested after 75 days of sowing. Leaves, roots and soils of each treatment were gently separated and for chemical properties and As concentration. The soils samples after harvesting were considered as 90 days after incubation counting from the previous incubation period without growing carrot (15 days).

6.2.4 Soil properties analysis

Before laboratory and pot experiments, Pangda soils were analyzed for chemical properties as follows; pH (1:1 soil: water) and available P. The methods of analysis were as described by the Department of Agriculture (DOA), (2005).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

6.2.5 Arsenic analysis of the soil samples

Determination of total As by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)

The total As content was analyzed by the Central Laboratory (Thailand) Co. Ltd. In brief, 0.5 g of dried soil was digested in 9 ml of concentrated nitric acid (HNO_3) and 3 ml hydrofluoric (HF) for 15 min using microwave according to EPA Method 3052. After cooling, the vessel contents were filtered, allowed to settle and then decanted, diluted to volume, and analyzed by Perkin Elmer Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, USA). Final results, obtained as mean values in triplicate, were expressed as mg/kg dry weight.

Determination of As species by Anodic stripping voltammetry (ASV)

Extraction of As species: the soil samples were air dried under room temperature and ground to the particle size less than 125 µm after sieving in plastic bag. A portion (400 mg) of the powdered samples was accurately weighed directly into a plastic test tube. MilliQ water (40 ml) was added and test tube was vortexes and placed in a sonicator bath for 15 min, twice. The mixture was then centrifuged at 5,000 rpm for 10 min and the supernatant was filtered (0.2 µm) before analysis (modified from Francesconi *et al.*, 2002).

Determination of As species was performed by Dr. Pipoon Bunpeng, Department of Chemistry, Faculty of Science, Chiang Mai University.

Analytical performance of the method

The reproducibility and the performance of reduction of As (V) with L-cysteine was evaluated. Standard As (V) solution at concentration 30, 60 and 80 µg/L were reduced by adding 0.0605 mg L-cysteine into 25.00 mL of 0.1 M HCl before heating at 70ºC for 30 min. The As concentration was determined by the standard addition method. The results obtained were 27±4.8, 55±4.0 and 80±4.5 µg/L, respectively (n=5) and the reduction percentage were in the range of 83 – 105%.

Preconcentration and separation

Anion exchange column

Solid phase extraction column with chloride-form strong anion exchange resin (AG1-X8) was applied for preconcentration and separation of the As [92-94]. The difference between the dissociation constant of arsenious acid (As (III)) (pK_a = 9.29) and

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

As acid (As (V)) (pK_{a1} = 2.25, pK_{a2} = 6.76 and pK_{a3} = 11.29) allows to separate these species on the basis of ion exchange at a given pH. At neutral pH, arsenous acid is present in the neutral form H_3AsO_3 and it is not retained by the anion exchange column. In contrast, As acid is dissociated to $H_2AsO_4^-$ and $HAsO_4^{2-}$ and the retention of these species should be expected.

Column and packing material for separation As (III) and As (V) from sample solution: 0.5 g of AG 1-X8 anion exchange resin was weighed and put into an Erlenmeyer flask and 150 ml of water was added. The slurry was poured into a plastic minicolumn. The resin was slightly pressed in the column, to remove any air bubbles. The column was washed with 10 ml of 1 M HNO_3. Then, the column was preconditioned with 20 ml of 2 M HCl and rinsed with 10 ml of water before use for preconcentration and separation.

Determination by Anodic stripping voltammetry (ASV), Metrohm 693 VA Processor voltammograph. Basically, a voltammetric system consists of an electrochemical cell equipped with a SPCE working electrode, a Pt auxiliary electrode, and a Ag/AgCl/3M KCl double junction reference electrode, a nitrogen gas purging tube, and a motor-driven PTFE sterring rod. In batch system, a mixture of sample/standard and supporting electrolyte solution (total volume of 10 mL) was placed in the electrochemical cell. The analysis steps are presented as the following program (deposition potential (-0.5 V), deposition time (60 s.), initial potential (-0.1 V), final potential (0.8 V), scan mode (differential pulse), step potential (10 mV), pulse amplitude (100mV), pulse time (40 ms)). Briefly, the procedure started with purging of the solution with nitrogen gas while stirring at 3000 rpm for 180 seconds, then the parameters of differential pulse mode were defined, and the deposition step was performed for 60 seconds with deposition potential of -500 mV. After that purging and stirring were stopped and waited for 5 seconds before the differential pulse was scanned from -100 to +800 mV at a scan rate of 33.33 mV/s. The procedure was repeated for 2 times for each concentration. The standard addition was applied for quantification.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

6.2.5 Growth measurement and chemical analysis

The carrot was carefully removed from each pot. Collected roots and leaves samples were cleaned with tap water, rinsed with deionized water and air-dried at room temperature for several days. The fresh weights of roots and leaves were measured prior to air drying. After air drying, the plant samples were oven dried at 60 °C until constant dry weights were recorded. Dried root and leaves were analyzed for nutrient contents as described by Bremner (1965) for total nitrogen (N), by Walinga *et al*. (1989) for total phosphorus (P), calcium (Ca), and magnesium (Mg) and by Kalra (1998) for total potassium (K).

6.2.6 Determination of total As by Inductively Coupled Plasma-mass spectrometry (ICP-MS)

Total As content was analyzed at the Central Laboratory (Thailand) Co. Ltd.). For plant samples, 0.20 g of each treatment was put in to the digestion container, then 2 ml of concentrated nitric acid (HNO_3) and 0.5 ml hydrogen peroxide (H_2O_2) were added. Digestion containers were placed in the microwave oven and start the digestion program, according to AOAC official Method 2013.06. After cooling, the vessel contents were filtered, allowed to settle and then decanted, diluted to volume, and analyzed by Agilent 7500c Inductively Coupled Plasma-mass spectrometry (ICP-MS) (Agilent Technologies, Tokyo, Japan). Final results, obtained as mean values in triplicate, were expressed as mg/kg dry weight.

6.2.7 Statistical analysis

Data were analyzed using a statistical analysis program, the Statistic 10 (SXW Tallahassee, FL, USA). The least significant difference (LSD) was used to interpret significant difference among the means ($P<0.05$).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

6.3 Results

6.3.1 Soil arsenic concentrations and properties

The arsenic level Pangda soils showed higher concentration than the standard level (3.9 mg/kg) with the value of 17.14 mg/kg (Table 6.2). The pH level of Pangda soils was considered strongly acid (pH 5.53). Optimum level of soil organic matter was recorded in Pangda soils (2.20%). The amount of available P and other plant nutrients of Pangda soils was higher than the optimum levels except for magnesium (Table 6.2).

Table 6.2 Soil properties and arsenic concentration of Pangda soils

Soil parameters	Value
Total As (mg/kg)	17.14
pH	5.53
OM (%)	2.20
Total N (%)	0.110
Available P (mg/kg)	264
Exchangeable K (mg/kg)	178
Exchangeable Ca (mg/kg)	1,553
Exchangeable Mg (mg/kg)	173

Each treatment of Pangda soils was incubated for 15 days. After 15 days of incubation, the soils were used to grow carrot for 75 days, thus in this study, the soils samples after harvesting were considered as 90 days after incubation. In general, the pH values of all the treatments were slightly increased after 15 days of incubation then after growing carrot for 75 days the pH values were slightly increased (Table 6.3). Without liming the pH values ranged from 5.37 - 5.70 after liming the pH values were increase with values of 6.37 - 7.15 (Table 6.3).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.3 pH change after 0, 15 and 90 days after incubation of Pangda soils

Treatment	pH		
	0 day	**15 days**	**90 days**[1/]
P1 (control)	5.57d[2/]	5.40b	5.70c
P2 (control + Lime)	6.66ab	6.58a	7.01b
P3 (BAs 7)	5.67c	5.37b	5.65c
P4 (BAs7 + Lime)	6.69a	6.66a	7.11ab
P5 (BAs11)	5.62cd	5.40b	5.62c
P6 (BAs11 + Lime)	6.70a	6.37a	7.15a
P7 (BAs29)	5.64cd	5.42b	5.61c
P8 (BAs29 + Lime)	6.61b	6.54a	7.13ab
F-test	**	**	**
C.V.	0.83	3.43	1.46

[1/]After 15 days of incubation, the soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation.
[2/] Mean with the same columns followed by different characters showed significant difference between treatment F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

The results of Pangda soils showed that, at 0 day, the available P values of the two controls (with and without liming) were similar (264 mg/kg). Pangda soil treated with BAs7 showed significantly lower P values than the controls (254.5 and 246.8 without and with liming, respectively). It was observed that Pangda soils treated with high arsenic resistant isolates BAs11 and BAs29, both with and without liming, gave significant lower P values than the controls and BAs7 (Table 6.4). The similar trend was observed at 15 days after incubation in that the P values obtained by amended with resistant isolates were significant lower than the controls and BAs7 inoculation. In contrast, the P values of inoculated treatments with liming (P4, P6 and P8) were significantly higher than the controls. However, on the average, the use of resistant bacterial isolates gave significant lower P values than the controls.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.4 Available P of Pangda soils after incubation with arsenic resistant bacteria

Treatment	Available P (mg/kg)[1/]		
	0 day	**15 day**	**90 day**[2/]
P1 (control)	264.7a	206.2abc	162.7bc
P2 (control + Lime)	264.2a	226.5a	171.0b
P3 (BAs 7)	254.5b	214.5ab	152.0c
P4 (BAs7 + Lime)	246.7b	228.0a	194.2a
P5 (BAs 11)	203.2d	190.0c	160.5bc
P6 (BAs 11 + Lime)	211.0cd	198.7bc	190.7a
P7 (BAs 29)	209.5d	197.0bc	152.5c
P8 (BAs 29 + Lime)	220.0c	209.0abc	200.2a
F-test	**	**	**
C.V.	2.66	7.77	4.56

[1/] Mean with the same columns followed by different characters showed significant difference between treatment F-test at P<0.05 the symbol "**" is significantly different at the P<0.05
[2/]After 15 days of incubation, the soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation.

6.3.2 Growth and nutrients uptake of carrot

After 75 days of growing in the pots, leaves, roots and soils of each treatment were gently separated. Fresh and dry weights and dry weight of carrot were recorded. It was observed that liming with or without bacterial inoculation significantly increased carrot biomass except for treatment P8. Treatment P6 (BAs11 + Lime) exhibited highest fresh and dry weight of storage roots with the values of 13.25 and 1.72 g, respectively (Table 6.5). The values of the root biomass of P6 was significantly higher than the rest of the treatments.

Nutrients uptake by carrot for all the treatments are shown in Table 6.6. The highest nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) uptake by roots was obtained in treatment P6 (BAs11 + Lime) with the values of 31.39, 4.30, 11.54, 1.38, and 2.24 mg/plant, respectively. It was observed that, in most cases, the nutrietns uptake by storage roots were significantly higher in treatments with liming as compare to the same treated soils without liming. The nutrients uptake in carrot leaves was highest in treatment P4 (BAs7 + Lime) with values of 13.45, 1.73, 6.33, 6.92 and 1.95 mg/plant, for N, P, K, Ca and Mg, respectively.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.5 Fresh and dry weights of carrot grown in Pangda soils after 75 days of growing

Treatment	Fresh weight (g) [1/]		Dry weight (g) [1/]	
	Root	leaves	Root	leaves
P1 (control)	7.90e	4.40d	1.03e	0.45e
P2 (control + Lime)	9.55c	6.40b	1.18d	0.67b
P3 (BAs 7)	8.68d	5.05c	1.10de	0.50d
P4 (BAs7 + Lime)	11.13b	7.08a	1.36c	0.74a
P5 (BAs 11)	11.25b	5.03c	1.50b	0.51d
P6 (BAs 11 + Lime)	13.25a	6.05b	1.72a	0.59c
P7 (BAs 29)	9.60c	6.08b	1.30c	0.60c
P8 (BAs 29 + Lime)	9.68c	5.05c	1.32c	0.52d
F-test	**	**	**	**
C.V.	4.83	4.74	4.67	4.75

[1/] Mean with the same columns followed by different characters showed significant difference between treatments F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

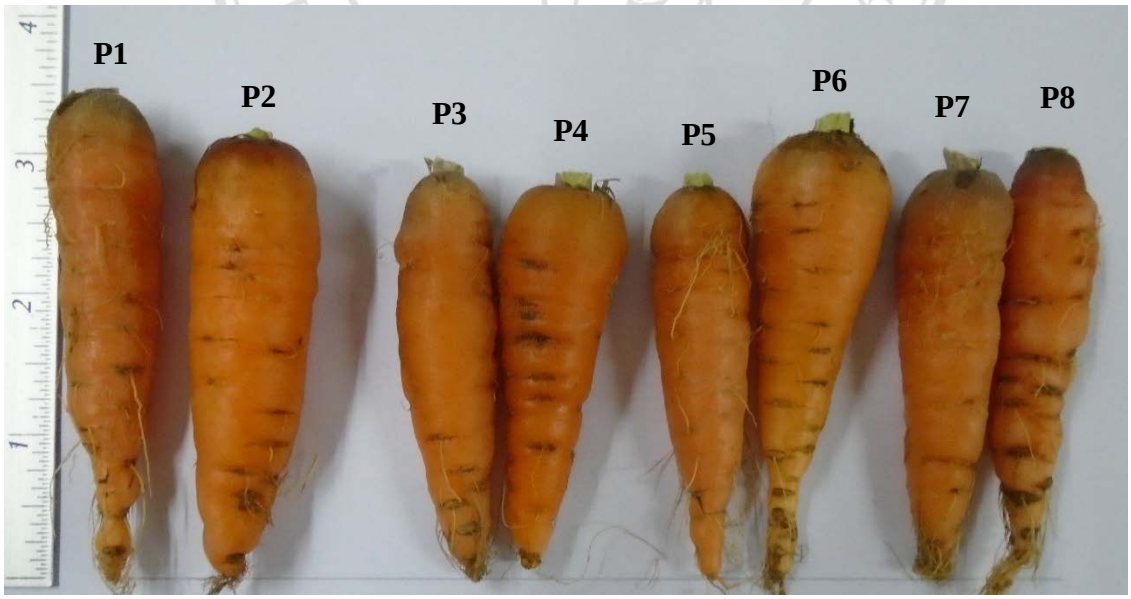

Figure 6.1 Carrot grown in Pangda soils after 75 days of growing

Table 6.6 Nutrients uptake by carrot grown in Pangda soils after 75 days of growing

Treatment	Nutrients uptake (mg/plant)[1/]									
	Total Nitrogen		Phosphorus		Potassium		Calcium		Magnesium	
	root	leaves	root	leaves	root	leaves	root	leaves	root	leaves
P1 control	12.94d	8.39d	1.85e	1.39c	6.42f	4.59d	0.92c	3.96c	1.13d	0.87f
P2 control + Lime	12.91de	10.83b	1.28f	1.58b	7.10de	6.32a	0.71e	5.82b	1.42c	1.66b
P3 BAs 7	11.65e	10.09c	2.28cd	1.38c	6.72ef	5.15bc	0.77de	3.55d	1.10d	1.24d
P4 BAs7 + Lime	18.60b	13.45a	3.40b	1.73a	8.78c	6.33a	0.91cd	6.92a	1.80b	1.95a
P5 BAs11	14.21c	10.04c	1.50f	1.17d	9.43b	5.33bc	1.08b	2.89e	1.54c	0.90f
P6 BAs11 + Lime	31.39a	11.39b	4.30a	0.96e	11.54a	5.52b	1.38a	3.11e	2.24a	1.58b
P7 BAs29	11.79de	8.85d	2.33c	1.49bc	7.42d	6.68a	0.78cde	3.20e	1.17d	1.09e
P8 BAs29 + Lime	19.67b	13.13a	2.04de	1.48bc	8.68c	5.05c	1.18b	3.80cd	1.94b	1.39c
F-test	**	**	**	**	**	**	**	**	**	**
C.V. (%)	5.22	4.58	7.57	6.01	4.95	4.87	10.63	5.09	8.73	5.20

[1/] Mean with the same columns followed by different characters showed significant difference between treatment F-test at P<0.05
the symbol "**" is significantly different at the P<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

6.3.3 Total arsenic concentration in Pangda soils

The initial concentration of total arsenic (As) in Pangda soils was 17.54 mg/kg. After mixing the soils for each treatment (0 day), the mixed soil should be air dried for a period of about 3 days and then ground for soil analysis. For this reason, the quantities of nutrients and total arsenic concentrations were not the same as the initial values (Table 6.7). The results of total As analysis at 0 day, showed that the P1 (control) maintained the highest values of 17.14 mg/kg while liming in P2 (control + Lime) and, P8 (BAs 29 + Lime) showed the lowest values of 16.14 and 16.09 mg/kg, respectively. After incubation for 15 days, the values of total As in Pangda soils in all the treatments were reduced as compared with the initial valued. The control without liming (P1) and inoculation with BAs7 (P3) gave the highest values of total As, 14.36 and 14.25 mg/kg, respectively. These two values were significantly higher than those of other treatments. Treatment P6 (BAs11 + Lime) gave significantly lower value of total As (12.96 mg/kg) than the rest of the treatments and gave the highest As reduction percentage of 24.39. After 15 days of incubation, the Pangda soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation. After 90 days of incubation, the values of total arsenic of all the treatments were slightly reduced as compared with the same treatment at 15 days. The highest values were obtained with P3 (BAs7), 13.76 mg/kg. The lowest value was obtained with bacterial inoculation plus liming in P4 (BAs7 + Lime) and P8 (BAs29 + Lime) with values of 12.92 and 12.88 mg/kg, respectively. These two values were significantly lower than the rest of the treatment except for P7. The As reduction percentage was highest in treatment P4 and P8 with values of 24.61 and 24.88%, respectively.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Arsenite (As(III)) is more mobile and more toxic than arsenate (As(V)) therefore the soils used in this study was also analyzed for As(V) content to evaluate the potential of arsenic-resistant isolates in As(III) transformation. At 0 and 15 days of incubation, the results indicated that BAs29 (P7) performed the highest ability to oxidize As(III) into As(V) followed by P8 (BAs29 + Lime), P5 (BAs11) and P6 (BAS11 + Lime) (Table 6.13). BAs29 and BAs29 + Lime gave the highest values of As(V) at 0 and 15 days of incubation with the values of 2.48 and 2.08 mg/kg, and 2.58 and 2.28 mg/kg, respectively.

However, at 90 days of incubation, the highest value of As(V) was obtained with BAs11 + Lime (0.79 mg/kg

Table 6.7 Total arsenic (As) concentration in Pangda soils after 0, 15 and 90 days of incubation

Treatment	0 day		15 days		90 days[1/]	
	Total As[2/] (mg/kg)	**As reduction** (%)	**Total As**[2/] (mg/kg)	**As reduction** (%)	**Total As**[2/] (mg/kg)	**As reduction** (%)
P1 (control)	17.14a	0.00	14.36a	16.25	13.06c	23.79
P2 (control + Lime)	16.14d	5.86	13.60c	20.65	13.13c	23.40
P3 (BAs 7)	16.82b	1.85	14.25a	16.88	13.76a	19.73
P4 (BAs7 + Lime)	16.20c	5.48	13.54c	21.03	12.92de	24.61
P5 (BAs 11)	16.89b	1.44	13.78b	19.60	13.41b	21.78
P6 (BAs 11 + Lime)	16.57c	3.35	12.96e	24.39	13.39b	21.89
P7 (BAs 29)	17.07a	0.44	13.32d	22.29	12.98d	24.29
P8 (BAs 29 + Lime)	16.09d	6.11	13.22d	22.86	12.88e	24.88
F-test	**		**		**	
C.V.	0.46		0.85		0.40	

[1/]After 15 days of incubation, the soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation.
[2/] Mean with the same columns followed by different characters showed significant difference between treatment
F-test at P<0.05 The symbol "**" is significantly different at the P<0.05

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.8 Arsenate As (V) concentration in Pangda soils after 0, 15 and 90 days of incubation

Treatment	**As (V)** (mg/kg)		
	0 day	**15 day**	**90 day**
P1 (control)	0.95f	0.83g	0.56b
P2 (control + Lime)	1.00e	1.30e	0.40f
P3 (BAs 7)	0.79g	1.40d	0.47d
P4 (BAs7 + Lime)	1.10d	1.20f	0.50c
P5 (BAs 11)	1.30c	1.20f	0.40f
P6 (BAs 11 + Lime)	0.99e	1.68c	0.79a
P7 (BAs 29)	2.48a	2.58a	0.45e
P8 (BAs 29 + Lime)	2.08b	2.28b	0.50c
F-test	**	**	**
C.V.	1.12	0.56	1.15

[1/]After 15 days of incubation, the soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation.
[2/] Mean with the same columns followed by different characters showed significant difference between treatment
F-test at P<0.05 The symbol "**" is significantly different at the P<0.

6.3.4 Total arsenic accumulation in carrot grown in Pangda soils

After 15 days of incubation, the Pangda soils were used to grow carrot for 75 days. Total arsenic accumulation in roots and leaves the soils samples after harvesting were considered as 90 days after incubation. The As concentration in all carrot roots and leaves was highest in treatment P6 (BAs11 + Lime) with the values of 0.048 and 0.080 mg/kg, respectively (Table 6.9). Arsecnic accumulation in leaves of P1 (control) was as high as P6 (0.046 mg/kg). Pangda soils treated with BAs29 gave the lowest value of As accumulations in leaves (0.025 mg/kg) while BAs29 + Lime gave the lowest value of As accumulations in roots. It was observed that liming the Pangda soil without bacterial inoculation could reduce about half As accumulation in leaves (0.027 mg/kg) as compared to the control without liming (0.046 mg/kg).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table 6.9 Arsenic accumulation in storage roots and leaves of carrot grown in Pangda soils

Treatment	**As accumulation in baby carrot** (mg As/kg DW)		
	Leaves	**Root**	**Total (Leaves + Root)**
P1 (control)	0.046b	0.033	0.079
P2 (control + Lime)	0.027e	0.039	0.066
P3 (BAs 7)	0.038c	0.032	0.070
P4 (BAs7 + Lime)	0.034d	0.038	0.072
P5 (BAs 11)	0.037c	0.025	0.062
P6 (BAs 11 + Lime)	0.048a	0.080	0.128
P7 (BAs 29)	0.025f	0.048	0.073
P8 (BAs 29 + Lime)	0.026ef	0.025	0.051
F-test	**	**	
C.V.	2.44	6.69	

[1/]After 15 days of incubation, the soils were used to grow carrot for 75 days, the soils samples after harvesting were considered as 90 days after incubation.
[2/] Mean with the same columns followed by different characters showed significant difference between treatment
F-test at P<0.05 The symbol "**" is significantly different at the P<0

6.4 Discussion

Food safety is now a major concern among Thai government and private sectors, consumers and thus the farmers. Arsenic contaminations in agricultural soils of northern Thailand (>3.9 – 40 mg/kg) has renewed the interest in studying the transformation of As in the soil and a potential risk of food chain contamination and/or a problem of crop yield loss. Soil microorganisms play an important role in arsenic transformation and may led to less toxicity to them and less uptake by crops. In the present study, after harvesting carrot in Panda soils (initial As value = 17.14 mg/kg), the use of As-resistant bacteria isolate BAs29 showed the highest values of arsenic reducing percentage (24.88%) and the sum values As(V) concentration (5.51 mg/kg) suggesting that the arsenite form (As(III)) in soils might be oxidized to the less toxic form As(V). The oxidation/reduction of arsenite/arsenate are important strategy for As detoxification since arsenite is about more toxic to living organisms and also more water-soluble than arsenate. Chang *et al*.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

(2007) concluded that *Pseudomonas putida* strain OS-5 completely oxidized As(III) to As(V) which was one of the arsenic detoxification systems. In addition, the results of the present study showed that liming the Panda soils with or without bacterial inoculation appeared to have higher reducing percentage of As than the unlime soils. Therefore, these results suggest that pH conditions can influence arsenic transformation.

The mechanisms of resistance are also due to reduced uptake of arsenate and increased concentrations of phosphate transport into bacterial cells (Duker *et al*., 2005) and might led to more phosphate uptake by plants. In the present study, the use isolate BAs29 in Pangda soil gave the highest P uptake in the storage roots of carrot while with liming, BAs11 gave the highest value of P uptake by roots. Our results indicated that, on the average, the use of As-resistant bacterial isolates gave significant lower P values in soils than the controls suggesting removal of arsenate and more P uptake by the storage roots of carrot. However, the lowest values of As accumulation in the roots was obtained when BAs29 was applied with liming. The values of total As accumulation in the storage roots 0.025 – 0.080 mg/kg which is appeared to be lower than the Thai standard values for foods (2.0 mg/kg) (Notification of the Ministry of Public Health, BE 2546). Gaw *et al*. (2008) found that radishes and lettuce grown in As contaminated soils, accumulated arsenic but in concentrations that were not exceeded the U.S. Food and Drug Administration (FDA) standard (2 mg/kg). However, the continuous consumption of As-contaminated foodstuffs such as rice, vegetables and fruit juice could pose a high risk on human health. When people are exposed to arsenic concentration above permissible limit i.e., 0.05 mg/kg (Khan *et al*., 2000), it causes various toxic effects on human health. Smith *et al*. (1992) stated that there is no threshold value when As becomes toxic to humans and exposure may give rise to cancer in liver, lung, skin, bladder and kidney even at low concentrations.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Besides, arsenic detoxification, the As-resistant bacterial isolates, particularly BAs29 also enhance carrot growth and plant nutrients uptake. The present study revealed the important roles of As-resistant bacteria in bioremediation and plant growth enhancement.

6.5 Conclusions

High arsenic resistant isolates BAs11 and BAs29 (*Bacillus altitudinis*) was used to evaluate their effects on arsenic transformation in soils and accumulation in carrot. Arsenic sensitive BAs7 was also used for comparison. The results indicated that the control without liming and inoculation with BAs7 gave the highest values of total As, 14.36 and 14.25 mg/kg, respectively (15 days after incubation). After harvesting carrot, the As reduction percentage was highest in treatment (BAs7 + Lime) and P8 (BAs29 + Lime) with values of 24.61 and 24.88%, respectively. At 0 and 15 days of incubation, the results indicated that BAs29 (P7) performed the highest ability to oxidize As(III) into As(V) followed by P8 (BAs29 + Lime), P5 (BAs11) and P6 (BAs11 + Lime). Pangda soils treated with BAs29 gave the lowest value of As accumulations in leaves (0.025 mg/kg) while BAs29 + Lime gave the lowest value of As accumulations in roots. The nutrients uptake in carrot leaves was highest in treatment P4 (BAs7 + Lime) with values of 13.45, 1.73, 6.33, 6.92 and 1.95 mg/plant, for N, P, K, Ca and Mg, respectively. Treatment P6 (BAs11 + Lime) exhibited highest fresh and dry weight of storage roots with the values of 13.25 and 1.72 g, respectively. The present study revealed that the use of As-resistant bacteria not only detoxify As but also enhance growth and nutrients uptake of carrot.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

REFERENCE

Bergqvist, C. 2011. Arsenic accumulation in various plant types. Licentiate of Philosophy Thesis, Department of Botany Stockholm University Sweden. 52 p.

Bremner, J.M. 1965. Total nitrogen. In: C.A. Black (Ed.), Methods of soil analysis. American Society of Agronomy. 9: 1149-1178.

Chang, J.S., I.H. Yoon and K.W. Kim. 2007. Isolation and ars detoxification of arsenite-oxidizing bacteria from abandoned arsenic-contaminated mines arsenic. Journal Microbiology Biotechnology 17(5): 812-821.

De la Fuente, C., R. Clemente, J.A. Alburquerque, D. Velez and M.P. Bernal. 2010. Implications of the use of As-rich groundwater for agricultural purposes and the effects of soil amendments on As solubility. Environmental Science, technology 44(24): 9463-9469.

De Temmerman, N., L. Alegria Mira, A. Vergauwen, H. Hendrickx and W.P. De Wilde. 2012. Transformable structures in architectural engineering. High Performance Structures and Materials VI.

Department of Agriculture. 2005. Manual of organic fertilizer analysis. Quick print off set: Bangkok. 45p. (in Thai)

Duker A.A., E.J.M. Carranza and Martin Hal, 2005 Arsenic geochemistry and health Environment International 31(5): 631-641

Francesconi, K., Visoottiviseth, P., Sridokchan, W., Goessler, W., 2002. As species in an As hyperaccumulating fern, Pityrogramma calomelanos: a potential phytoremediator of As-contaminated soil. Science of the Total Environment 284: 27-35.

Gaw, S.K., N.D. Kim, G.L. Northcott, A.L. Wilkins and G. Robinson. 2008. Uptake of ΣDDT, arsenic, cadmium, copper and lead by lettuce and radish grown in contaminated horticultural soils. Journal of Agricultural and Food Chemistry 56: 6584-6593

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Kalra, Y.P. 1998. Handbook of reference methods for plant analysis. London: CRC Press. 300 p.

Khan A.H., S.B. Rasul., A. Munir., M. Habibuddowla., M. Alauddin., S.S. Newaz. and A. Hussan. 2000. Appraisal of a simple arsenic removal method for groundwater of Bangladesh. Journal of Environmental Science and Health 35:1021-1041.

Kumpiene, J., I. Montesinos, A. Lagerkvist and C. Maurice. 2007. Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron treated soil. Chemosphere 67: 410-417.

Mayorga, P., A. Moyano, H.M. Anawar, A. García-Sánchez. 2013. Uptake and accumulation of arsenic in different organs of carrot irrigated with As-rich water. CLEAN– Soil Air Water 41:587-592.

McBride, M.B., T. Simon, G. Tam and S. Wharton. 2013. Lead and arsenic uptake by leafy vegetables grown on contaminated soils: effects of mineral and organic amendments. Water, Air, and Soil Pollution 224: 1-10.

Muñoz O., Díaz O., Leyton I., Nuñez N., Devesa V., Suñer M.A., Vélez D. and R. Montoro. 2002. Vegetables collected in the cultivated Andean area of Northern Chile: total and inorganic arsenic contents in raw vegetables. Journal of Agricultural and Food Chemistry 50: 642-647.

National Health and Family Planning of People's Republic of China (NFHPC). 2012. China Food Safety National Standard for Maximum Levels of Contaminants in Foods. http://www.cirsgroup.com/food/news/GB_27622012_maximum_levels_contaminants.html. Accessed 1 February 2015.

Notification of the Ministry of Public Health (No. 273) BE 2546. Food Standards Containing Contaminants (No. 2).

O'Neill, P. 1995. Arsenic. In Heavy Metals in Soils (B. J. Alloway, Ed.). Blackie Academic and Professional, London.

Sharma K.D., S. Karki., N.S. Thakur and S. Attri. 2012. Chemical composition, functional properties and processing of carrot - a review. Journal of Food Science and Technology 49(1): 22-32. DOI: 10.1007/ s13197-011-0310-7

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Shrestha, R.A., B. Lama., J. Joshi. and M. Sillanpaa. 2008. Effect of Mn(II) and Fe(II) on microbial removal of arsenic(III) Environmental Science and Pollution Research 15:303-307.

Shutsrirung, A. 2013. Selection of microorganism in highland for soil quality improvement in acid and high arsenic soils. Final report, Highland Research and Development Institute (Public Organization). Chiang Mai. 52 p.

Smith, A., C. Hopenhayn-Rich., M. Bates., H. Goeden., I. Hertz-Picciotto, H. Duggan., R. Wood., M. Kosnett and M. Smith. 1992. Cancer risks from arsenic in drinking water. Environmental Health Perspectives. 97: 259-267.

Smith, P.G., I. Koch and K.J. Reimer. 2008. Uptake, transport and transformation of arsenate in radishes (*Raphanus sativus*). Science of the total environment 390: 188-197.

Tu, S. and L.Q. Ma. 2004. Comparison of Arsenic and Phosphate Uptake and Distribution in Arsenic Hyperaccumulating and Non-hyperaccumulating Fern Journal Plant Nutrition 27:1227-1242.

Walinga, I., W.V. Vark, V.J.G. Houba and J.J. Vander Lee. 1989. Soil and Plant analysis a series of syllabi: part 7 plant analysis procedures. Department of Soil Science and Plant Nutrition. Wageningen Agricultural University, Netherland. 263p.

Woodruff, C.M., 1948. Determination of the exchangeable hydrogen and lime requirement of the soil by means of the glass electrode and a buffered solution. Soil Science Society of America, Proceedings 12: 141-142.

Woolson, E.A. 1983. Emissions, cycling and effects of arsenic in soil ecosystems. In Fowler, B. A. (Ed.), Biological and Environmental Effects of Arsenic. Topics in Environmental Effects of Arsenic. Topics in Environmental Health, 6, pp. 51-139. Elsevier, Amsterdam, 281 p.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved