

DISPERSION OF CADMIUM-RESISTANT BACTERIA IN CADMIUM -CONTAMINATED SOILS

AT MAE SOT DISTRICT, TAK PROVINCE

การกระจายตัวของแบคทีเรียต้านทานแคดเมียมในดินปนเปื้อนแคดเมียม  
บริเวณอำเภอสอด จังหวัดตาก

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## Abstract

Cadmium contamination in cultivated soils and rice grain grown in Mae Sot district, Tak Province, Thailand was brought to public attention in 2003. Cadmium is considered highly toxic to plants, animals and microbes. However, some bacteria have developed the ability to detoxify cadmium and other heavy metals there by conferring resistance on these microbes. The aims of this research were to study the quantities and dispersion of viable soil bacteria and cadmium-resistant found in cadmium contaminated soils at Mae Sot District. Cadmium-resistant bacteria were isolated and further investigated for their cadmium-resistance ability. Cadmium and zinc concentrations were found in 15 soil samples ranging from 0.20 to 1,021.75 mg/kg and 18.80

to 80,575.00 mg/kg, respectively. The maximum number of viable bacteria in soils collected in November 2007 and March 2008 were  $4.3 \times 10^8$  CFU/g and  $1.3 \times 10^7$  CFU/g soil, respectively. The smallest number of viable soil bacteria was found in soils containing a high concentration of cadmium. The number of cadmium-resistant bacteria that grew in TSA medium amended with 10 mM  $\text{CdCl}_2$  ranged from  $3.2 \times 10^4$  to  $1.3 \times 10^5$  CFU/g soil. 9 strains of cadmium resistant bacteria were selected for quantitative study of their level of resistance to cadmium toxicity. The results revealed that only 2 strains, TN6 and TM6, exhibited strong resistance to cadmium toxicity. 97.62% of TN6 and 84.62% of TM6 cells survived when placed in 3mM  $\text{CdCl}_2$ . These 2 strains of cadmium resistant bacteria, TN6 and TM6, were identified as *Alcaligenes* sp. and *Arthrobacter* sp.,

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respectively, through 16S rDNA sequencing.

**Keywords:** Cadmium-resistant bacteria, Cadmium-contaminated soil, *Alcaligenes*, *Arthrobacter*

## บทคัดย่อ

การปนเปื้อนของแคดเมียมในดินบริเวณพื้นที่เพาะปลูกและเมล็ดข้าวที่เก็บเกี่ยวจากอำเภอแม่สอด จังหวัดตากของประเทศไทย ได้รับความสนใจจากประชาชนในปี พ.ศ. 2546 แคดเมียมเป็นโลหะหนักที่มีความเป็นพิษสูงต่อพืช สัตว์ รวมทั้งจุลินทรีย์อย่างไรก็ตาม แบคทีเรียบางชนิดมีความสามารถในการต้านทานความเป็นพิษหรือกำจัดความเป็นพิษของโลหะหนักได้ ดังนั้นเป้าหมายของงานวิจัยนี้จึงทำการศึกษาจำนวนของแบคทีเรียที่มีชีวิตและแบคทีเรียที่ต้านทานแคดเมียมที่กระจายตัวอยู่ในดินที่ปนเปื้อนแคดเมียม บริเวณอำเภอแม่สอด จังหวัดตาก และนำแบคทีเรียต้านทานแคดเมียมที่คัดแยกได้มาทดสอบความสามารถในการต้านทานแคดเมียม ผลการทดลองพบว่า ตัวอย่างดินจำนวน 15 ตัวอย่าง มีปริมาณแคดเมียมและสังกะสีอยู่ในช่วง 0.20-1021.75 และ 18.80-80,575 มก./กก. ตามลำดับ ส่วนจำนวนแบคทีเรียที่มีชีวิตในดินที่เก็บในช่วงเดือนพฤศจิกายน 2550 และเดือนมีนาคม 2551 ที่พบมากที่สุดมีจำนวน  $4.3 \times 10^8$  และ  $1.3 \times 10^7$  CFU ต่อกรัมดินตามลำดับ และพบจำนวนแบคทีเรียในปริมาณต่ำในดินที่ปนเปื้อนแคดเมียมที่มีความเข้มข้นสูง สำหรับจำนวนแบคทีเรียต้านทานแคดเมียมที่สามารถเจริญได้ในอาหารเลี้ยงเชื้อ TSA ที่เติมแคดเมียมเข้มข้น 10 มิลลิโมลาร์อยู่ในช่วง  $3.2 \times 10^4$  ถึง  $1.3 \times 10^5$  CFU ต่อกรัมดิน และสามารถคัดเลือกแบคทีเรียต้านทานแคดเมียมได้จำนวน 9 สายพันธุ์ เมื่อนำมาศึกษาระดับการต้านทานความเป็นพิษต่อแคดเมียม พบว่ามีแบคทีเรียเพียง 2 สายพันธุ์คือ TN6 และ TM6 ที่มีความสามารถในการต้านทานความเป็นพิษของแคดเมียมได้ในระดับสูง โดยร้อยละของการรอดชีวิตต่อความเป็นพิษของแคดเมียมที่ความเข้มข้น 3 มิลลิโมลาร์ของแบคทีเรียสายพันธุ์ TN6 และ TM6 เท่ากับร้อยละ 97.62 และ 84.62 ตามลำดับ เมื่อทำการจำแนก

จีโนมแบคทีเรียทั้งสองสายพันธุ์โดยใช้วิธีการวิเคราะห์ลำดับเบส 16S rDNA พบว่า แบคทีเรียสายพันธุ์ TN6 และ TM6 จัดอยู่ในจีโนม *Alcaligenes* sp. และ *Arthrobacter* sp. ตามลำดับ

**คำสำคัญ:** แบคทีเรียต้านทานแคดเมียม, ดินปนเปื้อนแคดเมียม, *Alcaligenes*, *Arthrobacter*

## Introduction

Heavy metals are continuously released into the environment by natural means and as a result of human activity. Essential metals such as iron, copper, zinc, and manganese play important roles in biological systems. On the other hand, mercury, lead, and cadmium are non-essential elements. In contrast to these metals, which are toxic even in trace amounts, the essential metals tend to only produce toxic effects when the metal intake is in extreme excess<sup>(1)</sup>. High concentrations of heavy metals in the environment are known to be toxic to most organisms and these effects are increasingly being studied. In plants, such effects of heavy metal contamination may include growth inhibition, structural damage, and a decline of physiological and biochemical activities<sup>(2)</sup>.

In Mae Sot District of northwestern Thailand, cadmium contamination of cultivated soil and rice grain was brought to public attention by a joint investigation of International Water Management Institute (IWMI) and the Department of Agriculture (DOA) in 2003<sup>(3)</sup>. Soils in this area contain high concentrations of cadmium and zinc due to deposit of zinc ore. Cadmium is one of the

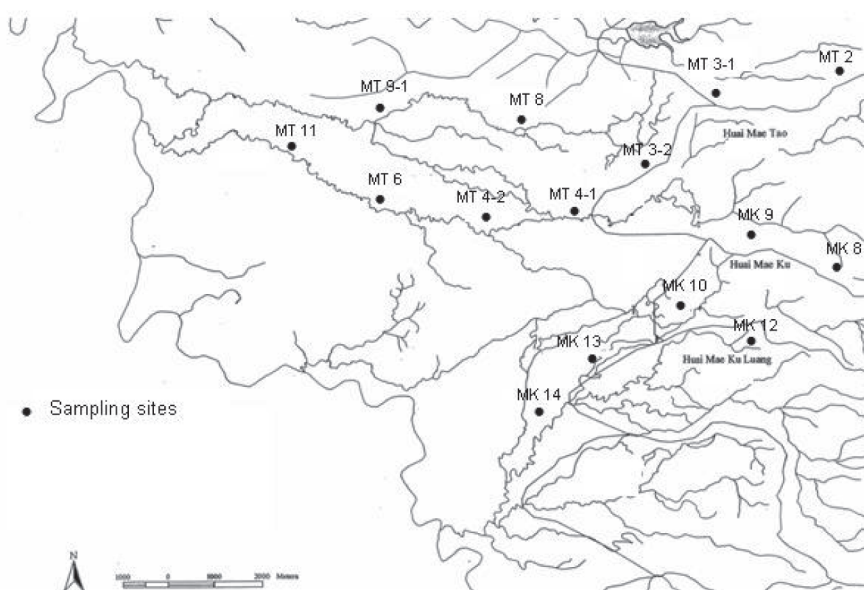
most toxic heavy metals to living organisms. In plants, generally speaking, and quite frequently in agriculturally important crops, Cadmium inhibits root and shoot growth, affects nutrient uptake and homeostasis, diminishes respiration activities through the inhibition of phosphatase and sulphatase, alters the microbial community structure, and reduces the level of dehydrogenase activity of and biomass produced by soil microbes <sup>(4, 5)</sup>. However, some bacteria have acquired resistance to the detrimental effects of Cadmium through heavy metal detoxification. Such microorganisms have evolved a number of heavy metal tolerance mechanisms including active efflux, extra- and intracellular complexation, extracellular precipitation, crystallization, and transformation<sup>(6)</sup>.

This study focused on assessing the distribution of viable bacteria and cadmium-resistant bacteria in cadmium-contaminated soils in Mae Sot District of Tak Province. Additionally, the level of cadmium resistance in isolated cadmium-resistant bacteria was investigated. Cadmium-resistant bacteria were utilized for microbial remediation of cadmium contamination in water, sediment and soils.

## Materials and Methods

### Study Area and Soil Sampling

The study areas included the flood plains of Mae Tao and Mae Ku Creeks in Mae Sot District, Tak Province, and neighboring areas, covering an area of approximately 110 square kilometers that encompassed the 3 sub-districts of Mae Ku, Mae Tao and Pra Taad Padaeng. The 15 soil samples were collected from different sites (Figure 1) based on the concentration of cadmium contamination in the soil. Soil samples were collected from 8 sites at Mae Tao Creek and 7 sites at Mae Ku Creek during the early of November 2007 and the late of March 2008 as the representative periods of rainy and summer seasons, respectively. Each 10g soil sample was collected with a sterilized spatula from a depth of 10 cm below the ground surface and stored in a sterilized bag for microbiological analysis. An additional 20g of each soil sample was collected at the same depth for analysis of their chemical and physical properties.



**Figure 1** Map of soil sampling sites at Mae Sot District, Tak Province

### **Analysis of Soil Physical and Chemical Properties**

Soil samples were dried in a hot air oven at 60°C for 12 hr. Subsequently, it was ground and filtered through a 2mm mesh sieve. Soil samples were tested for physical and chemical parameters, including soil texture and moisture by the hydrometer and saturation percentage methods, respectively<sup>(7, 8)</sup>. Soil pH was analyzed by glass electrode pH meter<sup>(7)</sup>. Soil organic matter was analyzed by the Walkley-Black method<sup>(9)</sup>. Soil Cation Exchange Capacity (CEC) was analyzed by the ammonium saturation method<sup>(10)</sup>. Soil samples were digested and analyzed for total concentrations of cadmium

and zinc by using Flame Atomic Absorption Spectrometer (FAAS).

### **Enumeration of Total Viable Bacteria and Cadmium Resistant Bacteria in Soils**

Soil samples were massed to 1g and suspended in 9.0mL of TSB medium. Each soil suspension was serially ten-fold diluted at  $10^{-1}$  to  $10^{-8}$ . Soil suspensions were collected at  $10^{-4}$ ,  $10^{-6}$  and  $10^{-8}$  and spread with a sterile glass rod onto TSA plate for enumeration of total viable bacteria. Soil suspensions were also collected at  $10^{-3}$ ,  $10^{-5}$  and  $10^{-7}$  and were spread onto TSA plate amended with 10mM  $\text{CdCl}_2$  for enumeration of cadmium-resistant bacteria. All plates were incubated at

28 °C in the dark for 24 hours. Colonies appearing on the agar plates were counted and calculated as CFU per gram of soil. This experiment was done in triplicates.

### Isolation and Screening of Cadmium Resistant Bacteria from Soils

5g soil samples were suspended in 15mL of TSB medium amended with 3mM  $\text{CdCl}_2$ . Then, the soil suspension samples were shaken on incubator shaker (150rpm) at 28 °C in the dark for 24 hr. Soil suspensions were transferred into 15mL of fresh TSB media amended with 5 and 10mM  $\text{CdCl}_2$ . Soil suspensions were spread with a sterile glass rod onto TSA plates amended with 10mM  $\text{CdCl}_2$ . Bacterial colonies appearing on the TSA plates amended with 10mM  $\text{CdCl}_2$  were selected for further study based on the differences in colony morphology. Bacterial colonies were streaked on fresh TSA plates amended with 5mM  $\text{CdCl}_2$  in order to maintain the cadmium resistant ability of isolated bacteria. A single colony of each isolate was re-streaked at least 2 times to ensure pure culture isolation. Pure culture of each isolate was sub-cultured on TSA slant and kept at 4 °C.

### Qualitative Analysis of Cadmium Resistance Levels in Isolated Bacteria from Soils

Cadmium resistance levels in selected bacteria from method No. 2.4 were determined by using growth inhibition zone and plate

sensitivity assays with some procedural modification<sup>(11, 12)</sup>. For the inhibition zone assay, exponential phase cells ( $\text{OD}_{600} \sim 0.5$  after 4 hours of growth) were mixed with 10mL of pre-warmed (5 °C) top agar (TSA agar containing 0.7% agar) and overlaid on top of TSA agar plates (14 cm-diameter Petri dishes and poured with 50 ml of TSA agar). The agar plates were left at room temperature for 15 minutes to let the top agar solidify. A 5 $\mu\text{L}$  aliquot of each 0.5, 1.0, and 2.0M  $\text{CdCl}_2$  was applied onto a 6-mm diameter of paper discs made from Whatman filter paper and subsequently placed on the lawn of bacterial cells. The diameters of growth inhibition zones in cadmium-resistant bacteria were measured after an overnight incubation at 28 °C. Both complete (inhibition zone including the 6-mm diameter of paper disc) and partial (less dense zone) growth inhibition zones were recorded.

For plate sensitivity assay, exponential phase cells ( $\text{OD}_{600} \sim 0.5$  after 4 hr of growth) were serially ten-fold diluted at  $10^{-1}$  to  $10^{-6}$ . A 10 $\mu\text{L}$  suspension sample at  $10^{-1}$  to  $10^{-6}$  was dropped onto a TSA plate (control) and TSA plates amended with 2 and 3mM  $\text{CdCl}_2$ . All plates were incubated at 28 °C in the dark for 24 hr. Colonies appearing after incubation on the agar plates were counted and the number of surviving cells was calculated. The percentage of surviving cells is defined as the number of colony forming units (CFU) per mL recovered after treatment divided by the number of CFU prior to treatment, all multiplied by 100. The survival curves were

determined by plotting the percentages of surviving cells versus the time of cultivation.

### **Morphological Study and Identification of Cadmium Resistant Bacteria**

Each bacterial strain was streaked on a fresh TSA plate and colony characteristics were observed including distance from surface, margin and color after incubation at 28 °C for 24-48 hours. Each strain was determined to be Gram-positive or Gram-negative by Gram stain and observed cell morphology by light microscope at 1,000X. Genus of isolated bacteria was determined by 16S rDNA sequencing analysis<sup>(13)</sup>. Approximately 1500-bp of 16S rDNA was amplified in a thermocycler by using universal primers of 27f (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3'). PCR product was directly sequenced by a BigDye terminator cycle sequencing kit on an ABI 310 automated DNA sequencer. Homology of the 16s rDNA sequence of isolate was analyzed by using BLAST program from *Genbank database*.

### **Statistical Analysis**

The physical and chemical characteristics of soil samples, growth curve of isolated bacteria, and cadmium resistance level in each

bacterial strain were statistically analyzed by using mean ( $\bar{X}$ ) and standard deviation (SD). T-test was used to compare the mean number of total viable bacteria in cadmium-contaminated soil in November 2007 versus March 2008. Statistical analysis of data was carried out with the SPSS statistical program.

## **Results and Discussion**

### **Physical and Chemical properties of soils**

Of the 15 soil samples collected and analyzed for their physical and chemical properties, most had the texture of clay and the moisture percentage ranged from 3.13% to 19.42% (Table 1). The mean pH of the soil samples was neutral while the percentages of organic matter contained in the samples were slightly low, ranging from 1.25%-6.72%. Cation exchange capacity of soil samples was moderately low to high (6.36-27.5 cmol/kg). Confirming the report from Al-Khashman *et al.*<sup>(14)</sup>, the upper part of the soil surface was highly enriched with heavy metals (iron, nickel, zinc, lead and copper). This might be attributed to the mobility of heavy metals, which is strongly influenced by many factors such as pH, Eh and the stability of minerals.

**Table 1** Physical and chemical properties of soils

Sampling site	Soil Texture	CEC (cmol/kg)	OM (%)	Moisture (%)	pH	Cadmium (mg/kg)	Zinc (mg/kg)
MT2	SCL	7.4	2.43	3.13	6.90	1021.75	65,925.00
MT3-1	L	27.5	6.72	14.40	7.02	550.25	80,575.00
MT3-2	C	24.9	3.77	11.19	6.91	2.15	695.00
MT4-1	SCL	8.1	2.90	8.38	7.83	17.08	1,085.00
MT4-2	SCL	8.1	3.12	3.62	7.73	62.75	2,582.50
MT6	C	18.4	3.47	13.67	7.58	130.30	7,747.50
MT8	SCL	17.7	2.72	8.25	6.62	17.45	742.50
MT9-1	C	9.7	2.49	4.37	7.96	45.78	1762.50
MT11	CL	17.0	3.02	10.29	6.12	5.28	296.25
MK8	C	6.36	1.60	10.41	6.36	0.40	31.25
MK9	C	7.15	1.53	16.74	7.15	0.70	94.50
MK10	C	7.84	2.12	19.42	7.84	0.80	243.50
MK12	C	7.42	1.25	14.35	7.42	0.40	18.80
MK13	C	6.79	1.25	19.26	6.79	0.20	29.55
MK14	SiCL	7.78	2.49	17.79	7.78	1.00	300.50

*Remark:* C = Clay; L = Loam; CL = Clay Loam; SCL = Sandy Clay Loam; SiCL = Silty Clay Loam

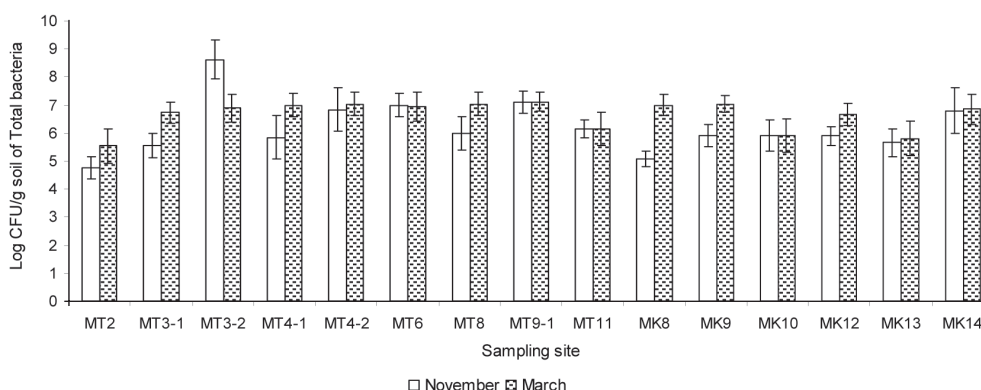
Cadmium and zinc concentrations in the 15 soil samples ranged widely from 0.20 to 1,021.75 mg/kg and 18.80 to 80,575.00 mg/kg, respectively. Soil sample cadmium concentrations from some sampling sites were found to be above the permissible limits of soil quality standard for agricultural use in Thailand (37 mg/kg)<sup>(15)</sup>. The highest cadmium concentration was found at sampling site MT2 at Mae Tao Creek. In terms of zinc, the highest soil concentration was found at sampling site MT3-1 at Mae Tao Creek and also exceed than the standard of zinc concentration in soil (600 µg/kg)<sup>(16)</sup>. In contrast, cadmium concentrations in soil collected from Mae Ku Creek sites (MK8-MK14) ranged from 0.2 to 1.0 mg/kg, well-below the standard.

#### **Quantity of Total Viable Bacteria and Cadmium Resistant Bacteria in Soils**

The number of total viable bacteria in 15 soil samples collected during the early of November 2007 and late-March 2008 ranged from  $5.6 \times 10^4$  to  $4.3 \times 10^8$  CFU/g soil and  $3.5 \times 10^5$  to  $1.3 \times 10^7$  CFU/g soil, respectively (Figure 2). The number of total viable bacteria found in highly

cadmium-contaminated soil (e.g., MT2) collected in the early of November 2007 and the late of March 2008 was  $5.6 \times 10^4$  CFU/g soil and  $3.5 \times 10^5$  CFU/g soil, respectively. In addition, there was no statistically significant difference between the number of heterotrophic culturable soil bacteria in November 2007 and March 2008 as assessed by T-test at  $p < 0.05$ . This result indicated that the season was no affecting the number of heterotrophic soil bacteria in cadmium-contaminated soils. The number of heterotrophic soil bacteria depend on some soil chemical and physical properties<sup>(6)</sup>. According Malik *et al.*<sup>(17)</sup>, the total aerobic heterotrophic count was found to be  $5.39 \times 10^7$  CFU/g of agricultural soil, whereas it was  $3.14 \times 10^7$  CFU/g of industrial soil. The number of total viable bacteria in soil contaminated with a high concentration of cadmium was low. As reported by Perkiomaki and Fritze<sup>(18)</sup>, the addition of cadmium to the soil can disturb the nutrient cycling of forest ecosystems because of its potential toxic effects on microbes. Cadmium had a greater inhibiting effect on microbes than did lead<sup>(19)</sup>.

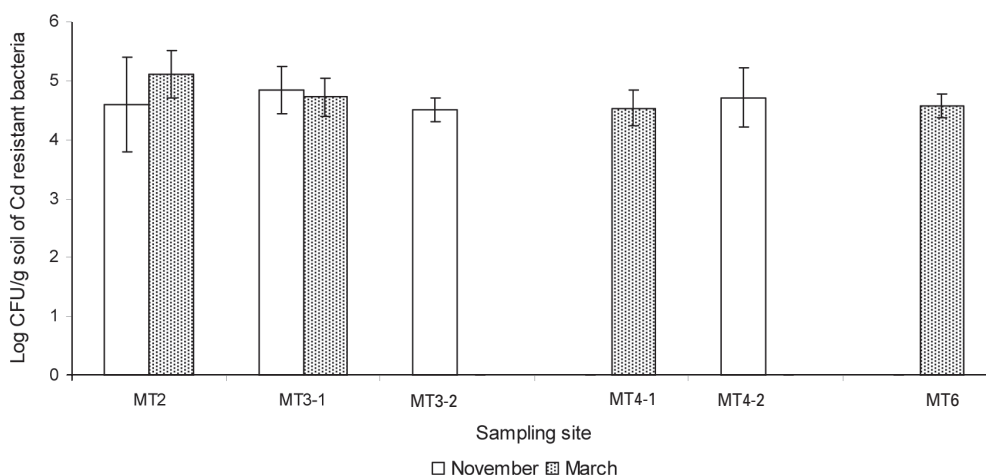




**Figure 2** The number of total viable bacteria in soils collected in November 2007 and March 2008

However, a huge number of total viable soil bacteria could survive in cadmium contaminated soils even though cadmium concentration in soils was higher than standard of soil quality. Microbes apply various types of resistance mechanisms in response to heavy metals <sup>(6)</sup>. The number of cadmium-resistant bacteria from 15 soil samples collected during the early of November 2007 and the late of March 2008 and grown on TSA amended with 10mM  $\text{CdCl}_2$  ranged from  $3.2 \times 10^4$  to  $7.0 \times 10^4$  CFU/g soil and  $3.4 \times 10^4$  to  $1.3 \times 10^5$  CFU/g soil, respectively (Figure 3). No cadmium-resistant bacteria were found in samples collected neither from all sampling sites in Mae Ku Creek (except sampling site MK8) nor some sampling sites in Mae Tao Creek (sampling site MT3-2, MT4-1, MT8 and MT11) due to the lower concentration of cadmium in soils. This finding reveals that soil bacteria in highly cadmium-contaminated soil had more tolerance to cadmium toxicity than soil bacteria found in soil with low cadmium-

contamination. Moreover, the large number of soil bacteria found to be resistant to cadmium toxicity ( $10\text{mM CdCl}_2$ ) was found in highly cadmium-contaminated soil (sampling site MT2) collected during both sampling periods. The study by Shuqing *et al.* <sup>(19)</sup> found that the number of soil microbes in cadmium concentrations of 0, 1, 5, 10, 50, 100 and  $200\text{mg/kg}$  were  $6.3 \times 10^{10}$ ,  $8.9 \times 10^{10}$ ,  $4.7 \times 10^{10}$ ,  $3.4 \times 10^{10}$ ,  $2.7 \times 10^{10}$ ,  $2.4 \times 10^{10}$ ,  $1.4 \times 10^{10}$  CFU/g, respectively. As reported by Ryan *et al.* <sup>(20)</sup>, the percentage of the number of metal-tolerant strains was calculated in relation to the total viable count. In the case of each site, 79% of strains isolated from site A (metal-contaminated site) were resistant to either copper, zinc or arsenic. The level of metal resistance at site B (metal-uncontaminated site) ranged from 30%-35% in the cases of copper, zinc and arsenic. In addition, the number of heavy metal-resistant bacteria could use for an indicator of the levels of heavy metal contamination <sup>(20)</sup>.



**Figure 3** The number of cadmium resistant bacteria grown on TSA plate supplemented with 10 mM  $\text{CdCl}_2$  in cadmium contaminated soils

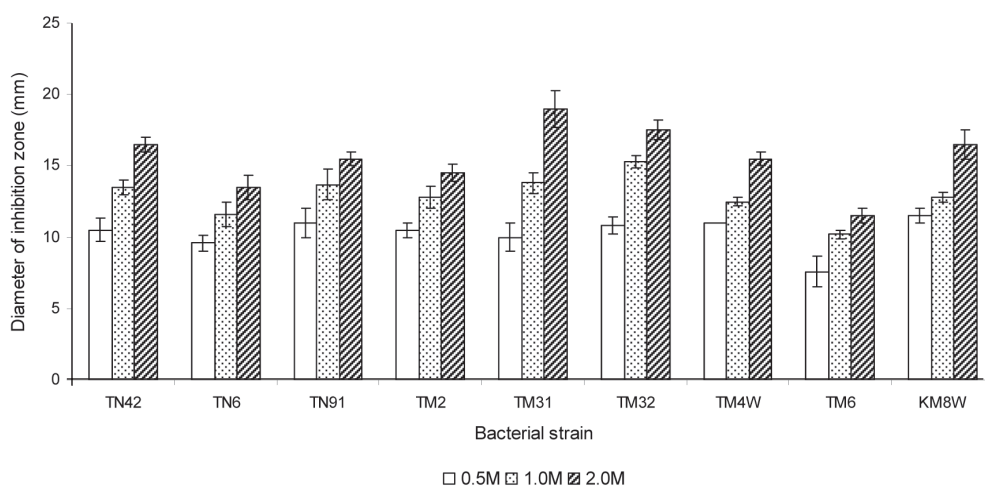
### Isolation of Cadmium Resistance Bacteria and their Resistance Levels against Cadmium Toxicity

The finding that cadmium resistant bacteria could survive in the soils that contaminated with high concentration of cadmium prompted us to isolate and screen the potent cadmium resistant bacteria from cadmium contaminated soils. A total of 9 bacterial strains, 3 of them isolated from soil samples collected during the early of November 2007 (sampling site MT4-2, MT6 and MT9-1) and 5 of them isolated from soils collected during the late of March 2008 (sampling site MT2, MT3-1, MT3-2 and MK8), were selected and assessed their resistance levels against cadmium toxicity by growth inhibition and plate sensitivity assays. Growth inhibition and plate sensitivity assays showed that most of the selected bacterial strains were isolated

from highly cadmium-contaminated soil. Two bacterial strains, TN6 and TM6, exhibited higher resistance to  $\text{CdCl}_2$  than other bacterial strains. Both TN6 and TM6 bacterial strains were isolated from cadmium contaminated soil (sampling site MT6) at Mae Tao Creek collected during the early of November 2007 and the late of March 2008. Soli sample in site MT6 contained high concentration of cadmium (130.30 mg/kg soil). For the same chemical, a bacterial strain with a larger clear zone correlated with higher sensitivity to the chemical as compared to strains with smaller clear zone diameter<sup>(21)</sup>. The inhibition zone diameters around each of the 6-mm discs containing 10  $\mu\text{L}$  of 2M  $\text{CdCl}_2$  of bacterial strains TN6 and TM6 were 13.5 mm and 11.5 mm, respectively (Figure 4). As reported by Chandy<sup>(22)</sup>, the relative sensitivity of marine bacteria isolated from the Al-Jubail coast to

different metals, in terms of the growth inhibition zone, was studied. At 10  $\mu\text{g}$  cadmium, a diameter of growth inhibition zone was about 10-15 mm. This finding indicates that some cadmium-resistant bacteria increase resistance under higher levels of cadmium toxicity because cadmium could induce the alteration of bacterial response or resistance mechanisms. However, cadmium-resistant bacteria could not alter or less

ability to alter their resistant potential against very high concentration of cadmium (sampling site MT2 and MT3-1) due to its toxic effect of cadmium. Heavy metal-contaminated site was the preferable environment for gene transfer of multiple metal resistant determinants in metal-resistant bacteria isolated to other bacterial isolates<sup>(20)</sup>.



**Figure 4** Diameter of growth inhibition zone against various concentrations of  $\text{CdCl}_2$  in cadmium resistant bacteria

In addition, experiments were then performed to find out the percentages of survival cells of the potent cadmium resistant bacteria against cadmium toxicity. The percentage of TN6 and TM6 cells surviving after cultivation on TSA plate amended with 3mM  $\text{CdCl}_2$  was 97.62% and 84.62%, respectively. This result indicated that bacterial strains TN6 and TM6 had the highest ability to resistant against cadmium toxicity. Both bacterial strains might use either

specific or non-specific resistance mechanism against cadmium toxicity.

### Genus Identification of the Potent Cadmium Resistant Bacteria

The genus of TN6 and TM6, two highly cadmium-resistant bacterial strains, were identified by 16S rDNA sequencing analysis as *Alcaligenes* sp. and *Arthrobacter* sp., respectively. The study by Trajanovska et al. reported that

resistance to a range of heavy metal ions was determined for lead-resistant and other bacteria that had been isolated from a battery-manufacturing site contaminated with high concentrations of lead<sup>(23)</sup>. Several isolates of Gram-positive (belonging to the genera *Arthrobacter* and *Corynebacterium*) and Gram-negative (*Alcaligenes* spp.) bacteria were resistant to lead, mercury, cadmium, cobalt, zinc and copper, although the levels of resistance to the different metal ions were specific to each isolate.

## Conclusion

This research focused on the quantity of viable soil bacteria and cadmium-resistant bacteria found in cadmium-contaminated soils at Mae Sot District, Tak Province. The resistance capabilities of selected soil bacteria against cadmium toxicity were also investigated. As compared to soil with a lower cadmium concentration a lower number of viable bacteria were found in soils containing high levels of cadmium (MT2) in the early of November 2007 ( $5.6 \times 10^4$  CFU/g soil) and the late of March 2008 ( $3.5 \times 10^5$  CFU/g soil) was found. The number of cadmium-resistant bacteria in high cadmium-contaminated soils (sampling site MT2) collected during November 2007 and March 2008 was  $3.9 \times 10^4$  and  $1.3 \times 10^5$  CFU/g soil, respectively. The results indicate that a high number of cadmium-resistant bacteria may be found in highly cadmium- contaminated soils. Most bacteria

isolated from such soil had a greater ability to resist cadmium toxicity than soil bacteria isolated from soil with low cadmium contamination. The bacterial strain TN6 and TM6 showed the strongest resistance ability against cadmium toxicity and they were identified as *Alcaligenes* sp. and *Arthrobacter* sp., respectively. This finding suggests that selected cadmium-resistant bacteria might be useful for microbial remediation of cadmium in contaminated soils.

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