CHAPTER IV

RESULTS

4.1 ISOLATION, SCREENING AND SELECTION OF COPPER-RESISTANT BACTERIA

4.1.1 Copper-Resistant Bacterial Isolates

Three hundreds and fifty bacterial strains resisted to 400 μg/ml Cu in 1/3-strength TSA medium were isolated. Resistances to 500, 600 and 700 μg/ml Cu were found in 86.57, 11.43 and 2 percent, respectively, of the resistant isolates as shown in **Table 4.1**. None of them was resistant to 800-1,000 μg/ml Cu. Seven Cu-resistant isolates were selected, namely CuR-4, CuR-14, CuR-24, CuR-25, CuR-32, CuR-38 and CuR-40. From **Table 4.2**, the first five bacterial strains were rod shape, gram-negative and identified as ***Pseudomonas* sp.**, and they were isolated from soils or sludges collected from metal industries or industrial treatment plants. CuR-38 strain which was isolated from soil collected from the copper rod industry, was rod shape, gram-negative and identified as ***Zoogloea* sp.**, and the last one, CuR-40, which was isolated from soil collected from the same place as CuR-38, was rod shape but gram-positive, spore-forming and identified as ***Bacillus* sp.**

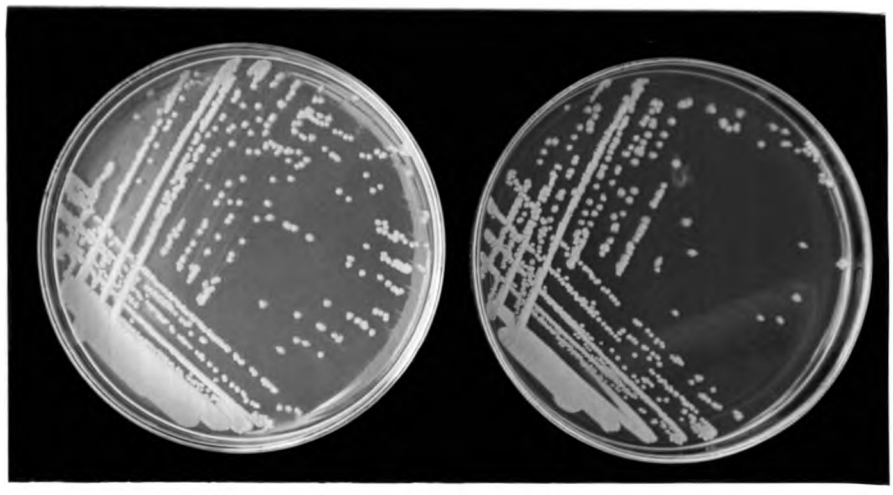
The colonial and cell characteristics of the seven selected bacterial isolates are shown in **Figure 4.1-4.7**.

Table 4.1 Copper resistance in 350 strains bacterial

Cu conc. (μg/ml)	No. of Strains	%
500	303	86.57
600	40	11.43
700	7	2.00
800-1,000	0	0
Total	350	100

Table 4.2 Some characteristics and identification of seven strains of the copper-resistant bacterial isolates

Bacterial Isolates	Sources (Sampling Site)	Characteristic of		Identified as
		Colony	Morphology	
CuR-4	Soil,(GENCO, Bangkok)	~3 mm in diameter, white and convex	Rod-shape, gram-negative, 0.5 by 1.5μm	***Pseudomonas* sp.**
CuR-14	Sludge,(Plating industry, Bangkok)	~4 mm in diameter, white and convex	Rod-shape, gram-negative, 0.7 by 3.0μm	***Pseudomonas* sp.**
CuR-24	Soil,(Plating industry, Bangkok)	~3 mm in diameter, white and convex	Rod-shape, gram-negative, 0.8 by 2.3μm	***Pseudomonas* sp.**
CuR-25	Soil,(Plating industry, Bangkok)	~3 mm in diameter, white and convex	Rod-shape, gram-negative, 1.0 by 1.5μm	***Pseudomonas* sp.**
CuR-32	Sludge,(Zip industry, Samutsakorn)	~3 mm in diameter, white and convex	Rod-shape, gram-negative, 0.8 by 1.7μm	***Pseudomonas* sp.**
CuR-38	**Soil,(Thai Cu rod industry, Samut Prakan)**	**~4 mm in diameter, white and convex**	**Rod-shape, gram-negative, 0.8 by 1.7μm**	***Zoogloea* sp.**
CuR-40	**Soil,(Thai Cu rod industry, Samut Prakan)**	**~4 mm in diameter, white and convex**	**Rod-shape, gram-positive, 0.7 by 1.6μm, endospore-forming**	***Bacillus* sp.**



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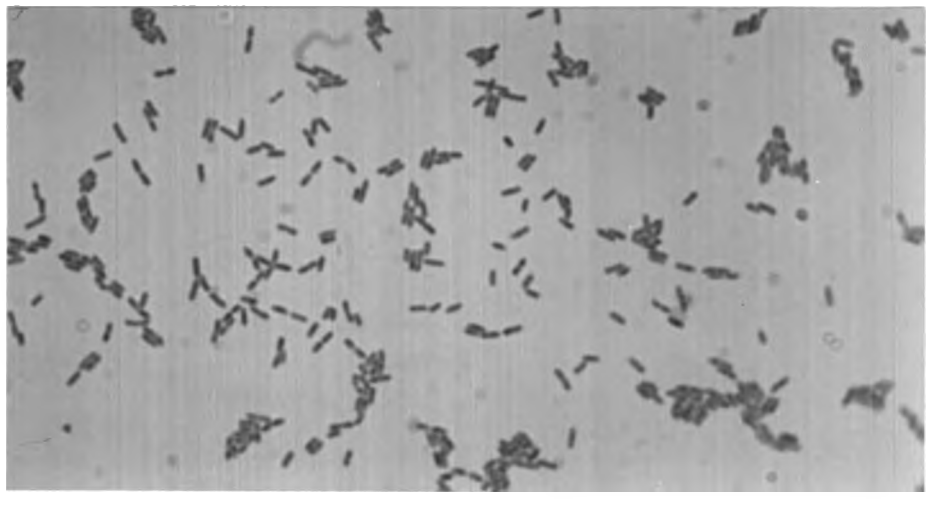
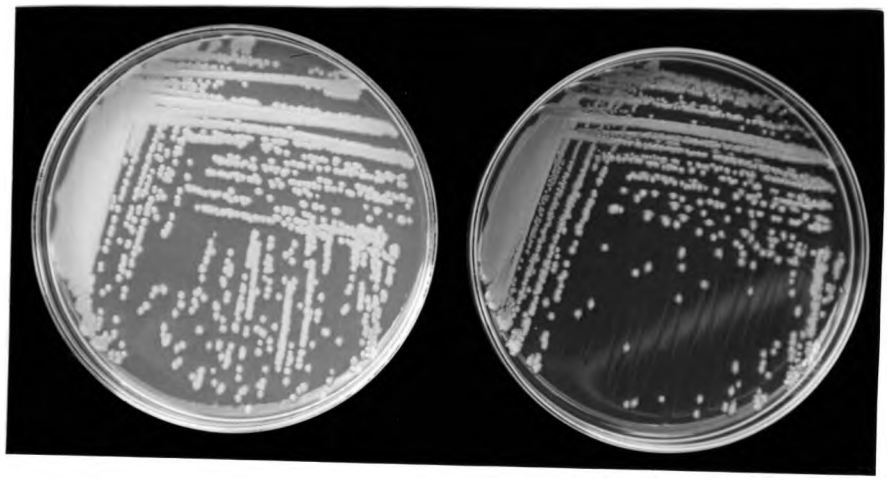


(c)

Figure 4.1 Colonial characteristics of copper-resistant bacterial strains CuR-4 (***Pseudomonas* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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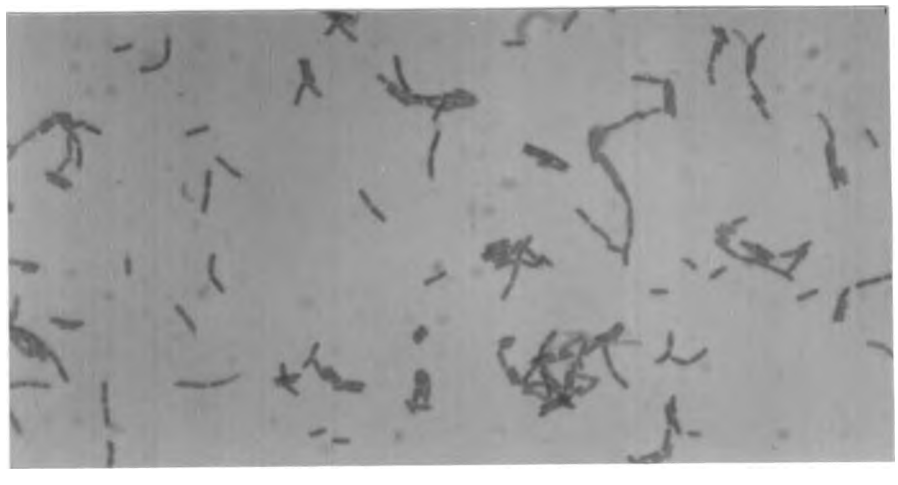
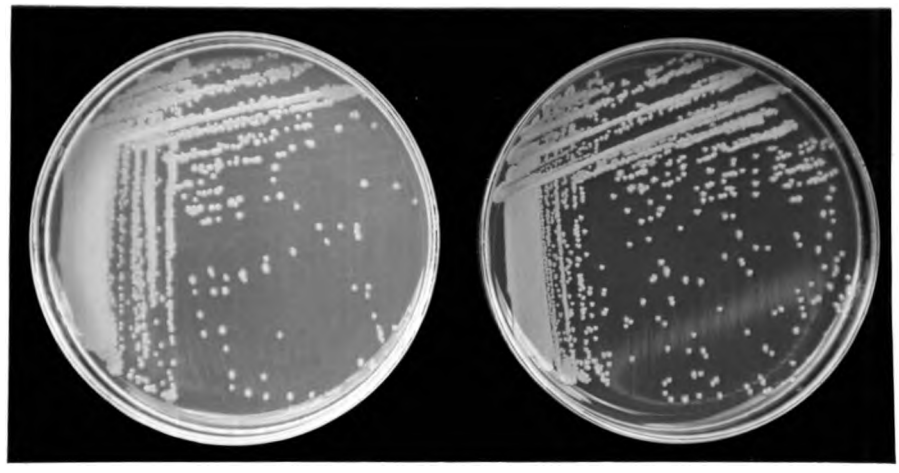


(c)

Figure 4.2 Colonial characteristics of copper-resistant bacterial strains CuR-14 (***Pseudomonas* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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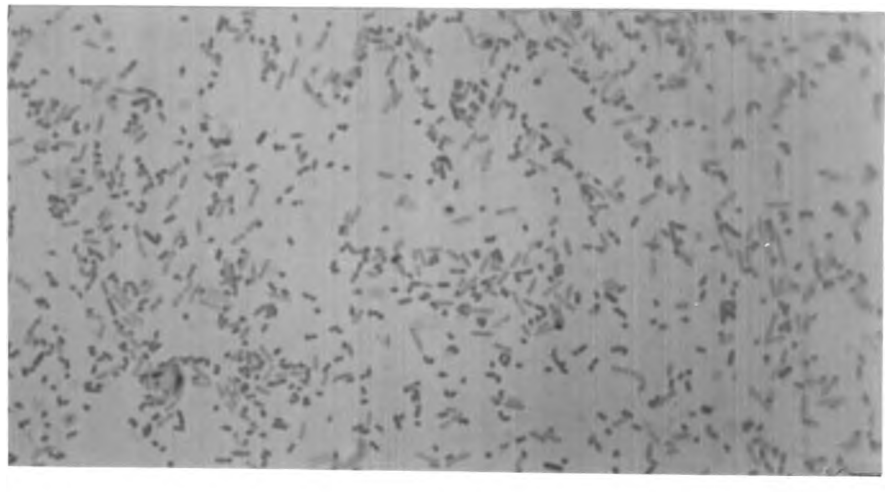

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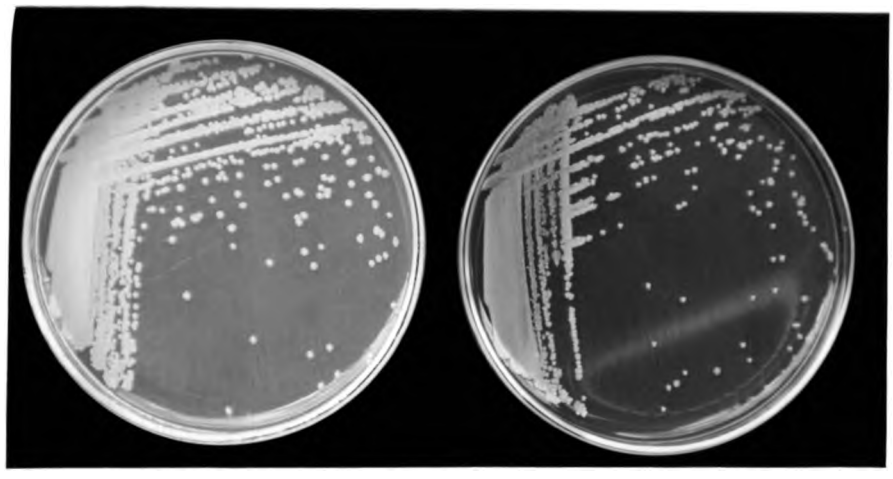
Figure 4.3 Colonial characteristics of copper-resistant bacterial strains CuR-24 (***Pseudomonas* sp.**) grown on TSA (a) and TSA containing 700 µg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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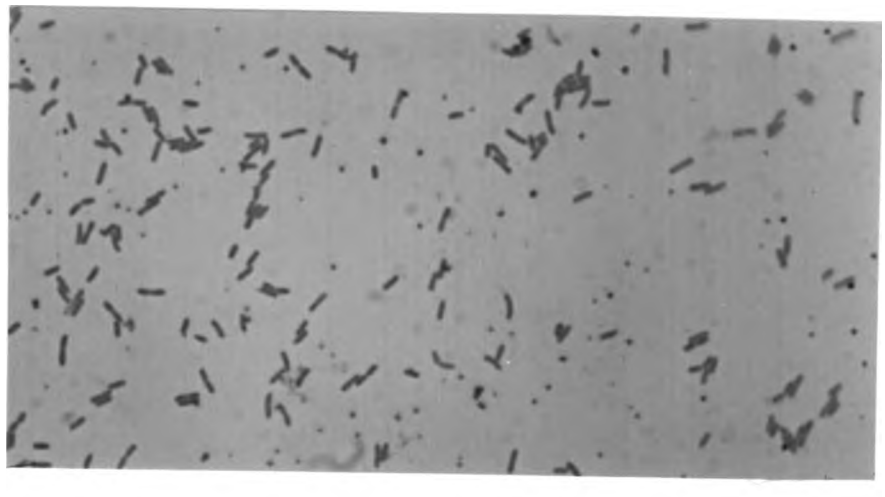
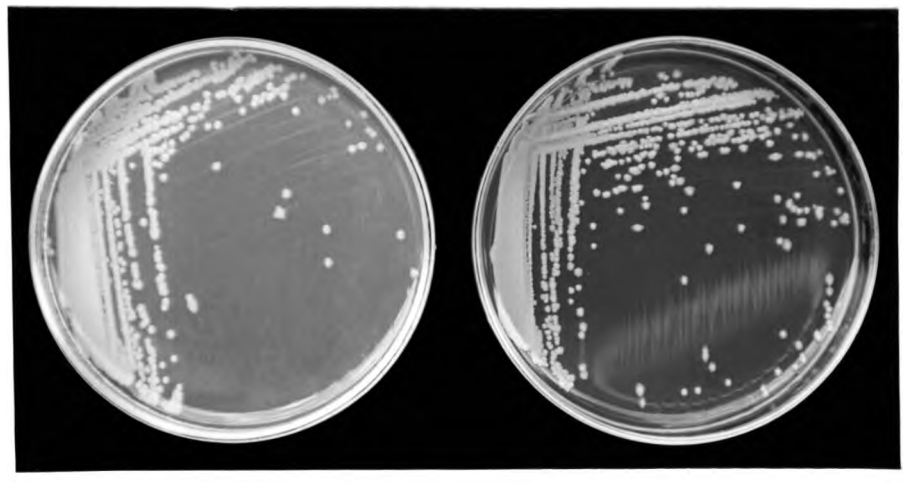


(c)

Figure 4.4 Colonial characteristics of copper-resistant bacterial strains CuR-25 (***Pseudomonas* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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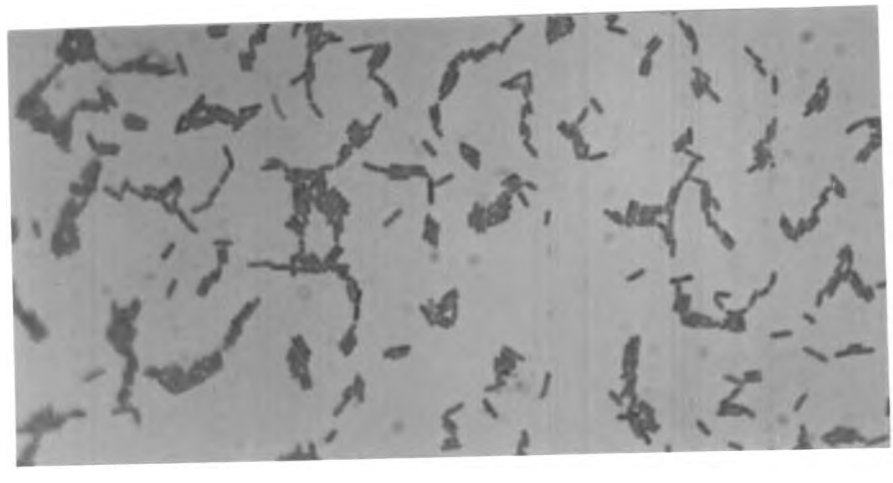
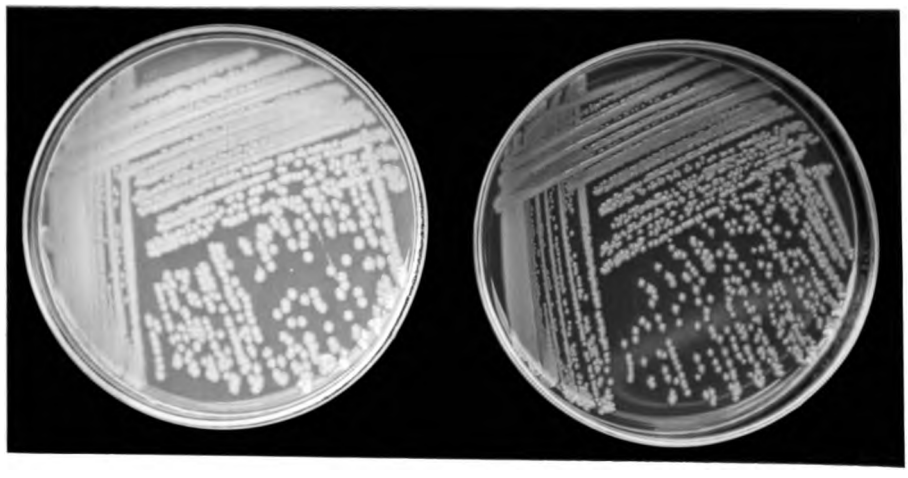


(c)

Figure 4.5 Colonial characteristics of copper-resistant bacterial strains CuR-32 (***Pseudomonas* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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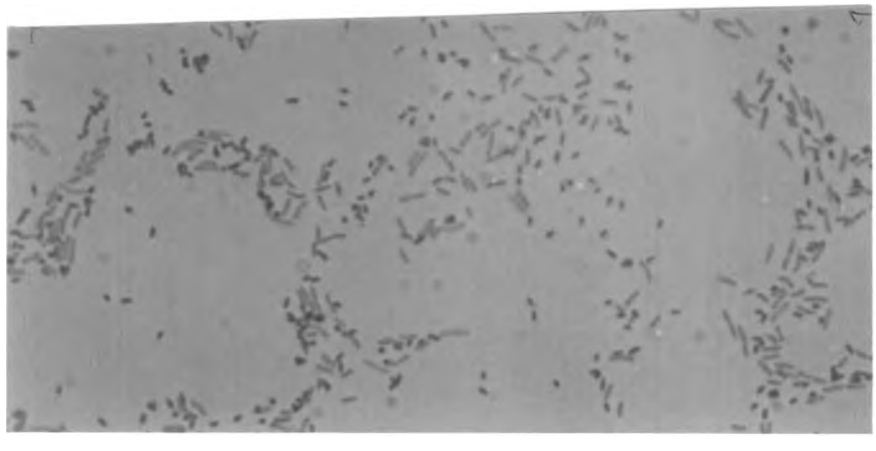
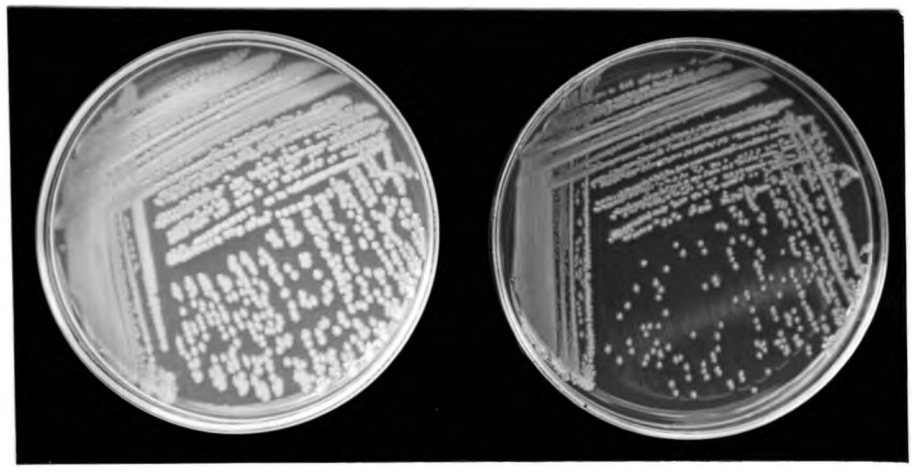


(c)

Figure 4.6 Colonial characteristics of copper-resistant bacterial strains CuR-38 (***Zoogloea* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)



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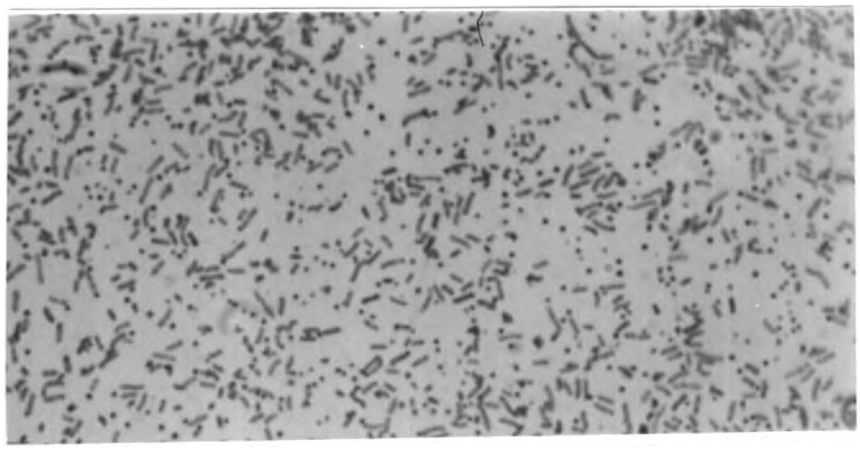

(c)

Figure 4.7 Colonial characteristics of copper-resistant bacterial strains CuR-40 (***Bacillus* sp.**) grown on TSA (a) and TSA containing 700 μg/ml copper (b), incubated at 37°C for 24 hr., and gram staining (c)

4.1.2 Stability of Copper Resistance and EPS Production of the Selected Bacterial Isolates

The results of the stability of Cu resistance and EPS production of the seven selected bacterial isolates are briefly summarized in **Table 4.3**. All of the test bacterial isolates were able to maintain the highest Cu resistance (700 μg/ml) after, at least, 20 times of subculturing in TSA containing small amount of Cu. All of the five selected isolates identified as ***Pseudomonas*** **sp.**, were not able to produce exopolysaccharide, but CuR-38 or ***Zoogloea*** **sp.** and CuR-40 or ***Bacillus*** **sp.** were able to produce EPS.

4.2 RESISTANCE OF COPPER-RERSISTANT BACTERIAL ISOLATES TO OTHER METAL IONS

All of seven strains of the Cu-resistant bacterial isolates were found to be resistant to a number of other heavy metals, i.e., Zn, Mn and Cd, but none of them were able to resist to Ag, Ni and Cr; detailed result is summarized in **Table 4.4**. The bacterial strains CuR-4, CuR-14, CuR-24, CuR-25 and CuR-32 (all are ***Pseudomonas*** **sp.**) were resistant to the same resistance levels of Zn, Mn and Cd, i.e., 500, 300 and 500 μg/ml, respectively; strain CuR-14 resisted Zn, Mn and Cd at the same resistance level, i.e., 500 μg/ml. Similarly, CuR-38 and CuR-40 were able to grow in medium containing Zn or Mn or Cd, individually, at the same concentration, i.e., 300 μg/ml. Both of them were isolated from the same site (copper rod industry), but different in identification, i.e., ***Zoogloea*** **sp.** and ***Bacillus*** **sp.**

Table 4.3 Stability of copper resistance after 20 times of repeated subculturing and EPS production in seven strains of the copper-resistant bacterial isolates

Bacterial Isolates		Resistance to Copper (μg/ml)	Stability of resistance*	EPS production
Strains	**Identified as**			
CuR-4	***Pseudomonas* sp.**	700	Positive (20)	Negative
CuR-14	***Pseudomonas* sp.**	700	Positive (>20)	Negative
CuR-24	***Pseudomonas* sp.**	700	Positive (20)	Negative
CuR-25	***Pseudomonas* sp.**	700	Positive (20)	Negative
CuR-32	***Pseudomonas* sp.**	700	Positive (20)	Negative
CuR-38	***Zoogloea ramigera***	**700**	**Positive (>20)**	**Positive**
CuR-40	***Bacillus licheniformis***	**700**	**Positive (>20)**	**Positive**

* After at least 20 times of repeated subculturing

Table 4.4 Resistance to other heavy metals was found in the bacterial isolates

Strains	Resistant Concentration of Heavy Metal (μg/ml)						
	Cu	**Zn**	**Mn**	**Cd**	**Ag**	**Ni**	**Cr**
CuR-4	700	500	300	500	-	-	-
CuR-14	700	500	500	500	-	-	-
CuR-24	700	500	300	500	-	-	-
CuR-25	700	500	300	500	-	-	-
CuR-32	700	500	300	500	-	-	-
CuR-38	**700**	**300**	**300**	**300**	**-**	**-**	**-**
CuR-40	**700**	**300**	**300**	**300**	**-**	**-**	**-**

4.3 EFFECT OF pH AND TEMPERATURE ON VIABLE COUNTS OF THE BACTERIAL ISOLATES

The optimum pH and temperature of those selected bacterial isolates were found to be 7 and 37°C, respectively (summarized in **Table 4.5**). All pH level 10, the reduction of number of viable cells was higher than at the pH level 4, it may imply that the effect of alkaline condition on growth is stronger than acidic condition, but the effect of temperature at higher (40°C) or lower (30°C) levels was not as strong as pH level.

In EPS-producing bacterial isolates (CuR-38 and CuR-40), the effects of pH and temperature were shown in **Figure 4.8-4.9**.

4.4 GROWTH OF COPPER-RESISTANT AND EPS PRODUCING BACTERIAL ISOLATES IN COPPER-CONTAINING MEDIUM

The results of growths of the Cu-resistant and EPS-producing bacterial strains, CuR-38 and CuR-40, were shown in **Figure 4.10** and **Figure 4.11**. Comparing growth on TSB (pH 7), TSB (pH 4) and TSB containing 700 μg/ml Cu (pH 4), it was found that growth of both test bacterial strains in the last medium was the lowest. Its is possible to say that, in acidic condition of growth medium, selected bacterial strains in this study (***Zoogloea*** and ***Bacillus*** **sp.**) seems to be acidic tolerant not acidophiles.

Table 4.5 Effects of pH and temperature on growth of the bacterial isolates

Strains	Initial no. of organisms (cellsx10^6/ml)	No. of organisms (cellsx10^8/ml)									
		pH							Temp (°C)		
		4	5	6	7	8	9	10	30	37	40
CuR-4	3.7	1.8	2.1	3.3	4.5	2.1	1.4	1.2	4.2	4.5	4.1
CuR-14	3.2	1.4	2.8	3.9	4.4	3.2	1.9	1.3	3.7	4.4	4.0
CuR-24	3.4	1.4	2.3	3.2	3.9	2.6	2.3	1.0	3.6	3.9	3.8
CuR-25	3.5	1.6	2.1	2.6	4.0	2.1	1.4	0.9	3.3	4.0	3.9
CuR-32	3.1	1.3	2.5	2.7	3.8	2.6	1.4	0.9	2.7	3.8	3.4
CuR-38	**3.8**	**1.2**	**1.8**	**3.2**	**4.8**	**2.9**	**1.2**	**0.8**	**4.4**	**4.8**	**3.8**
CuR-40	**3.7**	**1.7**	**2.6**	**3.2**	**4.7**	**3.0**	**2.3**	**1.4**	**3.9**	**4.7**	**4.2**

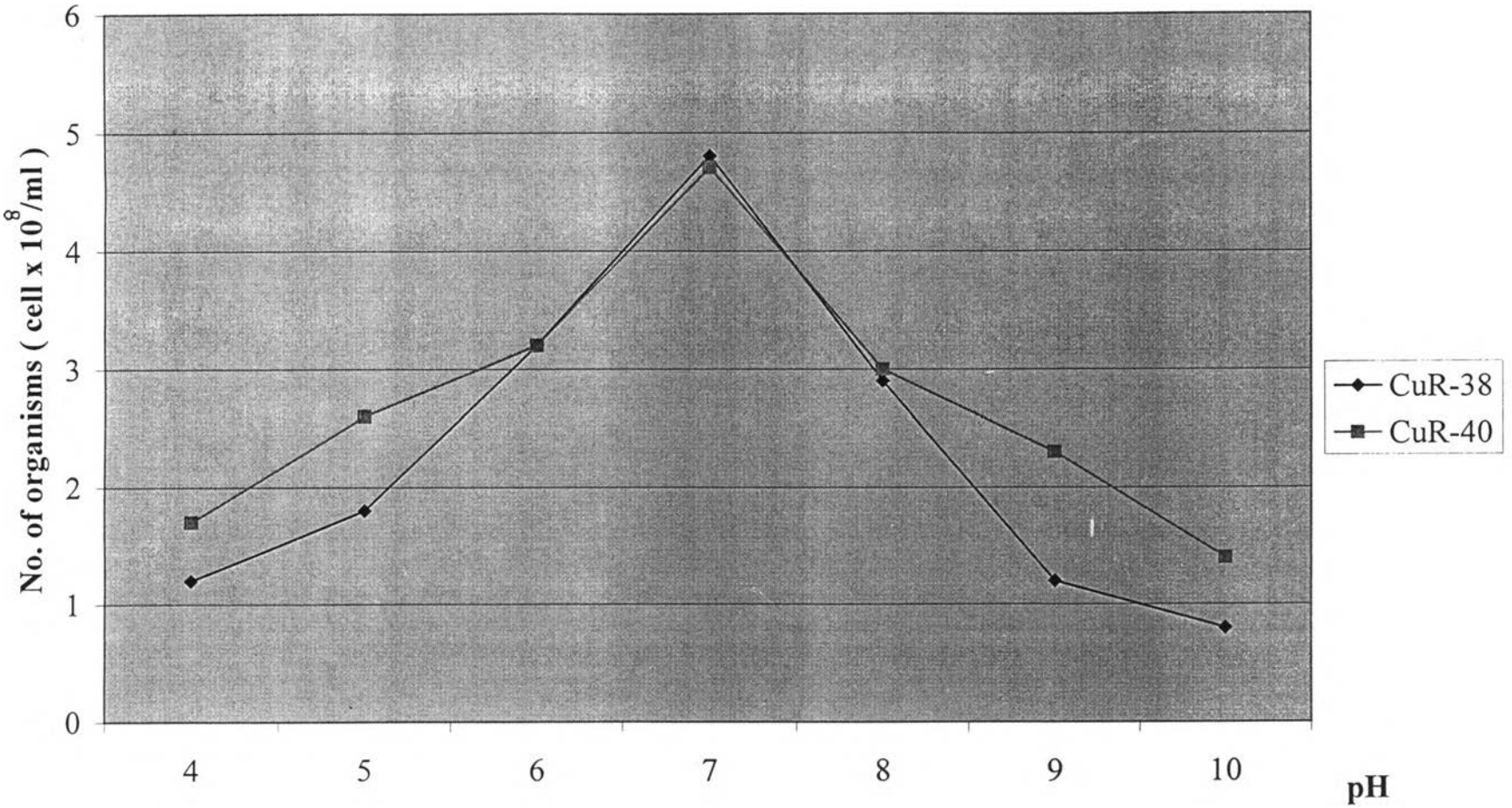

Figure 4.8 Effect of pH on growth of the EPS - producing bacterial isolates

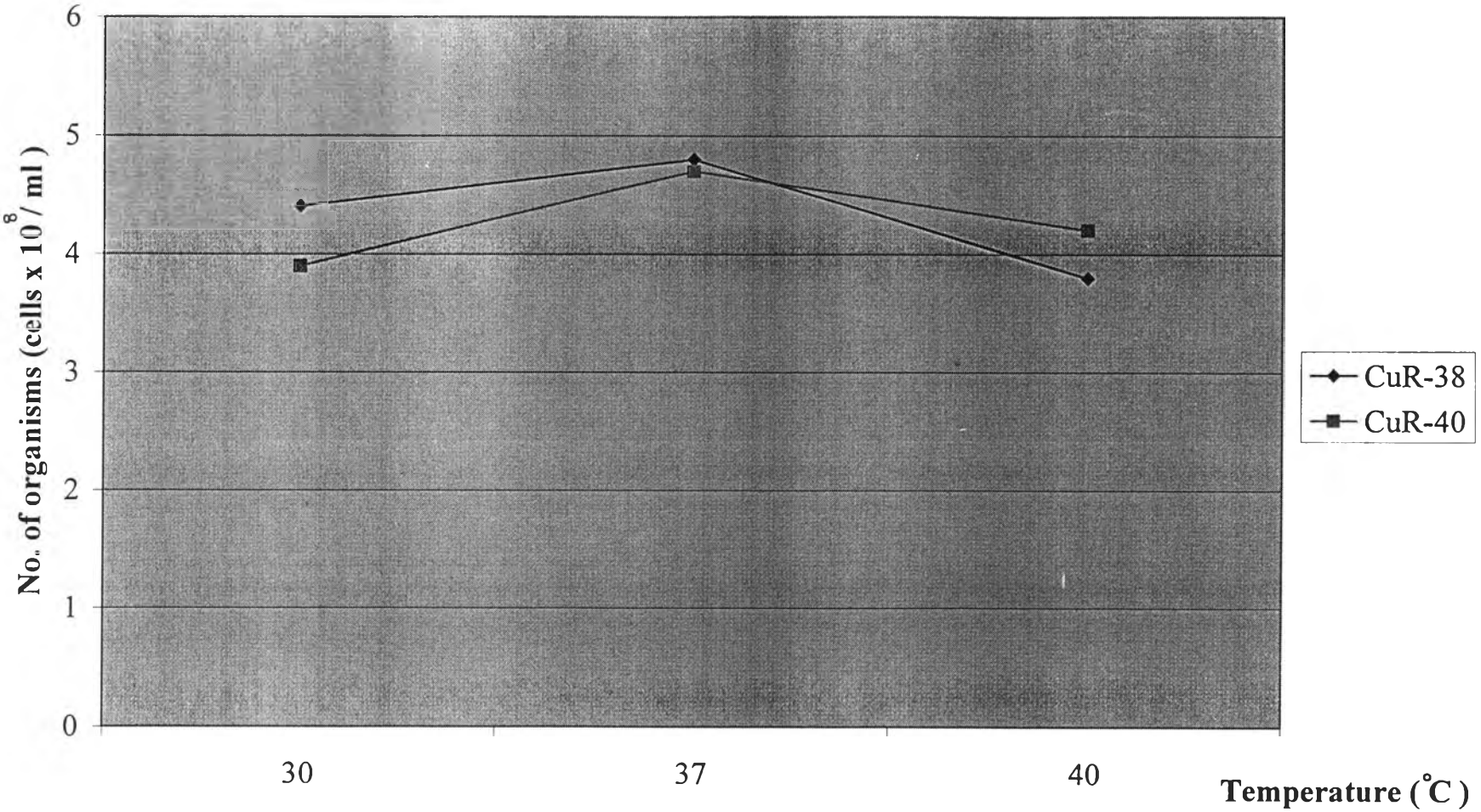

Figure 4.9 Effect of temperture on growth of the EPS - producing bacterial isolates

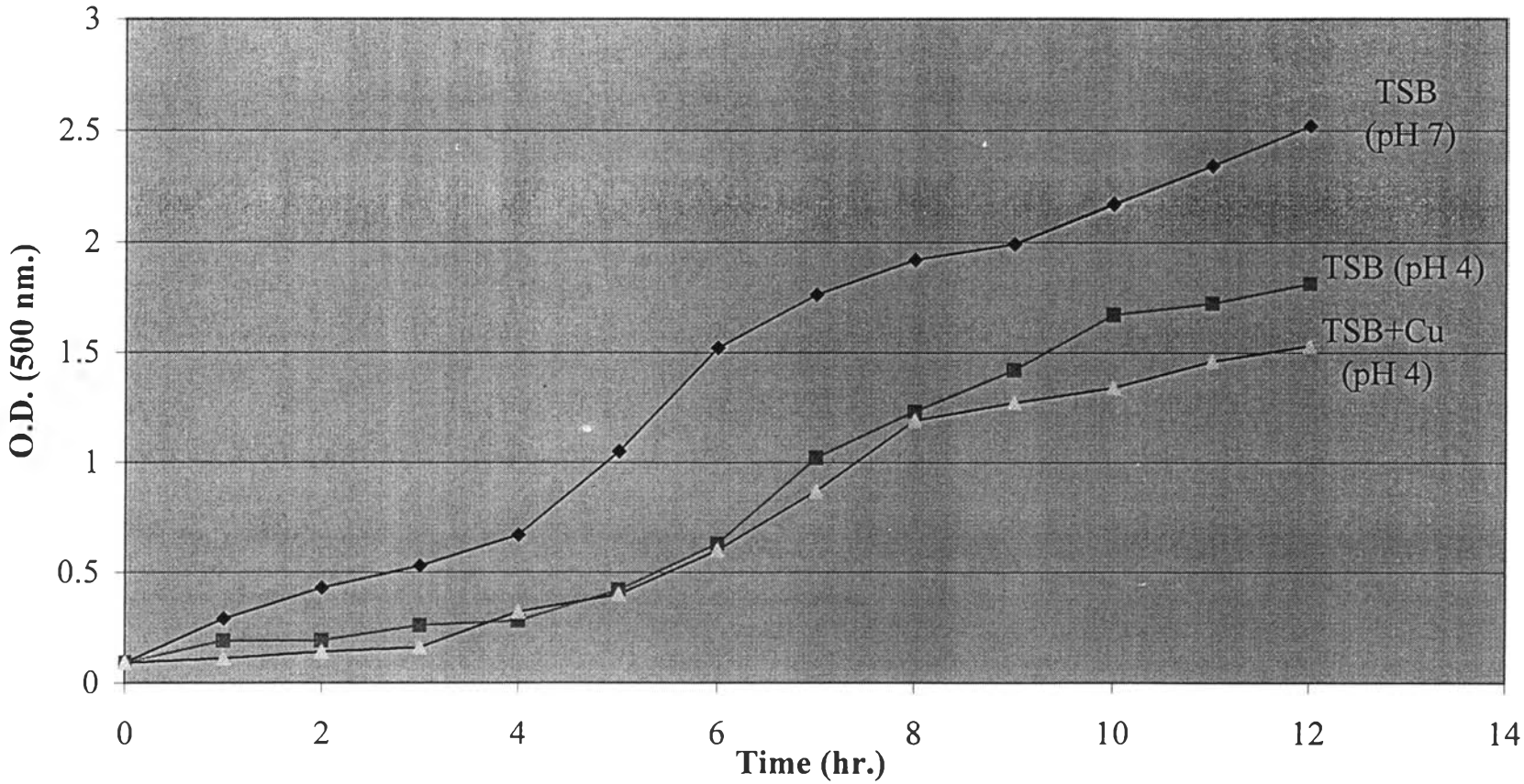

Figure 4.10 Growth of CuR-38 in TSB (pH 7), TSB (pH 4) and TSB containing 700 µg/ml Cu (pH 4) media, incubated at 37°C for 12 hr.

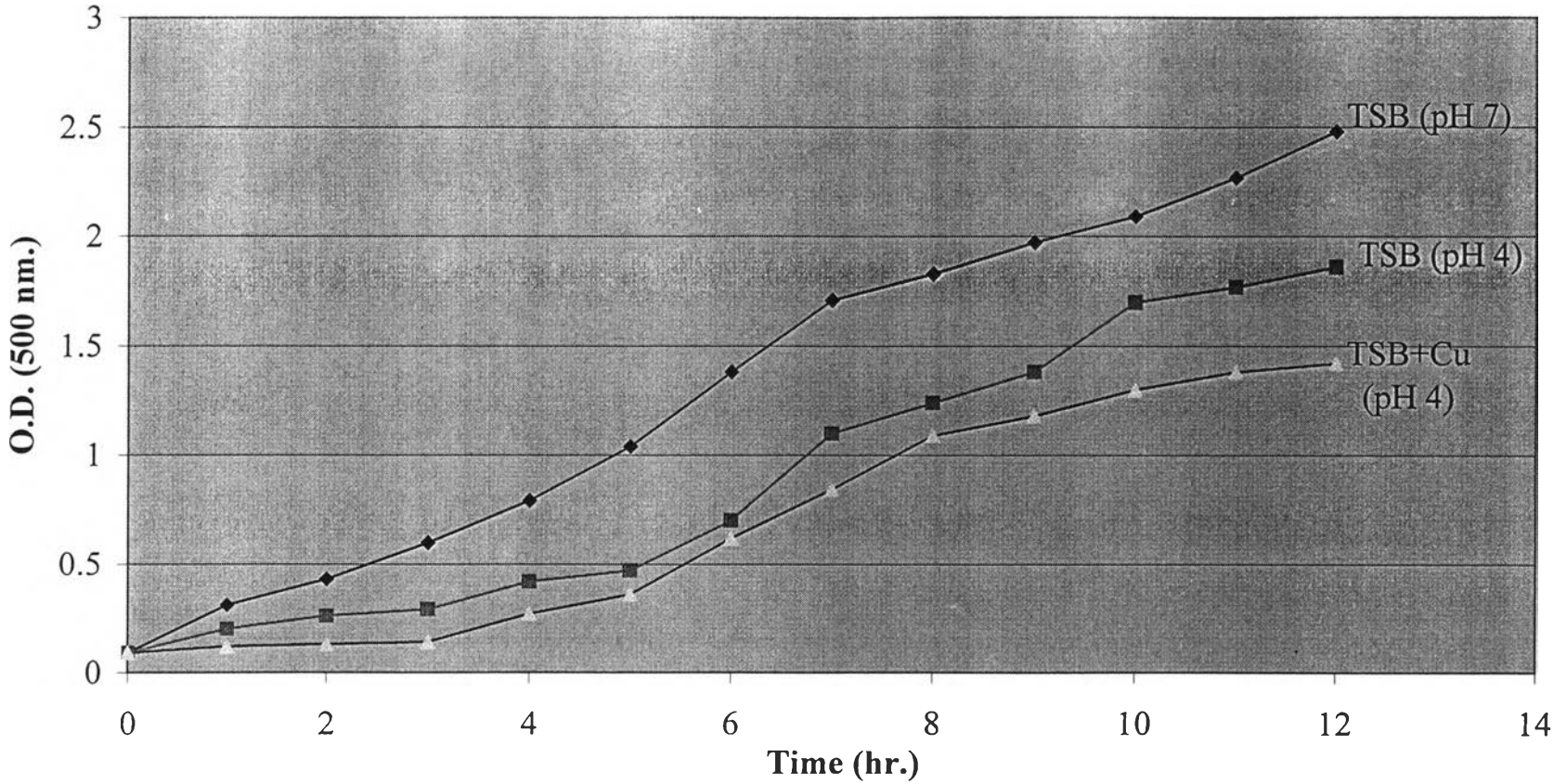

Figure 4.11 Growth of CuR-40 in TSB (pH 7), TSB (pH 4) and TSB containing 700 µg/ml Cu (pH 4) media, incubated at 37°C for 12 hr.

4.5 EFFECTS OF pH, TEMPERATURE AND INCUBATION PERIOD ON EPS PRODUCTION OF THE SELECTED BACTERIAL ISOLATES

Optimum pH for growth and EPS production of both bacterial isolates (CuR-38 and CuR-40) are neutral or at 7 (**Table 4.6**). At pH 7, the dry weight amounts of EPS and cells and the proportion of EPS and cells of ***Zoogloea*** **sp.** were 64.39 mg/125 ml, 18.85 mg/125 ml and 3.416, respectively; and of ***Bacillus*** **sp.** were 59.25 mg/125 ml, 17.09 mg/125 ml and 3.467, respectively. No growth of any selected bacterial isolate was found in TSB adjusted to extreme pH (4 or 10). It is possible to say that high yields of EPS production and cell proliferation were found in the pH range 6-7.

Optimum temperature for growth and EPS production of CuR-38 and CuR-40 was 37°C; summarized briefly in **Table 4.7**. At 37°C, the dry weight amounts of EPS and cells and also the proportion of EPS and cells of ***Zoogloea*** **sp.** were 64.39 mg/125 ml, 18.85 mg/125 ml and 3.416, respectively, and for ***Bacillus*** **sp.** were 59.25 mg/125 ml, 17.09 mg/125 ml and 3.467, respectively.

Optimum incubation period for growth and EPS production was found to be 48 hours or two days; summarized briefly in **Table 4.8**. After 48-hr. incubation, the dry weight amounts of EPS and cells and also the proportion of EPS and cells of ***Zoogloea*** **sp.** were 69.86 mg/125 ml, 18.59 mg/125 ml and 3.758, respectively; and of ***Bacillus*** **sp.** were 66.33 mg/125 ml, 17.88 mg/125 ml and 3.710, respectively.

Table 4.6 Effect of pH on EPS production of CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	pH	Dry weight mg of		Proportion of EPS (mg) / Cells (mg)
		EPS	Cells (without EPS)	
CuR-38	3	0	0	0
	4	0	9.87	0
	5	33.77	16.11	2.096
	6	55.04	18.22	3.021
	7	64.39	18.85	3.416
	8	29.83	16.30	1.830
	9	17.01	10.17	1.673
	10	0	6.33	0
CuR-40	3	0	0	0
	4	0	8.76	0
	5	20.01	13.79	1.455
	6	48.03	14.23	3.375
	7	59.25	17.09	3.467
	8	12.86	12.33	1.043
	9	8.77	8.93	0.982
	10	0	6.84	0

Table 4.7 Effect of temperature on EPS production of CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	**Temperature (°C)**	**Dry weight mg of**		**Proportion of EPS (mg) / Cells (mg)**
		EPS	**Cells (without EPS)**	
CuR-38	30	58.18	18.97	3.067
	37	64.39	18.85	3.416
	40	62.43	19.29	3.236
CuR-40	30	57.11	16.68	3.383
	37	59.25	17.09	3.467
	40	59.23	17.37	3.410

Table 4.8 Effect of incubation periods on EPS production of CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	**Incubation periods (hr.)**	**Dry weight mg of**		**Proportion of EPS (mg) / Cells (mg)**
		EPS	**Cells (without EPS)**	
CuR-38	24	64.39	19.85	3.244
	48	69.86	18.59	3.758
	72	35.37	13.93	2.539
CuR-40	24	59.25	17.09	3.467
	48	66.33	17.88	3.710
	72	20.14	12.69	1.587

In conclusion, the optimal pH, temperature and incubation period for growth and EPS production of the selected bacterial strains, i.e., CuR-38 and CuR-40, should be 7, 37°C and 48 hr., respectively. Thereafter growth condition of those selected bacterial isolates for further studies should be conducted.

4.6 EFFECT OF COPPER ON INDUCTION OF EPS PRODUCTION IN THE SELECTED BACTERIAL ISOLATES

Table 4.9 has shown that copper added into the culture medium was not able to induce the EPS production in the selected bacterial isolates. The highest amount of dry weight EPS produced by CuR-38 and CuR-40 grow in TSB alone (no copper adding), pH 7, incubation at 37°C for 48 hours were found to be 67.86 and 60.18 mg, respectively. On Contrary, less amount of EPS was produced in the culture system containing copper. CuR-38 and CuR-40 produced EPS spontaneously. It is possible to say that there is no effect of copper on the induction of EPS production in those selected bacterial isolates in this study.

Table 4.9 Copper has no effect on the induction of EPS production in CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	Copper Concentration (μg/ml)	Dry weight mg of		Proportion of EPS (mg) / Cells (mg)
		EPS	Cells (without EPS)	
CuR-38	0	67.86	12.78	5.310
	10	62.48	13.11	4.766
	20	59.27	13.07	4.535
CuR-40	0	60.18	13.55	4.441
	10	56.75	13.47	4.213
	20	55.88	12.96	4.312

4.7 ACCUMULATION OF COPPER AND OTHER HEAVY METALS

4.7.1 Contact Time of Copper Accumulation by EPS

The results indicated that the equilibrium was taken within 15 minutes and prolonged exposure time did not increase copper accumulation from the solution (summarized in **Figure 4.12**). Maximum copper, uptake by EPS isolated from CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**) were 270 and 248 μg/ml, respectively, from the initial copper concentration (500 μg/ml) in the solution. At 30, 45 and 60 minutes of exposure time, the Cu uptake by exopolysaccharide isolated from each strain of the test bacteria are equal to 15-min. uptake.

4.7.2 Effect of Soluble Copper Concentration on Copper Accumulation by EPS

The results of copper accumulation by EPS isolated from CuR-38 and CuR-40 are briefly summarized in **Table 4.10**. In the lowest copper concentration in the solution (100 μg/ml), the highest percentage of Cu accumulation by CuR-38 and CuR-40 exopolysaccharides was found, i.e., 93 and 92, respectively. Cu accumulation was gradually increased (93-252 μg/ml for CuR-38 EPS and 92-222 μg/ml for CuR-40) when soluble Cu accumulation was also increased (100-400 μg/ml). However, it was found that Cu accumulation was kept constantly (270 μg/ml for CuR-38 EPS and 248 μg/ml for CuR-40 EPS) when soluble Cu concentration was increased (500-700 μg/ml). Evidence of the higher in soluble Cu concentration, and the lower in percentage of Cu accumulation in both EPS was shown.

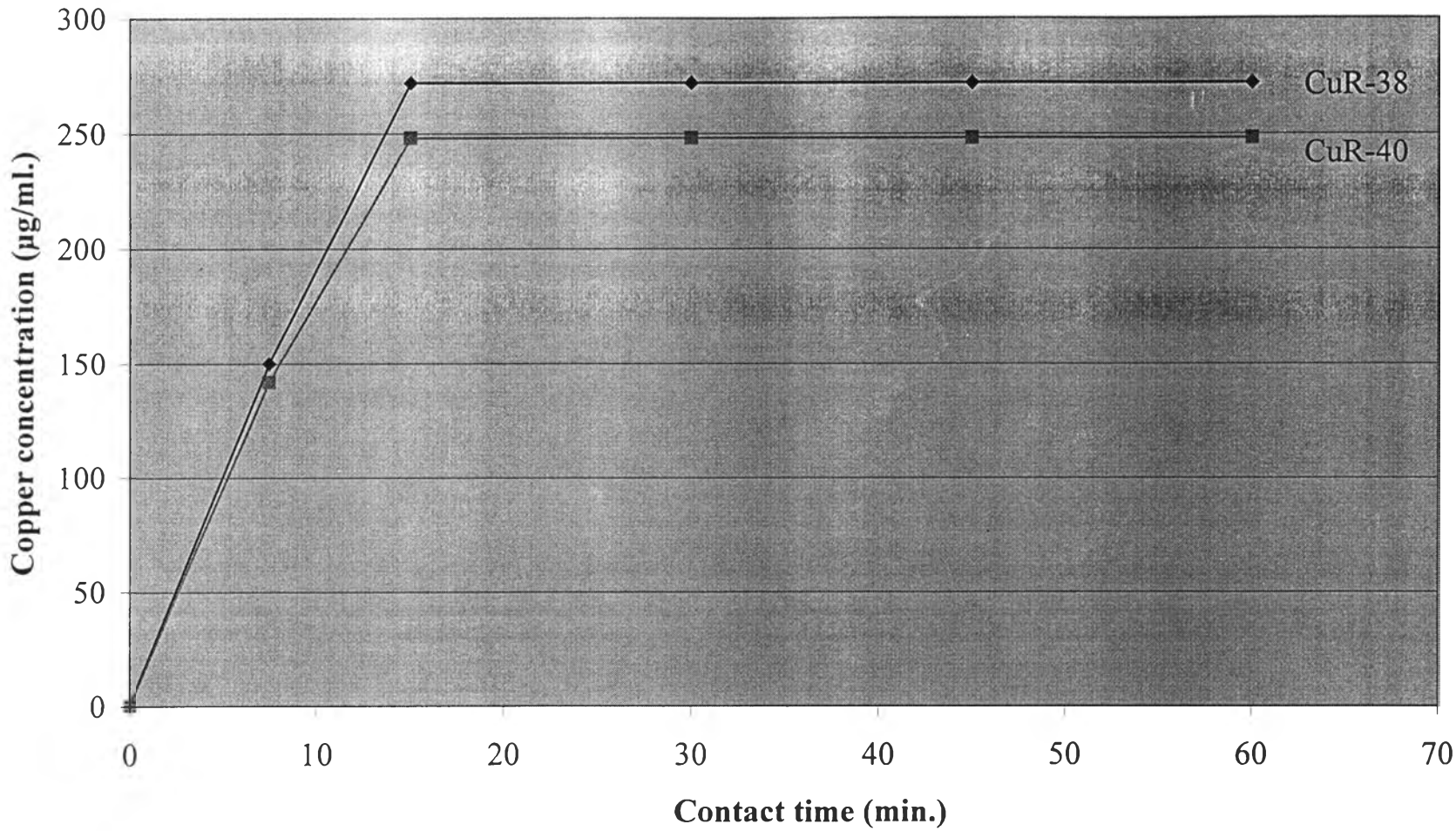

Figure 4.12 Contact time of the uptake copper by EPS.

Table 4.10 Effect of soluble copper concentration on copper accumulation by EPS isolated from CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	**Initial Copper Concentration (μg/ml)**	**Copper accumulation (μg/ml)**	**Percentage of accumulation**
CuR-38	100	93	93.00
	200	178	85.00
	300	225	75.00
	400	252	63.00
	500	270	54.00
	600	270	45.00
	700	270	38.75
CuR-40	100	92	92.00
	200	157	78.50
	300	209	69.66
	400	222	55.55
	500	248	48.16
	600	248	41.33
	700	248	35.42

4.7.3 Copper Accumulation by Wet EPS and by Wet Whole Cells

The results of copper accumulation by EPS and whole cells were presented in **Table 4.11**. After 15-min. exposure time, Cu accumulation by EPS of CuR-38 and CuR-40 was found to be 94.9 and 89.6 μg/ml / 5 g wet weight, respectively, which was removed from 112 μg/ml of soluble copper concentration. The results indicated that the accumulation of Cu by EPS was higher than by whole cells of the same organisms, and the percentage of Cu accumulation by CuR-38 or ***Zoogloea* sp.** was higher than by CuR-40 or ***Bacillus* sp.**, both in EPS and whole cells (**Figure 4.13**).

4.7.4 Copper Accumulation by Dried Whole Cells, Dried EPS and Dried Cells without EPS

Similar to the wet EPS and wet whole cells (see **Section 4.7.3**), Cu accumulation by dried EPS was higher than by dried whole cells of the same test organisms and also higher than by dried cells without EPS (**Table 4.12**). The result has shown that EPS may contribute significantly to copper adsorption or biosorption. After 15-min. exposure time, maximum copper accumulation by EPS, by whole cells and by cells without EPS of CuR-38 were 92.3, 86.8 and 17.2 μg/ml, respectively, whereas of CuR-40 were 90.1, 81.5 and 32.9 μg/ml, respectively. Percentage of copper accumulation by EPS, by whole cells and by cells without EPS of CuR-38 (***Zoogloea* sp.**) was a little bit higher than of CuR-40 (***Bacillus* sp.**); as shown in **Figure 4.14**.

Table 4.11 Copper accumulation by wet EPS and wet whole cells of CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	Initial Copper Concentration (μg/ml)	Wet Weight g of		Cu accumulation (μg/ml)		Percentage of accumulation	
		Whole cells	EPS	Whole cells	EPS	Whole cells	EPS
CuR-38	112	5.0	5.0	90.3	94.9	80.63	84.73
CuR-40	112	5.0	5.0	86.2	89.6	76.96	80.00

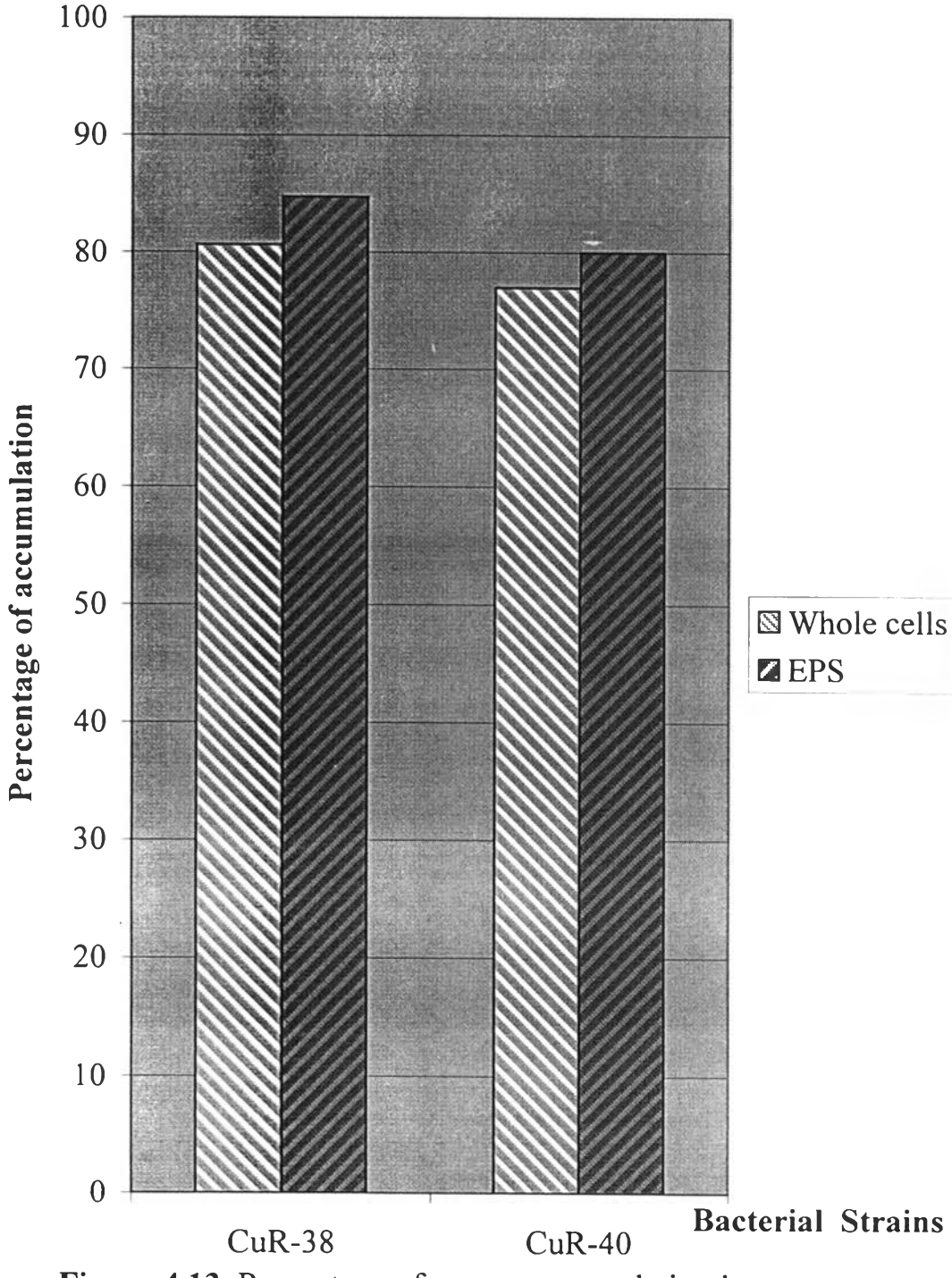

Figure 4.13 Percentage of copper accumulation in CuR-38 (***Zoogloea*** **sp.**) and CuR-40 (***Bacillus*** **sp.**), comparision between EPS and the Whole cells.

Table 4.12 Copper accumulation by dried whole cells, dried EPS and dried cells without EPS of CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	Initial Cu Conc. (μg/ml)	Dry weight g of			Cu accumulation (μg/ml)			% of accumulation		
		Whole cells	EPS	Cells (with-out EPS)	Whole cells	EPS	Cells (with-out EPS)	Whole cells	EPS	Cells (with-out EPS)
CuR-38	112	1.0	1.0	1.0	86.5	92.3	17.2	77.23	82.41	15.39
CuR-40	112	1.0	1.0	1.0	81.5	90.1	23.9	72.77	80.45	21.34

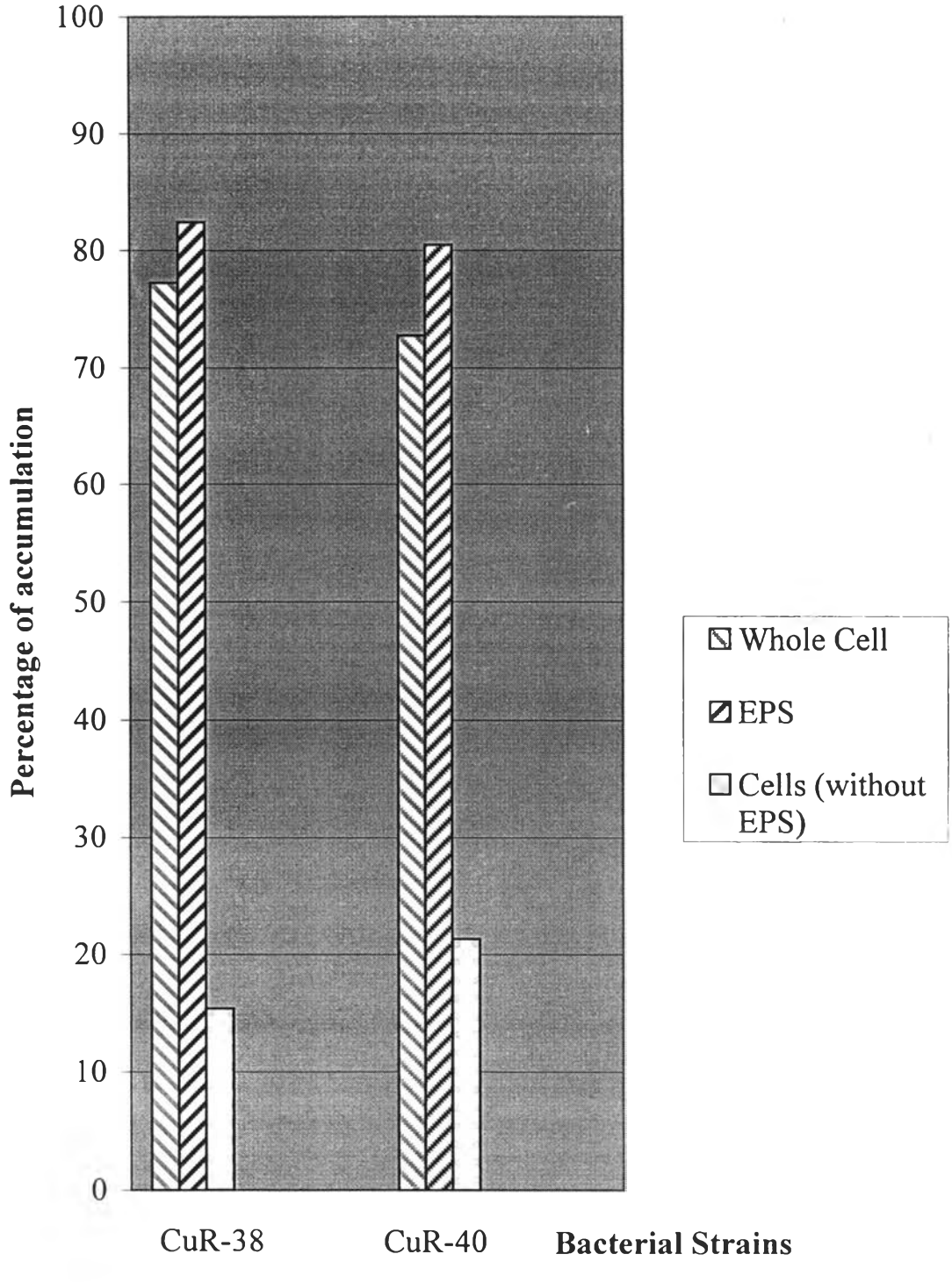

Figure 4.14 Percentage of Cu accumulation by dried whole cells, dried EPS and dried cells without EPS of CuR-38 (***Zoogloea*** **sp.**) and CuR-40 (***Bacillus*** **sp.**)

4.7.5 Accumulation of Some Heavy Metals by EPS

This experiment attempted to present that EPS of strains CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**) could be utilized to adsorb other metals as well as copper. The accumulation of each of those test metals, i.e., Zn, Mn and Cd (see **Section 4.2**) by EPS was quite high compared with Cu, especially, Zn and Cd (**Table 4.13**). The percentages of those metal accumulation or binding by EPS of the test strains were found in **Figure 4.15**. The accumulation of each heavy metal in metal mixture (Cu, Zn, Mn and Cd) was shown in **Table 4.14** and the percentage of accumulation of each metal in metal mixture was found in **Figure 4.16**.

4.8 RECOVERY OF BOUND METAL IONS

Acid treatment was used to elute metal(s) from EPS. Effectively, bound metal ions can be separated from EPS by increasing in acidity, e.g., 77.8 and 68.0% of Cu were removed when the total amounts of adsorbed Cu were 98.4 and 93.1 μg/ml for strains CuR-38 and CuR-40, respectively. Percentages of metal recovery from EPS of CuR-38 and CuR-40 were shown in **Figure 4.17** and **Figure 4.18**, respectively. Acid treatment brought about the efficient metal recovery. It may be a good chelator for copper, zinc and cadmium (**Table 4.15**). Fortunately, prolonged exposure time increased the metal recovery.

Table 4.13 Accumulation of some heavy metals, i.e., Cu, Zn, Mn and Cd, individually, by EPS isolated from CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	Initial conc. of heavy metals (μg/ml)				Accumulation of heavy metals (μg/ml)				% of accumulation			
	Cu	Zn	Mn	Cd	Cu	Zn	Mn	Cd	Cu	Zn	Mn	Cd
CuR-38	112	114	109	111	97.5	94.2	83.6	95.9	87.05	82.63	76.70	86.40
CuR-40	112	114	109	111	92.7	83.5	73.0	89.5	82.77	73.25	66.97	80.63

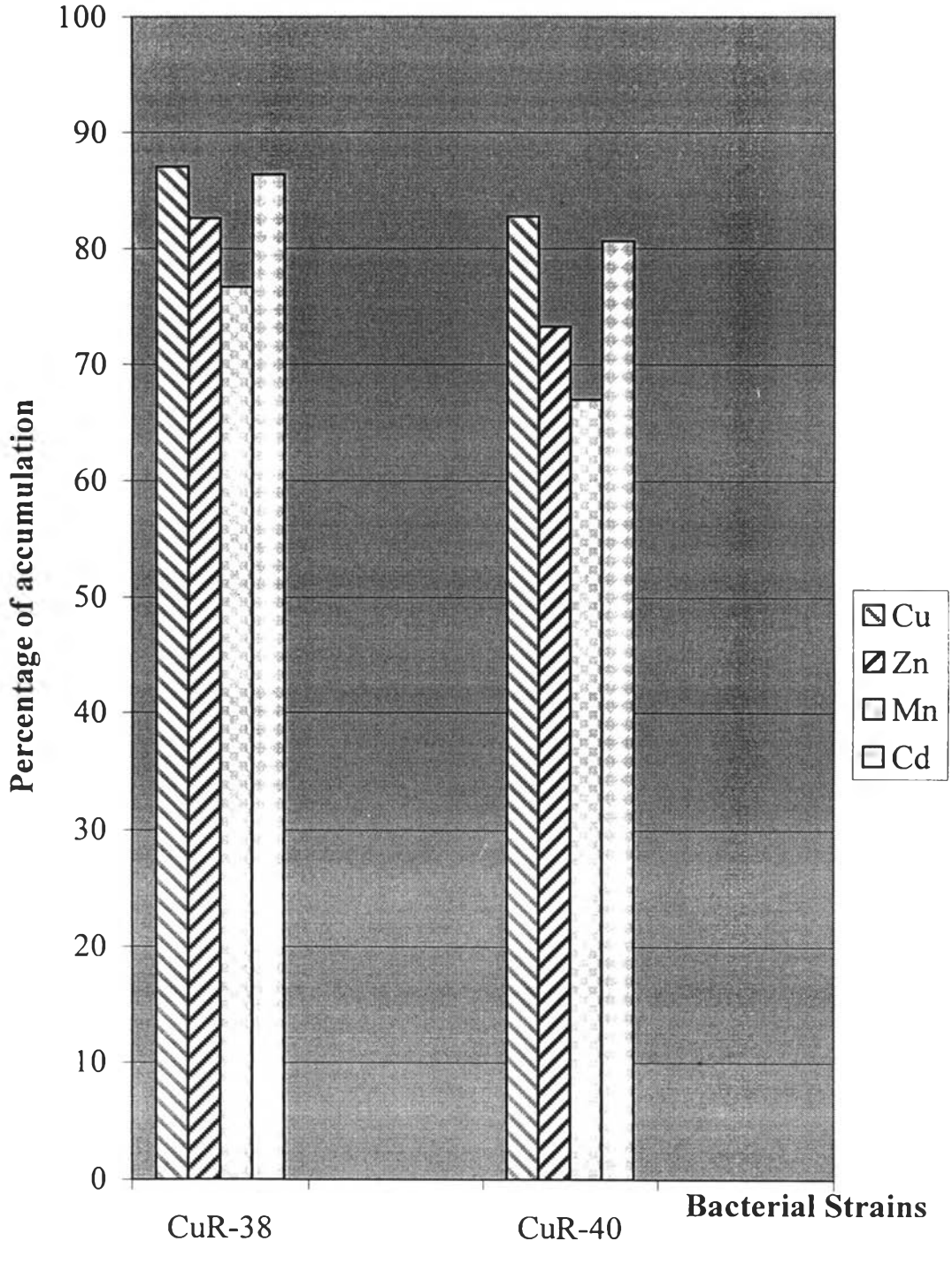

Figure 4.15 Percentage of heavy metal accumulation , individually, by EPS isolated from CuR-38 (***Zoogloea*** **sp.**) and CuR-40 (***Bacillus*** **sp.**)

Table 4.14 Accumulation of metal in metal mixture (Cu, Zn, Mn and Cd) by EPS isolated from CuR-38 (***Zoogloea* sp.**) and CuR-40 (***Bacillus* sp.**)

Selected Strains	Initial conc. of heavy metals (μg/ml)				Accumulation of heavy metals (μg/ml)				% of accumulation			
	Cu	**Zn**	**Mn**	**Cd**	**Cu**	**Zn**	**Mn**	**Cd**	**Cu**	**Zn**	**Mn**	**Cd**
CuR-38	112	114	109	111	24.6	23.4	20.4	23.2	21.97	20.50	18.69	20.88
CuR-40	112	114	109	111	23.3	20.9	17.1	22.3	20.78	18.33	15.71	20.09

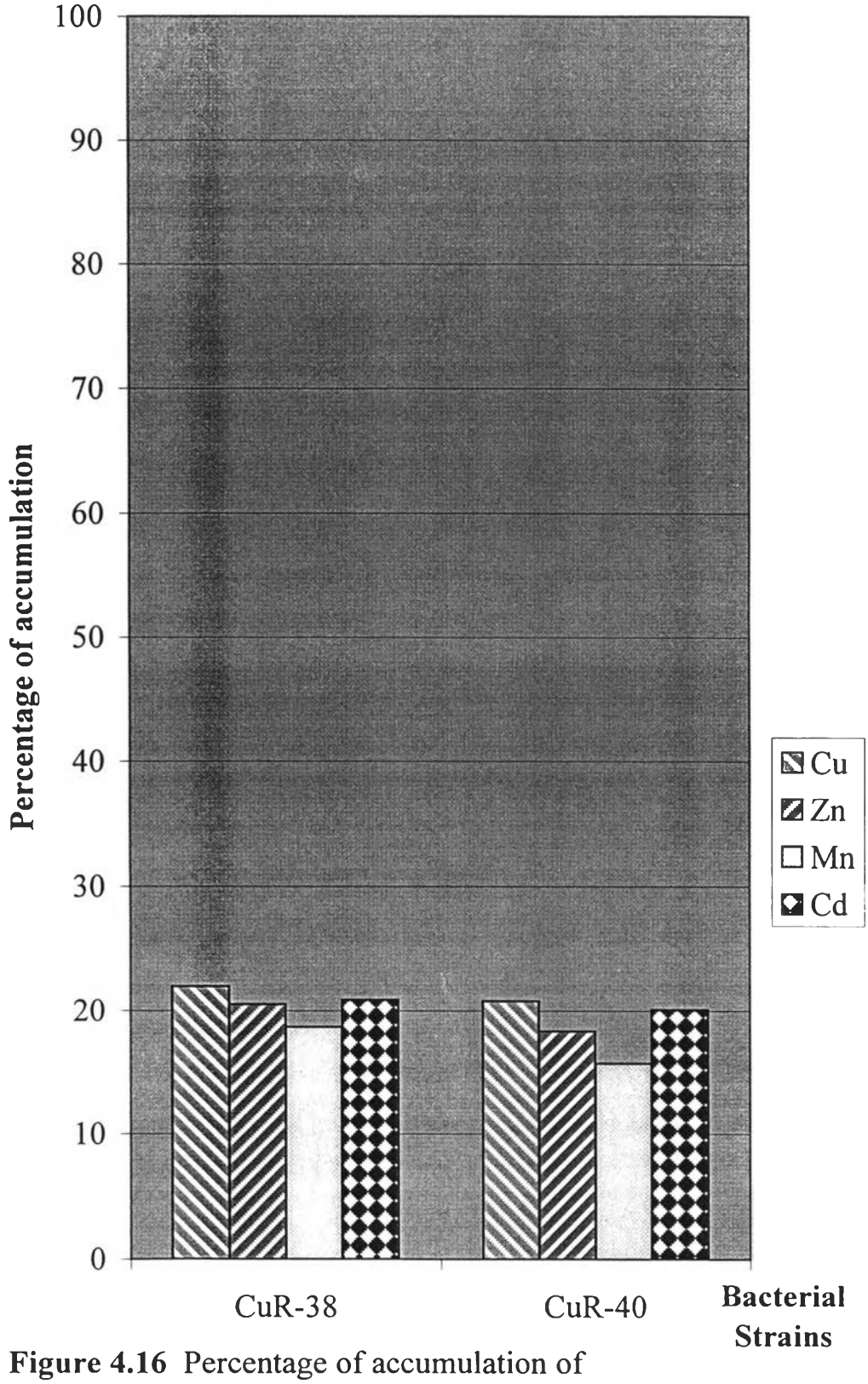

Figure 4.16 Percentage of accumulation of metal in metal mixture (Cu, Zn, Mn and Cd) by EPS isolated from CuR-38 (***Zoogloea*** **sp.**) and CuR-40 (***Bacillus*** **sp.**)

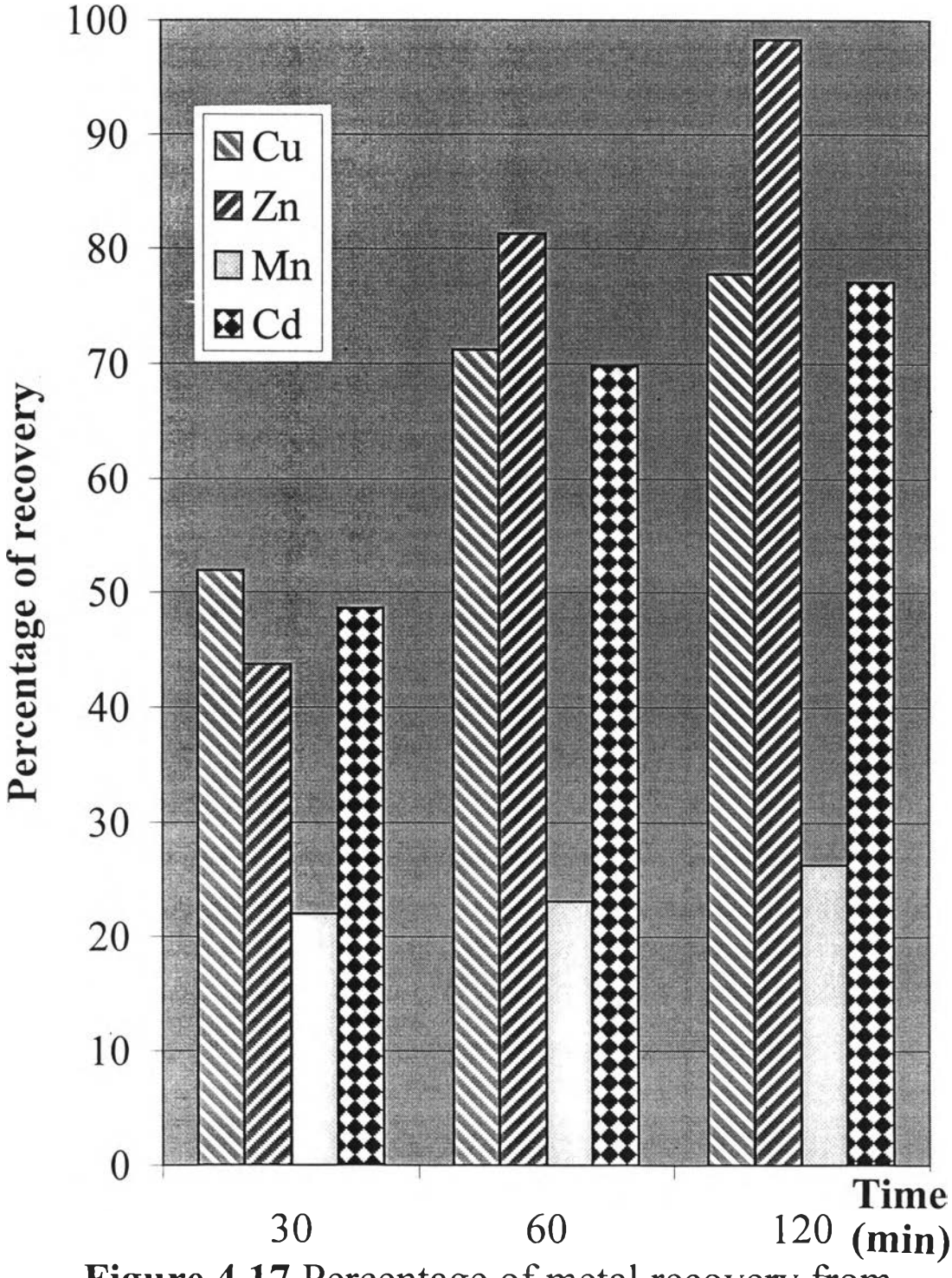

Figure 4.17 Percentage of metal recovery from EPS of CuR-38 with acid treatment at different intervals of time.

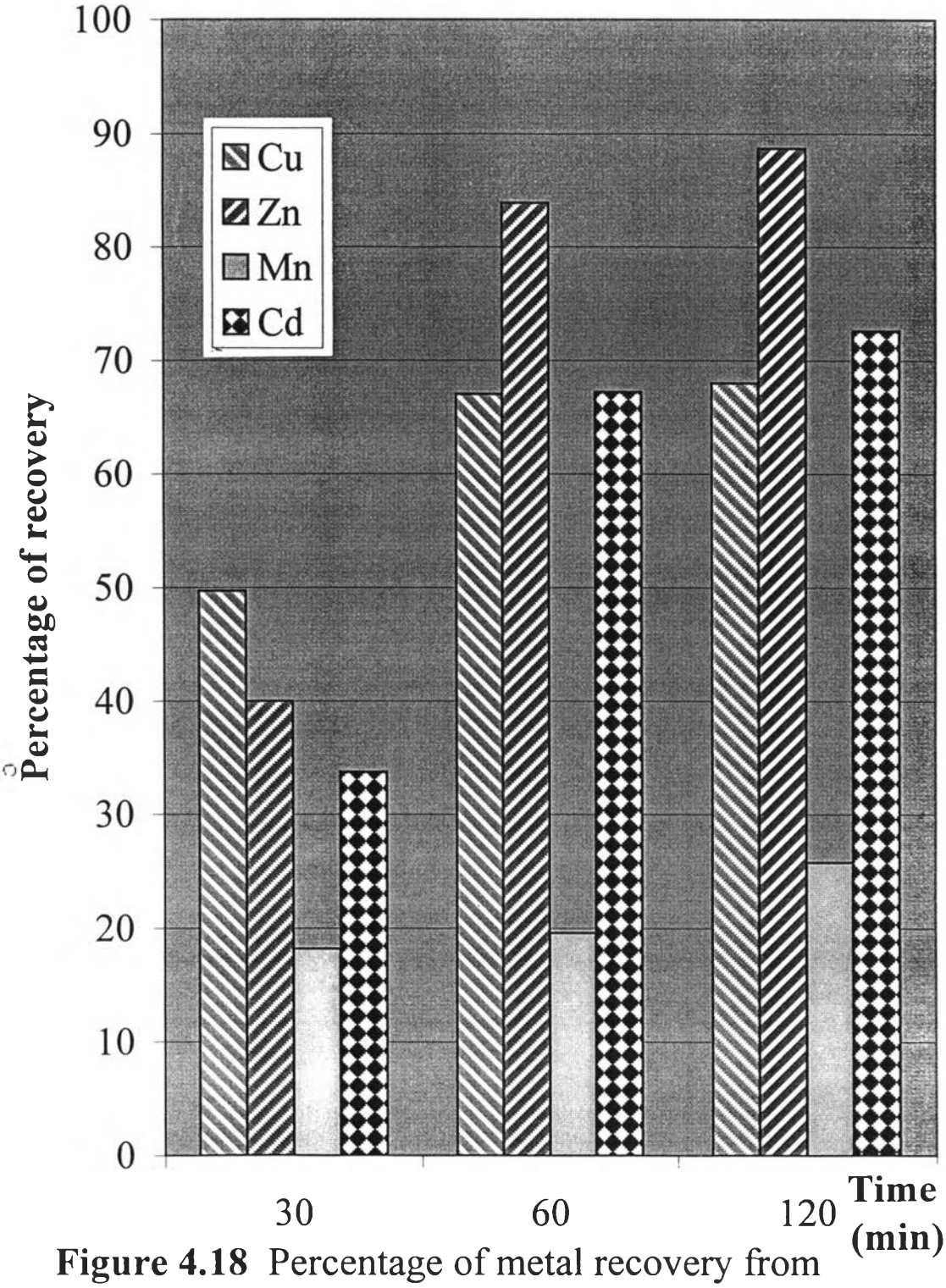

Figure 4.18 Percentage of metal recovery from EPS of CuR-40 with acid treatment at different intervals of time

Table 4.15 Recovery of Cu, Zn, Mn and Cd, individually, from EPS by acid treatment

Selected Strains	Time (min.)	Percentage of recovery*			
		Cu	Zn	Mn	Cd
CuR-38	30	51.9	43.7	22.0	48.6
	60	71.2	81.3	23.1	69.8
	120	77.8	98.3	26.3	77.1
CuR-40	30	49.7	40.0	18.2	33.7
	60	67.0	83.9	19.6	67.2
	120	68.0	88.7	25.8	72.6

* Determined as amount of each metals recovered, divided by the amount of each metals adsorbed

Treatment of EPS with acid after exposed to metal mixture (Cu, Zn, Mn and Cd) showed that copper and zinc was recovered efficiently and prolonged exposure time increase the metal recovery (**Table 4.16**), and the percentage of metal recovery from EPS of CuR-38 and CuR-40 after exposed to metal mixture were shown in **Figure 4.19** and **Figure 4.20**.

After regeneration of EPS with acid treatment, the percentage of copper accumulation were 76.5 and 63.0 at the first regeneration and decreased gradually to 65.0 and 54.0 at the fifth regeneration for CuR-38 and CuR-40, respectively (**Table 4.17**). The percentages of Cu accumulation on a number of regeneration were shown in **Figure 4.21**.

Table 4.16 Recovery of metal from EPS after exposure to metal mixture (Cu, Zn, Mn and Cd) by acid treatment

Selected Strains	Time (min.)	Percentage of recovery*			
		Cu	Zn	Mn	Cd
CuR-38	30	50.8	43.7	17.1	18.5
	60	70.3	87.1	23.2	43.5
	120	77.7	98.2	25.2	45.2
CuR-40	30	43.6	39.8	16.1	17.8
	60	61.4	85.4	19.4	11.9
	120	65.3	89.7	26.3	27.3

* Determined as amount of each metals recovered, divided by the amount of each metals adsorbed

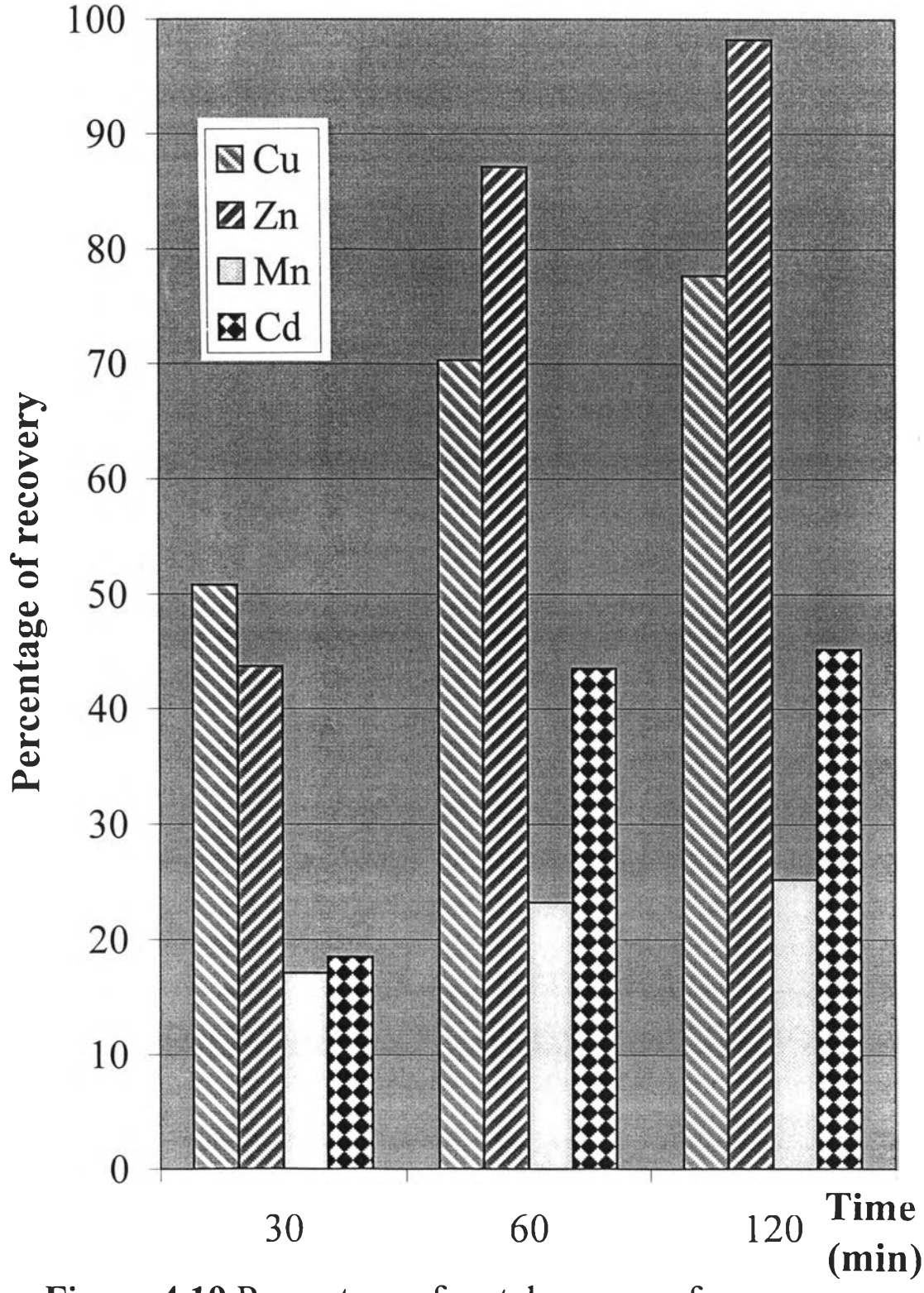

Figure 4.19 Percentage of metal recovery from EPS of CuR-38 after exposed to metal mixture by acid treatment at different imterval of time

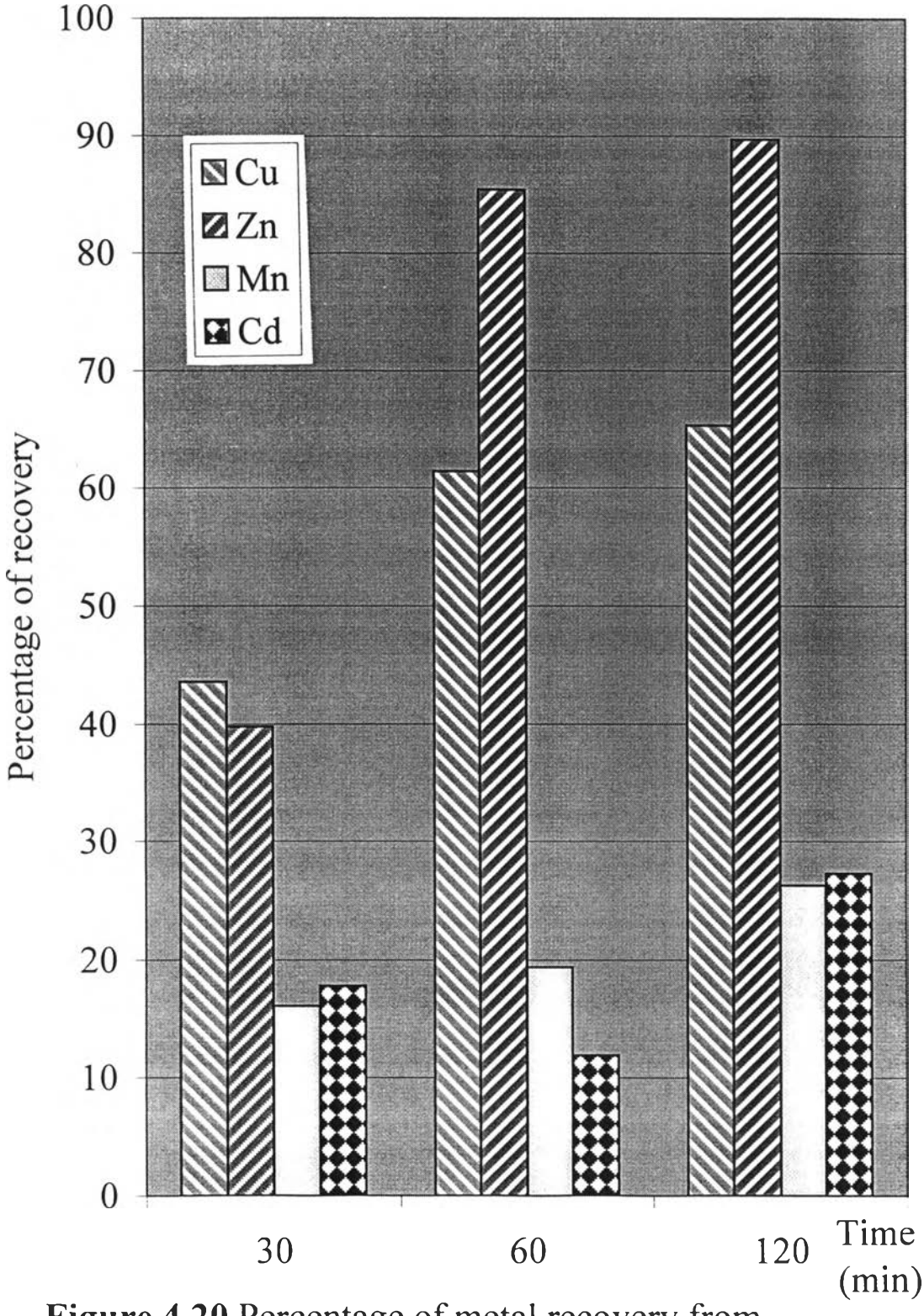

Figure 4.20 Percentage of metal recovery from EPS of CuR-40 after exposed to metal mixture by acid treatment at different interval of time.

Table 4.17 Accumulation of copper by EPS after regeneration from the first time to the fifth time

No. of regeneration	Percentage of accumulation	
	CuR-38	**CuR-40**
1	76.5	63.0
2	73.5	59.5
3	73.5	55.5
4	73.0	55.0
5	65.0	54.0

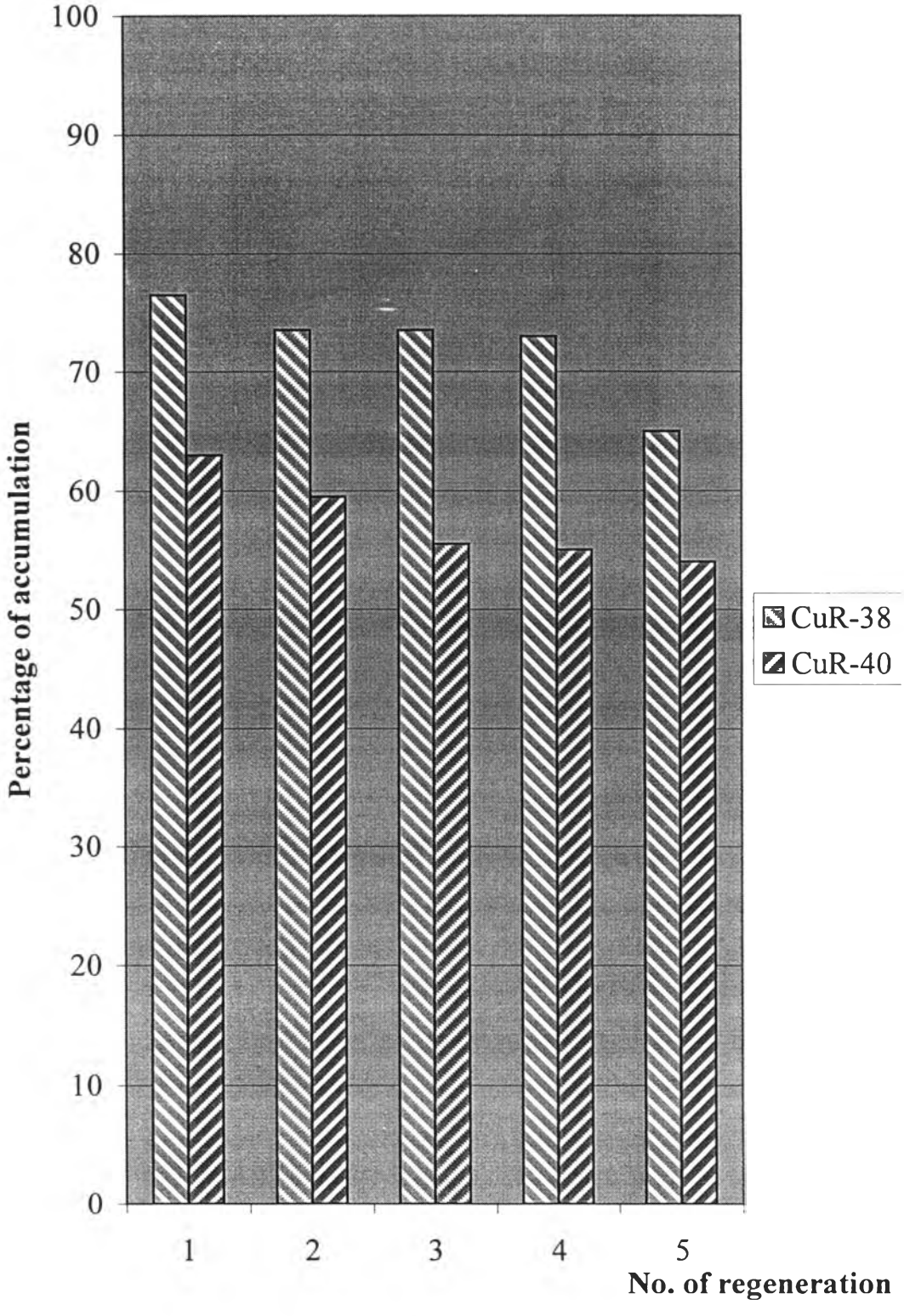

Figure 4.21 Percentage of copper accumulation on a number of regeneration.