CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary experiment

4.1.1 Solubility test

It was often accepted that many biosorbents have high uptake capacities for heavy metals at high pH because acidic condition at low pH usually causes competition between proton (H^+) and heavy metals (M^{2+}) at the binding site of biosorbents (Volesky, 1990: Sungkhum, 2003). However, metal precipitation takes place at high pH and this would interfere with the adsorption characteristics. Therefore it is important that a suitable pH for the adsorption be identified and, in this research, the suitable pH was defined as the highest pH that all four heavy metals were still dissolved but not precipitated. The test results were compared with prediction from the MINEQL program and the calculation using the solubility product values (K_{sp}) as shown in Figures 4.1.1 to 4.1.4. It is noted that these solubility curves were obtained from experiments with an initial metal concentration of 100 mg/l. Hence, the maximum solubility obtained was limited at only 100 mg/l whereas the actual solubility might be higher than 100 mg/l. The pH where a drop of solubility was first observed is summarized below:

Heavy metal	PH		
	K_{sp}	MINEQL	Experiment
Cu	6	5.3	5.94
Cd	8.4	8.35	6.27
Pb	7.5	5.8	7.02
Zn	6.9	6.5	> 7

The results suggested that the pH of the solution be controlled at 5 ± 0.2 to ensure a complete dissolution of all metals and this pH was maintained for all subsequent experiments.

4.1.2 Determination of the component in pure algae

The inorganic compositions of *Caulerpa lentillifera* determined by microwave digester and following by ICP analysis were shown in Figure 4.2 and summarized in the following tabulation.

	Metal in alga (μmol-metal/g-alga)											
	Ag	Al	B	Ba	Bi	Ca	Cd	Cr	Cu	Fe	Ga	K
Average	0.23	453.23	5.19	0.92	0.14	42.44	0.04	0.24	0.37	113.81	0.16	42.28
%RSD*	38.44	10.62	19.32	16.06	48.22	0.65	29.16	22.85	57.5	8.06	12.72	24.54
	Li	Mg	Mn	Na	Ni	Pb	Sr	Tl	Zn			
Average	153.68	41.6	35.33	3.9	0.83	0.04	0.7	0.01	4.42			
%RSD*	67.9	6.94	4.3	41.98	58.1	45.31	7.5	40.61	12.16			

* %RSD = Percentage of Relative Standard Deviation

The results showed that main inorganic components comprised alkaline, alkaline earth (calcium, potassium, lithium, magnesium, etc.) and some metal transitions (iron and manganese). The main element observed in this test was aluminum, at about 450 μmol per gram algae. Aluminum might be one of the main constituents of this algal species or it might be possible that the watersource which was the source of this alga contained large quantity of Al. Hence, this alga could have uptaken this metal species in large quality before it was collected for this study. The main divalent metals were magnesium and calcium. This was because this alga contained chlorophyll and the magnesium is a major inorganic component in chlorophyll molecule and calcium may be a major component in cell wall structure of this alga. These divalent metals may be the important key for the adsorption of heavy metals as they might play a significant role in the ion exchange process. The alga was also found to have trace amounts of the four heavy metals of interest, i.e. copper (0.37 μmol/g-algae), cadmium (0.04 μmol/g-algae), lead (0.04 μmol/g-algae), and zinc (4.42 μmol/g-algae). Among these, zinc seemed to be the most abundant species. However, they were only present in tiny amount conpound with the quantity adsorpbed into the alga as will be discussed shortly.

4.2 Biosorption of single component heavy metal

4.2.1 Removal efficiency and isotherm

The removals for all heavy metals examined in this research were found to depend inversely on their initial concentrations as illustrated in Figures 4.3.1 and 4.3.2. In Figures 4.3.2, the metal concentrations for the four metals were not in the same range. It is reminded here that the range of metal concentrations was specified in "mass" unit where

the concentration of each heavy metal varied from 0 – 100 mg/l. However the reported units in this figure is in molar and the conversion of this "mass" to "molar" unit resulted in an inequality of metal concentration range. Among the four metals, Cd exhibited the lowest adsorption efficiency at high initial concentration. Figures 4.4.1 to 4.4.4 display the isotherm of each heavy metal. The isotherm curve in Figure 4.4.5 also supports this finding as Cd exhibited lowest adsorption capacity when compared with other heavy metals. Cu, Pb, and Zn presented similar adsorption characteristics.

The parameters of Langmuir and Freundlich isotherm models for all metals examined in this experiment can be summarized as follows:

Heavy Metal	Langmuir isotherm	Freundlich isotherm
Cu	q_{max} = 0.0852 mmol/g* (or 5.41 mg/g**)	K = 0.1642 mmol basis*** (or 0.8848 mg basis****)
	b = 10.87 l/mmol (or 0.17 l/mg)	$1/n$ = 0.5943 unitless
	r^2 = 0.8789	r^2 = 0.8457
Cd	q_{max} = 0.0255 mmol/g* (or 2.86 mg/g**)	K = 0.0570 mmol basis*** (or 0.7469 mg basis****)
	b = 44.51 l/mmol (or 0.39 l/mg)	$1/n$ = 0.4551 unitless
	r^2 = 0.8514	r^2 = 0.9114
Pb	q_{max} = 0.0296 mmol/g* (or 6.13 mg/g**)	K = 0.1981 mmol basis*** (or 1.7135 mg basis****)
	b = 146.94 l/mmol (or 0.71 l/mg)	$1/n$ = 0.5955 unitless
	r^2 = 0.9588	r^2 = 0.9072
Zn	q_{max} = 0.0817 mmol/g* (or 5.34 mg/g**)	K = 0.1171 mmol basis*** (or 0.8762 mg basis****)
	b = 10.96 l/mmol (or 0.17 l/mg)	$1/n$ = 0.5186 unitless
	r^2 = 0.8978	r^2 = 0.8877

* mmol-heavy metal/g-algae, ** mg-heavy metal/g-algae,

*** mmol basis is the unit of K obtained from the isotherm with q as a y-axis in a unit of mmol/g and C_e as an x-axis in a unit of mmol/l. The actual unit of K in mmol basis is $(\text{mmol-metal})^{1-1/n} \cdot l^{1/n}/\text{g-algae}$.

****mg basis is the unit of K obtained from the isotherm with q as a y-axis in a unit of mg/g and C_e as an x-axis in a unit of mg/l. The actual unit of K in mg basis is $(\text{mg-metal})^{1-1/n} \cdot l^{1/n}/\text{g-algae}$.

The results showed that the adsorption's equilibrium for these metals in the concentration range employed in this work could well be described by both Langmuir and Freundlich isotherms ($r^2 > 0.85$). Langmuir isotherm suggested that maximum sorption capacity (q_{max}) of copper was the greatest among the four metals. The order of q_{max} can be prioritized from high to low as: Cu > Zn > Pb > Cd while the affinity constant (b) of Pb was the greatest indicating that Pb was the most easily bonded component to the binding sites of this algae. The maximum adsorption capacity, for Pb in Langmuir isotherm might not be of great accuracy as the range of concentration employed in this work was rather

narrow and did not cover the high concentration range. A higher range of concentration was not considered here as it is not applicable to biosorpton technology. Hence, the maximum adsorption capacity for Pb should not be used for further evaluation and only "b" can be used with great confidence as it provided the information within the range of this experiment. The "b" value indicated that Pb was the fastest adsorbed species. This agreed with the comparison using parameters from Freundlich isotherm model (K and 1/n) where the order of adsorption capacities from high to low was: Pb > Cu > Zn > Cd at high initial concentration.

Note that the comparison using Freundlich parameters is rather complicated. While the parameters obtained from Langmuir isotherm model (q_{max} and b) can be considered individually as they are independent and refer to two adsorption characteristics, the two parameters from Freundlich isotherm model are dependent function in the graph of isotherm model. Thus the comparison must be considered simultaneously by plotting the power graph ($y = ax^b$; a,b is constant) .

In addition, during the adsorption, the pH of solution tended to increase steadily and pH adjustment was needed to maintain the pH at 5 ± 0.2. This indicated that there was a competition between H^+ and metal species in binding themselves onto the binding sites of algae. As H^+ was adsorbed, the relative concentration of OH^- increased and this resulted in a higher pH level.

4.2.2 Relationship between functional groups and metal sorption

The functional group is one of the keys to understand the mechanism of metal binding on the algal surface. FT-IR was used to analyze the functional groups in the pure algae. The results of FT-IR transmission spectra are shown in Figures 4.5.1 to 4.5.5. Table 4.1 shows the dominant peak obtained from transmission spectra. There were several functional groups found in the structure of *Caulerpa lentillifera* such as carboxylic acid, amine, amide, amino, sulfonyl, and sulfonate groups.

In the comparison between spent algae with pure algae, it was observed that there was a shift (more than 10 cm^{-1}) in wave number of dominant peaks associated with the loaded metal. This shift in the wavelength is generally known to indicate that there was a metal binding process taking place on the surface of the alga (Matheickal, 1998).

The carboxylic group in this alga contains the following minor groups: O-H stretching, O-H Bending, and C-O stretching. The O-H Bending group was observed to shift clearly at a wave number of 1414 cm^{-1} for all four heavy metals while the other groups did not seem to have shifts of the wave number of the peak. These indicated that there was

a high potential that O-H Bending groups from carboxylic acid involved with heavy metals binding.

For amine group, there were changes in wave number for N-H stretching, in the Cu and Cd sorptions. The C-N stretching was found to be a clear peak at 1324 cm^{-1} for the sorptions of Cd and Pb, while the sorption of Cu and Zn saw a large shift to the wave number of 1077 cm^{-1}. The N-H Bending group did not seem to change for all metals. These can be interpreted that N-H stretching in amine group was employed for Cu and Cd sorption and C-N stretching was for all four metal sorption while N-H Bending were thought not to involve in the metal binding by this biomass.

The observation for amide group revealed that N-H stretching was shifted by Cd sorption where the new peak occurred at a wave number of 3440 cm^{-1} and 3330 cm^{-1} for Cu and Pb sorption, respectively. For this case, the new peak occurred while the old peak still existed. This might lead to the conclusion that this N-H stretching was available in excess quantity for the adsorption of Cu and Pb. The occupied N-H stretching band might show its transmission peak at the new shifted wave number whereas the un-occupied was at the normal wave number (the same as in the fresh algae). The C-O stretching group in this amide group did not show shift in wavelength which suggested that this was not involved with the adsorption in this work.

On the other hand, C-O in amino group seemed to play an important role for all metal sorption a shift in the wavelength was always found. The N-H Bending in this amino group, however, was not found to involve with the adsorption of Cu, Cd, and Pb but involved for Zn.

The S=O stretching in the sulfonyl and sulfonate groups involved with all four metal sorption since the peak in sulfonate group at 1366 cm^{-1} disappeared and occurred at 1384 cm^{-1} in sulfonyl group. The S-O stretching in sulfonate group was found to have a new peak at 800 cm^{-1} in the sorption of Zn. This mean S=O stretching was employed for all four metals sorption while S-O stretching was employed for Zn sorption. This result agreed with Matheickal's work in 1998, for which he reported that sulfonate group had a strongly acidic characteristic, which enabled the heavy metal adsorption even at low pH.

In shorts, the possible functional groups that may be employed for each metal binding by *Caulerpa lentillifera* can be concluded in the following tabulation:

	Cu	Cd	Pb	Zn
Carboxylic				
O-H Bending	*	*	*	*
Amine				
N-H Stretching	*	*		
C-N Stretching	*	*	*	*
Amide				
N-H Stretching	*	*	*	
Amino				
C-O	*	*	*	*
N-H Bending				*
Sulfonyl				
S=O Stretching	*	*	*	*
Sulfonate				
S=O Stretching	*	*	*	*
S-O Stretching				*

It should be noted that FT-IR resuts in this work did not rather provide quantitative analysis nor the level of affinity to each metal of the functional group presented in the alga. Hence, although there might be several potential functional groups for the metal binding, this did not reflect the ability of the alga in sorping the heavy metals. They only presented the possibility of the coupling between the metal species and the functional groups in the alga.

4.3 Biosorption of binary components heavy metal.

The adsorption experiment for binary heavy metal mixtures was carried out in a similar manner as the single metal experiment. Two heavy metals were selected from the four heavy metals, i.e. Cu, Cd, Pb, and Zn, with the following combinations: Cu-Cd, Cu-Pb, Cu-Zn, Cd-Cu, Cd-Pb, Cd-Zn, Pb-Cu, Pb-Cd, Pb-Zn Zn-Cu, Zn-Cd, and Zn-Pb. The mixture solution was then prepared with a primary heavy metal concentration varied from 10, 25, and 50 mg/l and the secondary heavy metal concentration was fixed at 50 mg/l.

4.3.1 Copper as primary metal

In this series, Cu was set as a primary component and Cd, Pb or Zn as secondary components. Figure 4.6 display the isotherm of Cu in binary component system compared with that in the single component system. The observation from the binary sorption isotherm found that the adsorption capacity of the alga for Cu was only about 45.5% (with Cd), 44.5% (with Pb), and 40.7% (with Zn) of the capacity obtained when the solution only contained Cu ion, respectively. To compare the total adsorption capacity of the alga, all metals adsorpbed onto the surface of the alga must be considered. This was achieved by adding the total moles of primary and secondary metal species adsorbed onto the alga, and

the results are summarized in the tabulation below. It is clear that the total adsorption capacity for the heavy metals seemed to be lower in the binary mixture cases than the single component system. This indicates strongly that there was a competitive adsorption of these four metals on the surface of the alga and the presence of one extra metal species reduced the total adsorption capacity of the alga.

Mixture	qCu for single system mmol/g	qCu for binary system mmol/g	q secondary metal for binary system mmol/g	Total q in binary (q Cu + q secondary metal) mmol/g
Cu with Cd	0.0680	0.0310	0.0099	0.0409
Cu with Pb	0.0680	0.0303	0.0116	0.0419
Cu with Zn	0.0680	0.0277	0.0078	0.0355

The second column in the above tabulation illustrates the adsorption capacity for the single component system whereas the last column represents the total combined adsorption capacity for the binary systems. The comparison between these two columns illustrated that the removal efficiency at all copper concentrations for binary systems were considerably less than that from a single component system. Zn at 50 mg/l seemed to be most effective in reducing Cu adsorption capacity in the binary system. This may be because Zn was present in the highest quantity (in molar concentration). The conversion of concentration units from mass to mole revealed that 50 mg/l of Zn was 0.76 mmol/l, whereas 50 mg/l of Pb was 0.24 mmol/l, and Cd was 0.44 mmol/l. This high quantity of Zn could then affect the adsorption in a greater extent than other metals. It is interesting to note that, although Pb was present in a small quantity, it interfered more with the adsorption of Cu than Cd. This indicated that Pb might be more easily adsorbed to the binding site of algae than Cd.

4.3.2 Cadmium as primary metal

In this case, Cd was set as a primary component and Cu, Pb or Zn as secondary components. Figure 4.7 display the isotherm of Cd in binary component compared with that in the single component system. The results revealed that the adsorption capacity of the alga for Cd in the binary system was only about 31.1% (with Cu), 22.6% (with Pb), and 24.8% (with Zn) of the capacity obtained when the solution only contained Cd ion. The next tabulation displays total sorption capacities for Cd in binary mixtures along with the single component adsorption and the adsorption for the secondary metal in each particular case.

Mixture	qCd for single system mmol/g	qCd for binary system mmol/g	q secondary metal for binary system mmol/g	Total q in binary (q Cd + q secondary metal) mmol/g
Cd with Cu	0.0318	0.0099	0.0310	0.0409
Cd with Pb	0.0318	0.0072	0.0116	0.0188
Cd with Zn	0.0318	0.0079	0.0128	0.0207

Again the total adsorption capacity for the heavy metals seemed to be lower in the binary mixture cases than in the single component system. There was, however, one exception for the adsorption of Cd with Cu where the total adsorption was found to be higher than the adsorption for Cd alone. This indicated that the some of the binding sites for Cd might not be the same as these for Cu. On the other hand, Section 4.3.1 showed that Cd reduced Cu sorption capacity which indicated that there might exist some common binding sites for Cd and Cu, and the adsorption of Cu was more favorable than Cd for those binding sites. Considering the isotherm for Cd in the binary solution of Cd with Cu at 50 mg/l (0.78 mM), Pb at 50 mg/l (0.24 mM), and Zn at 50 mg/l (0.76 mM), the results demonstrated that the presence of other metal tended to reduce Cd removal. Pb was found to be the most effective in reducing Cd removal whereas Cu was found to have more effect than Zn. This suggested again that Pb might be more easily adsorbed to the binding site of algae than Cu and Zn, respectively.

4.3.3 Lead as primary metal

In this section, Pb was set as a primary component and Cu, Cd or Zn as secondary components. Figure 4.8 display the isotherm of Pb in binary component system compared with that in the single component system. The results revealed that the adsorption capacity of the alga for Pb was only about 28.5% (with Cu), 28.5% (with Cd), and 22.6% (with Zn) of the capacity obtained when the solution only contained Pb ion. The next tabulation displays total sorption capacities for Pb in binary mixtures along with the single component adsorption and the adsorption for the secondary metal in this particular case.

Mixture	qPb for single system mmol/g	qPb for binary system mmol/g	q secondary metal for binary system mmol/g	Total q in binary (q Pb + q secondary metal) mmol/g
Pb with Cu	0.0407	0.0116	0.0303	0.0419
Pb with Cd	0.0407	0.0116	0.0072	0.0188
Pb with Zn	0.0407	0.0092	0.0099	0.0191

Again the total adsorption capacity for the heavy metals seemed to be lower in the binary mixture cases than in the single component system. There is, however, one

exception for the adsorption of Pb with Cu where the total adsorption was found to be higher than the adsorption for Pb alone. This indicated that the some of the binding sites for Pb might not be the same as those for Cu. On the other hand, Section 4.3.1 showed that Pb reduced Cu sorption capacity which indicated that there might exist some common binding sites for Pb and Cu. Considering the percentage for Pb in the binary solution of Pb with Cu at 50 mg/l (0.78 mM) , Cd at 50 mg/l (0.44 mM), and Zn at 50 mg/l (0.76 mM), the results demonstrated that the presence of other metal tended to reduce Pb removal. Zn was found to be the most effective in reducing Pb removal whereas Cu and Cd were found to have similar effect.

It is reminded here that, in the experiment for a single metal system, the adsorption of Pb did not reach its maximum adsorption capacity. The isotherm for the binary component system emphasized here again that this Pb adsorption was not at the saturation point. This might be the reason why Pb adsorption capacity was lower than Cu.

4.3.4 Zinc as primary metal

In this part, Zn was set as a primary component and Cd, Pb or Zn as secondary components. Figure 4.9 display the isotherm of Zn in binary component system compared with that in the single component system. The observation from the binary sorption isotherm found that the adsorption capacity of the alga for Zn was only about 11.8% (with Cu), 19.4% (with Cd), and 15.0% (with Pb) of the capacity obtained from the solution only contained Zn ion, respectively. The next tabulation displays total sorption capacities for Zn in binary mixtures along with the single component adsorption and the adsorption for the secondary metal for this case.

Mixture	qZn for single system mmol/g	qZn for binary system mmol/g	q secondary metal for binary system mmol/g	Total q in binary (q Zn + q secondary metal) mmol/g
Zn with Cu	0.0659	0.0078	0.0277	0.0355
Zn with Cd	0.0659	0.0128	0.0079	0.0207
Zn with Pb	0.0659	0.0099	0.0092	0.0191

The results illustrated that the sorption capacity for binary systems were considerably less than that from a single component system. Cu at 50 mg/l seemed to be most effective in reducing Zn adsorption capacity in binary system. This may be because Cu was present in the highest quantity (in molar concentration). The conversion of concentration units from mass to mole revealed that 50 mg/l of Cu, Cd, and Pb was 0.78 mmol/l, 0.44 mmol/l, and 0.24 mmol/l, respectively. This high quantity of Cu could then affect the adsorption in a greater extent than other metals. It is interesting to note that,

although Pb was present in a small quantity, it interfered more with the adsorption of Zn than Cd. This indicated that Pb could be more easily adsorbed to the binding site of algae than Cd. This agrees with the result in Section 4.3.1.

4.3.5 Adsorption of binary mixture heavy metal solution.

To investigate the effect of the secondary component on the overall adsorption capacities of the alga, the experiment was set to have the overall initial concentration of all heavy metals at 50 mg/l. The data on adsorption capacities are summarized below:

Mixture (1^{st} metal with 2^{nd} metal)	q of 1^{st} metal at C_i = 50 mg/l for single system (mmol/g)	q of 1^{st} metal at C_i = 50 mg/l for binary system (mmol/g)	q of 2^{nd} metal at C_i = 50 mg/l for single system (mmol/g)	q of 2^{nd} metal at C_i = 50 mg/l for binary system (mmol/g)
Cu with Cd	0.0497	0.0310	0.0218	0.0099
Cu with Pb	0.0497	0.0303	0.0199	0.0116
Cu with Zn	0.0497	0.0277	0.0445	0.0078
Cd with Pb	0.0218	0.0072	0.0199	0.0116
Cd with Zn	0.0218	0.0079	0.0445	0.0128
Pb with Zn	0.0199	0.0092	0.0445	0.0099

The results showed that the sorption capacitiy of each metal in binary mixture decreased when compared with that of the single component. These indicated that all of metals were mainly bonded on the same of binding sites and this agrees with the results from the determination of functional groups for metal binding in Section 4.2.2. The capability of metal to bond with these binding sites was believed to depend on the affinity of each metal to those binding sites. However, there must be same other binding sites for each specific metal species, but might be at lesser quantity than this main site.

4.3.6 Concluding remarks for binary sorption.

From the results above, the effect of secondary metal species on reducing the adsorption capacity for the primary metal could be summarized as:

Primary heavy metal	Effect of secondary metal on reducing adsorption capacity for primary metal
Cu	Pb > Cd
Cd	Pb > Cu > Zn
Pb	Cannot conclude the order of the effect
Zn	Pb > Cd, Pb > Cu

Pb seemed to be the component with the highest affinity to the binding sites of algae since it dominated in the binary sorption and it was always the most easily adsorbed

component than other metals. This result agreed well with the isotherm model from previous section that Pb was with the greatest affinity constant (b) in the Langmuir model and also the greatest for K and 1/n from the Freundlich model. The order of affinity to the binding site of this alga observed from the binary sorption systems might then be finalized as follows: Pb > Cu > Zn > Cd according to the order obtained from Freundlich model in Section 4.2.1. All results in binary sorption systems were statistically proven by t-test to check for the accuracy of the results. Testing results indicated that experimental findings were accepted at the level of significance was 0.05 ($\alpha = 0.05$).

4.4 Biosorption of ternary component heavy metals

The adsorption experiment for ternary heavy metal mixtures was carried out in a similar fashion with single and binary metal cases. Three heavy metals were selected from the four heavy metals: Cu, Cd , Pb, and Zn with the following combinations: Cu-Cd-Pb, Cu-Cd-Zn, Cu-Pb-Zn, Cd-Pb-Zn. The mixture solution was then prepared with equimolar heavy metals at 0.1, 0.2 , and 0.3 mmol/l.

4.4.1 Cu-Cd-Pb Series

Figures 4.10.1 and 4.10.2 illustrate isotherms of ternary component adsorption system and the relationship between the removal percentage and initial concentration for the three components: Cu, Cd, and Pb. The detail can be summarized as follows:

Initial conc	Cu		Cd		Pb	
(mM)	%Removal	SD	%Removal	SD	%Removal	SD
0.1	92.86	1.86	80.74	1.53	100	0
0.2	88.39	0.70	67.62	5.80	100	0
0.3	84.93	1.68	55.35	3.42	100	0

The results showed that Pb was totally adsorbed (100%) while Cu could be adsorbed in a lower extent but still more than Cd. The total sorption capacities for ternary component system were compared with those of single component systems and the results are:

Single component system			Ternary component system.			
q of Cu C_i = 0.3 mM (mmol/g)	q of Cd C_i = 0.3 mM (mmol/g)	q of Pb C_i = 0.3 mM (mmol/g)	q of Cu C_i = 0.1 mM (mmol/g)	q of Cd C_I = 0.1 mM (mmol/g)	q of Pb C_i = 0.1 mM (mmol/g)	Total q (mmol/g)
0.0148	0.0134	0.0174	0.0056	0.0048	0.0060	0.0164

The result revealed that the total sorption capacity of the three metals in ternary component solution was always more than that of the single component system but there was only one exception for the Pb case where the sorption capacity in the single sorption system was higher than that in the ternary system. This implied that there was a difference in binding site for each heavy metal and the quantity of binding site for Pb might be higher than Cu and Cd. Interestingly, Pb had the highest sorption capacity in single and ternary component systems. This ensured that Pb was the most favorable for the binding site of this alga compared with Cu and Cd.

Note that the concentration range for the ternary component experiment was still in the low concentration range where the isotherm did not reach the maximum capacity level. In this range, the relationship between adsorption capacity and the equilibrium concentration was still first order. Experiments at high concentration range might give different results, as the adsorption capacity might be zero order with equilibrium concentration.

4.4.2 Cu-Cd-Zn Series

Figures 4.11.1 and 4.11.2 illustrate isotherm of ternary component and the relationship between the removal percentage and initial concentration for the ternary mixture sorption of Cu, Cd, and Pb. The detail can be summarized as follows:

Initial conc	Cu		Cd		Zn	
(mM)	%Removal	SD	%Removal	SD	%Removal	SD
0.1	92.75	0.37	84.8	1.18	90.95	1.37
0.2	88.44	0.85	76.8	1.03	72.38	1.18
0.3	86.04	0.19	60.96	0.72	55.77	0.52

The results showed that Cu was adsorbed more than Cd and Zn at each initial concentration. The total sorption capacities for ternary component system were compared with those of single component systems and the results are:

Single component system			Ternary component system.			
q of Cu	q of Cd	q of Zn	q of Cu	q of Cd	q of Zn	Total q
C_i = 0.3 mM	C_i = 0.3 mM	C_i = 0.3 mM	C_i = 0.1 mM	C_i = 0.1 mM	C_i = 0.1 mM	
(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)
0.0148	0.0134	0.0100	0.0056	0.0051	0.0054	0.0161

Again the result revealed that the total sorption capacity of three metals in ternary component solution is always more than that of the single component system. This implied that there was a different binding site for each heavy metal. However, the adsorption capacity for Zn in the single and ternary componnent systems were generally found to have the lowest sorption capacity compared with Cu and Cd. It was possible that Zn had the least affinity for this alga and therefore the capacity of Zn was quite low at the range of concentration employed in this work.

4.4.3 Cu-Pb-Zn series

Figures 4.12.1 and 4.12.2 illustrate isotherm of ternary component and the relationship between the removal percentage and initial concentration for the ternary mixture sorption of Cu, Pb, and Zn. The detail can be summarized as follows:

Initial conc	Cu		Pb		Zn	
(mM)	%Removal	SD	%Removal	SD	%Removal	SD
0.1	90.41	1.51	100	0	85.64	6.87
0.2	8.49	2.11	99.93	0.11	55.72	1.83
0.3	84.37	2.08	97.36	0.74	41.26	1.54

The results showed that Pb were the greatest adsorbed species (96 - 100%) followed by Cu and Zn accordingly. The total sorption capacities for ternary component system were compared with those of single component systems and the results are:

Single component system			Ternary component system.			
q of Cu	q of Pb	q of Zn	q of Cu	q of Pb	q of Zn	Total q
C_i = 0.3 mM	C_i = 0.3 mM	C_i = 0.3 mM	C_i = 0.1 mM	C_i = 0.1 mM	C_i = 0.1 mM	
(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)	(mmol/g)
0.0148	0.0174	0.0100	0.0054	0.0060	0.0051	0.0165

The result revealed that the total sorption capacity of three metals in ternary component system was always more than that of the single component system. There was, again, one exception for the Pb case where the sorption capacity for Pb alone was found to be higher than the total sorption capacity for ternary sorption system. Moreover, Pb had the highest sorption capacity in single and ternary component systems. This emphasized the conclusion that Pb had the highest quantity of binding site in the alga and had the most affinity to the binding site of this alga compared with the other metals, while Zn was the least adsorbed species (lowest affinity) on this alga in the range of low metal concentration.

4.4.4 Cd-Pb-Zn series

Figures 4.13.1 and 4.13.2 illustrate isotherm of ternary component and the relationship between the removal percentage and initial concentration for the ternary mixture sorption of Cd, Pb, and Zn. The detail can be summarized as follows:

Initial conc	Cd		Pb		Zn	
(mM)	%Removal	SD	%Removal	SD	%Removal	SD
0.1	79.98	2.09	100	0	80.86	1.13
0.2	72.06	1.85	100	0	66.97	3.54
0.3	59.10	1.82	99.00	0.49	58.81	2.67

The results showed that again Pb were best adsorbed by the alga at removal percentage about 99 - 100 while the Cd and Zn had similar adsorption capacity. The total sorption capacities for ternary component system were compared with those of single component systems and the results are:

Single component system			Ternary component system.			
q of Cd C_i = 0.3 mM (mmol/g)	q of Pb C_i = 0.3 mM (mmol/g)	q of Zn C_i = 0.3 mM (mmol/g)	q of Cd C_i = 0.1 mM (mmol/g)	q of Pb C_i = 0.1 mM (mmol/g)	q of Zn C_i = 0.1 mM (mmol/g)	Total q (mmol/g)
0.0134	0.0174	0.0100	0.0048	0.0060	0.0048	0.0156

The result revealed that the total sorption capacity of the three metals in the ternary component solution was always more than that of the single component system but there are exception for Pb where the sorption capacity for Pb alone was found to higher than that in ternary sorption system. Furthermore, Pb had the highest sorption capacity both in single and ternary sorption systems. This, again, concluded that there was a difference in binding site responsible for the binding of each heavy metal and the binding site for Pb seemed to be present in the largest quantity and the affinity for Pb was also the most active.

4.4.5 Concluding remarks for ternary sorption.

It can be observed from these experimental results that the removal efficiencies for all heavy metals examined in this research decreased with an increase in initial concentration like that obtained from the single component system. The order of metal affinity to the binding site of algae for each series can be summarized as follows:

Series	Order of metal affinity
Cu-Cd-Pb	Pb > Cu > Cd
Cu-Cd-Zn	Cu > Cd.> Zn.
Cu-Pb-Zn	Pb > Cu > Zn.
Cd-Pb-Zn	Pb > Cd ≅ Zn.

These results indicated the affinity for each heavy metals by this alga *Caulerpa lentillifera* could be ordered from high to low as: Pb > Cu > Cd > Zn. This result was in contrast with that obtained from the single component system where Cd exhibited the lowest adsorption characteristics. It was possible that, in this experiment, the range of concentration was rather low and fell in the region where Cd adsorption was better than Zn. In a single component experiment, the concentration range was rather high and, in this region, Zn adsorption was better than Cd. All results in ternary sorption systems were statistically proven by t-test to check for the accuracy of the results. Testing results indicated that experimental findings were accepted at the level of significance was 0.05 ($\alpha = 0.05$).

4.5 Biosorption of four-components heavy metals

The adsorption experiment for four heavy metal mixtures was carried out in a similar fashion with the ternary component where each heavy metal was prepared at the same concentrations of 0.1, 0.2, and 0.3 mmol/l.

4.5.1 Removal percentage and total sorption capacities of four component mixtures

Figures 4.14.1 and 4.14.2 illustrate the isotherm of the four components and the relationship between removal percentage and initial concentration for four-component mixture sorption of Cu, Cd, Pb, and Zn, respecrively. The detail can be summarized as follows:

Initial	Cu		Cd		Pb		Zn	
Conc.	%Removal	SD	%Removal	SD	%Removal	SD	%Removal	SD
0.1	90.17	1.63	81.94	1.35	100	0	85.57	3.16
0.2	84.96	2.20	65.97	1.47	100	0	55.26	4.72
0.3	85.27	0.49	51.46	1.15	99.4	0.55	47.19	1.26

The results showed that the increae in initial concentration resulted in a decrease of removal percentage, similar finding as in the single component, binary component, and ternary component systems. The preferentia order of heavy metals to the binding site of algal surface was: Pb > Cu > Cd > Zn. This agrees with results in ternary component

system. The total sorption capacities for the four-component system were compared with those of single component systems and the results are:

Single component system				Four-component system.				
q of Cu $C_i = 0.4$ mM (mmol/g)	q of Cd $C_i = 0.4$ mM (mmol/g)	q of Pb $C_i = 0.4$ mM (mmol/g)	q of Zn $C_i = 0.4$ mM (mmol/g)	q of Cu $C_i = 0.1$ mM (mmol/g)	q of Cd $C_i = 0.1$ mM (mmol/g)	q of Pb $C_i = 0.1$ mM (mmol/g)	q of Zn $C_i = 0.1$ mM (mmol/g)	Total q (mmol/g)
0.0200	0.0172	0.0231	0.0135	0.0054	0.0051	0.0060	0.0049	0.0214

The result revealed that the total sorption capacity of three metals in four component system was always more than that of the single component system. There was, again, one exception for the Pb case where the sorption capacity for Pb alone was found to be higher than the total sorption capacity for the ternary sorption system. Moreover, Pb had the highest sorption capacity in single and ternary component systems. This confirmed the conclusion that Pb had the highest quantity of binding site in the alga and had the most affinity to the binding site of this alga compared with the other metals. Nevertheless Zn was found to have the lowest sorption capacity both in single and in four component systems. This confirmed the conclusion that Zn was the least adsorbed species (lowest affinity) on this alga in the range of low metal concentration. Again, all results in four component system had already statistically proven by t-test to check for the accuracy of the results. Testing results indicated that experimwntal findings were accepted at the level of significance was 0.05 ($\alpha = 0.05$).

4.6 Concluding remarks

The removal efficiencies for all heavy metals examined in this research decreased with an increase in initial concentration. The results from ternary and four component systems supported the conclusion of the order for algae's affinity for each heavy metal. Specifically, the affinity could be ordered from high to low as: Pb > Cu > Cd > Zn at low concentration range and Pb > Cu > Zn > Cd at high concentration range. The total sorption capacity seemed to lower than those of single systems but except for Zn.

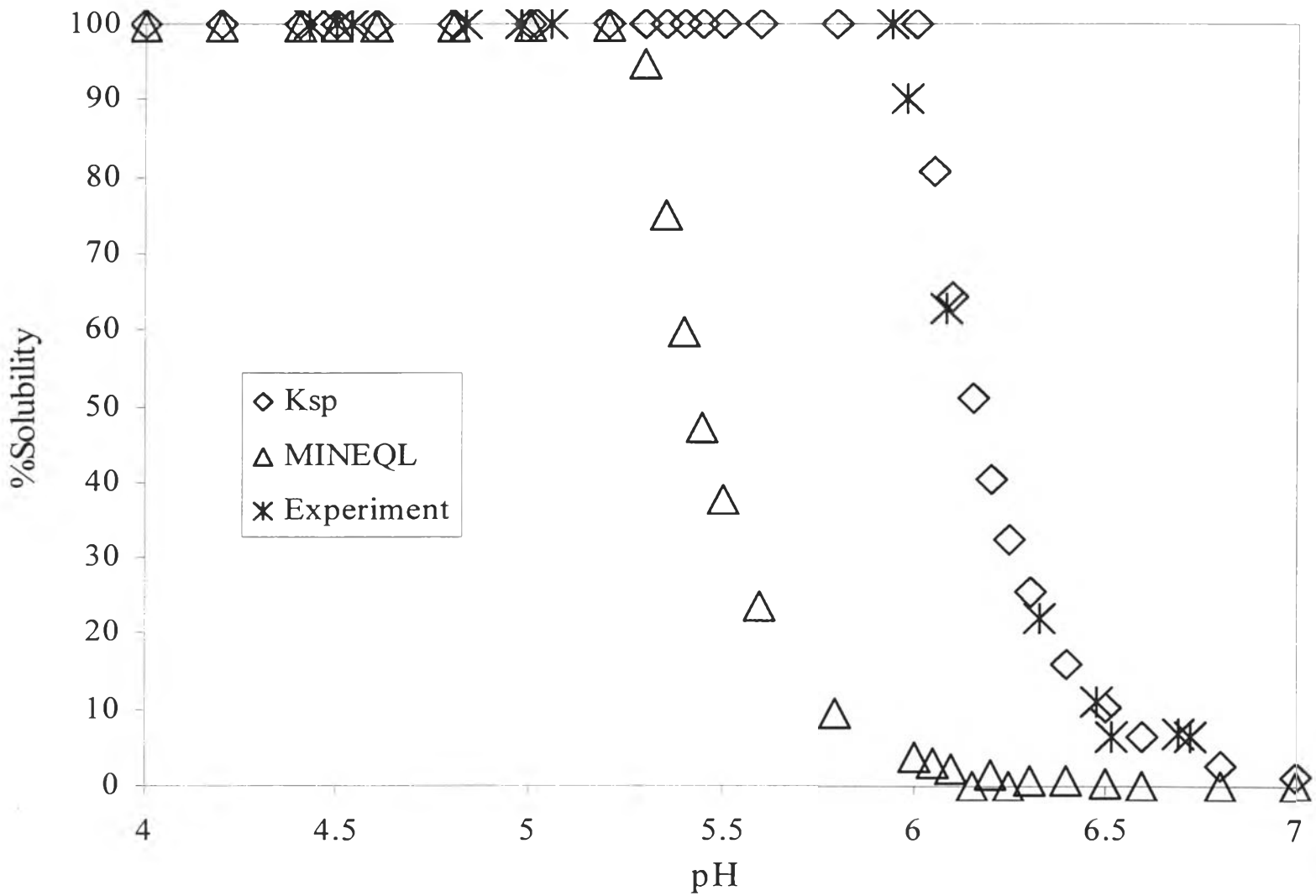

Figure 4.1.1 Solubility of copper (II) ion with initial concentration of 100 mg/l

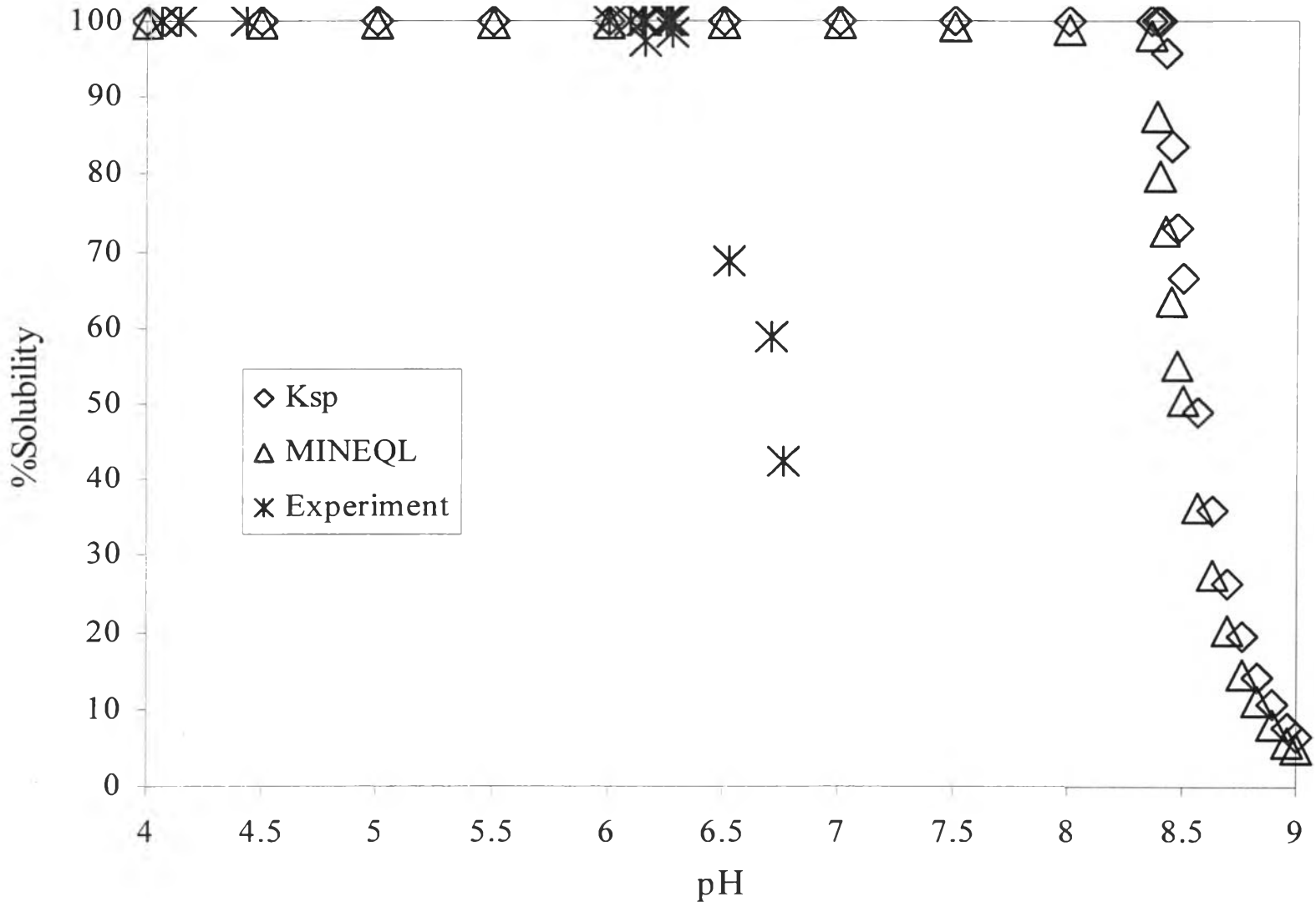

Figure 4.1.2 Solubility of cadmium (II) ion with initial concentration of 100 mg/l

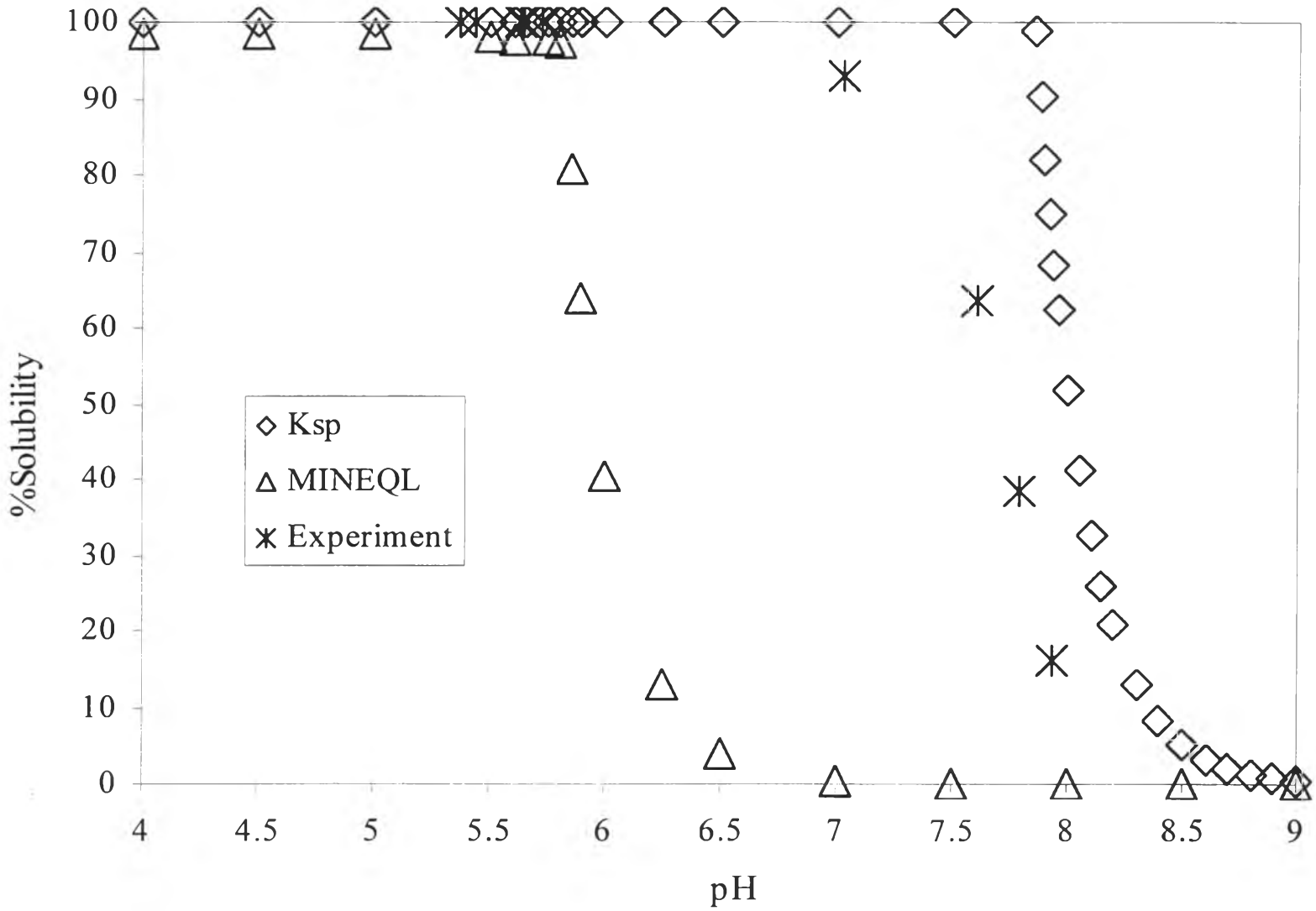

Figure 4.1.3 Solubility of lead (II) ion with initial concentration of 100 mg/l

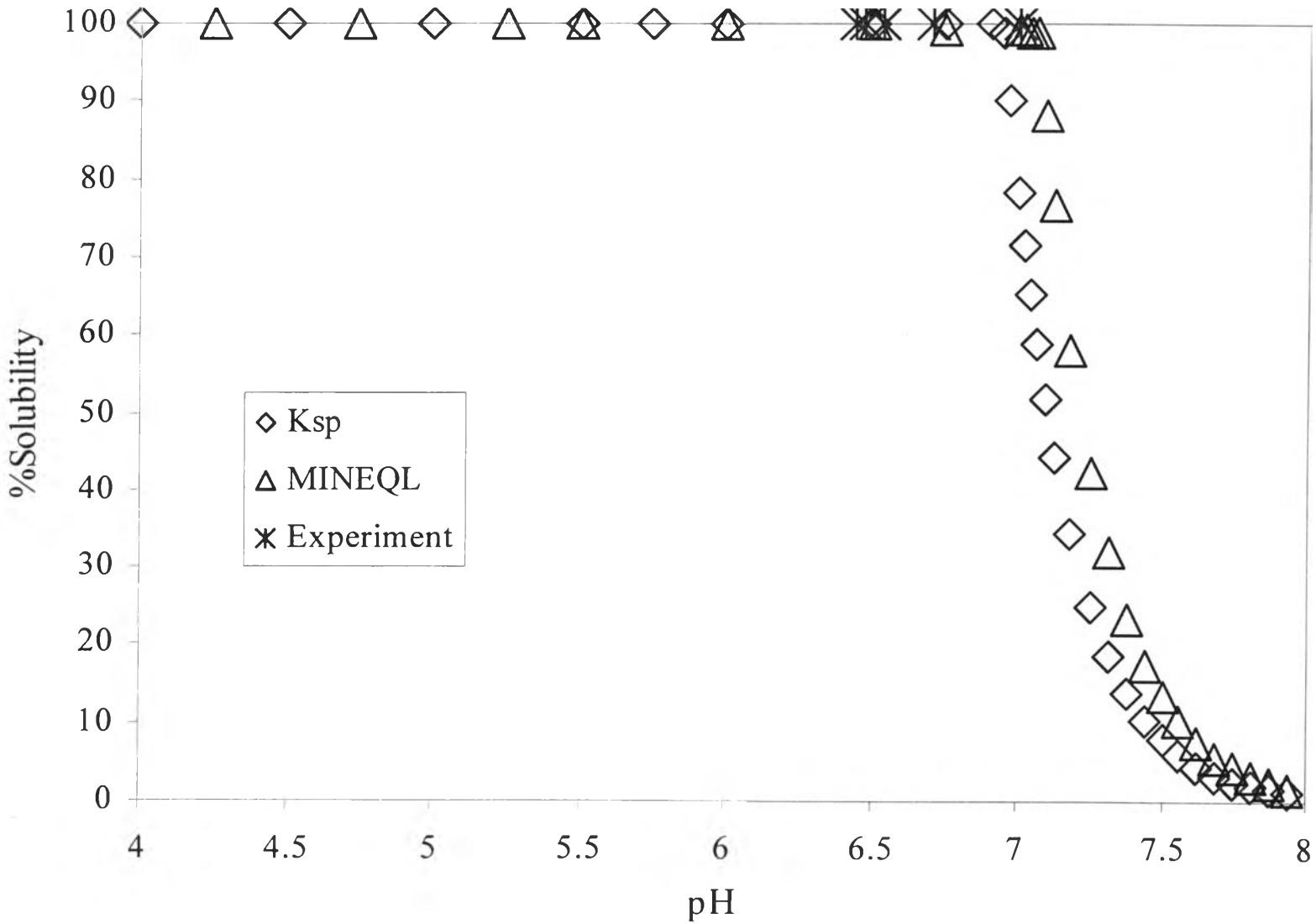

Figure 4.1.4 Solubility of zinc (II) ion with initial concentration of 100 mg/l

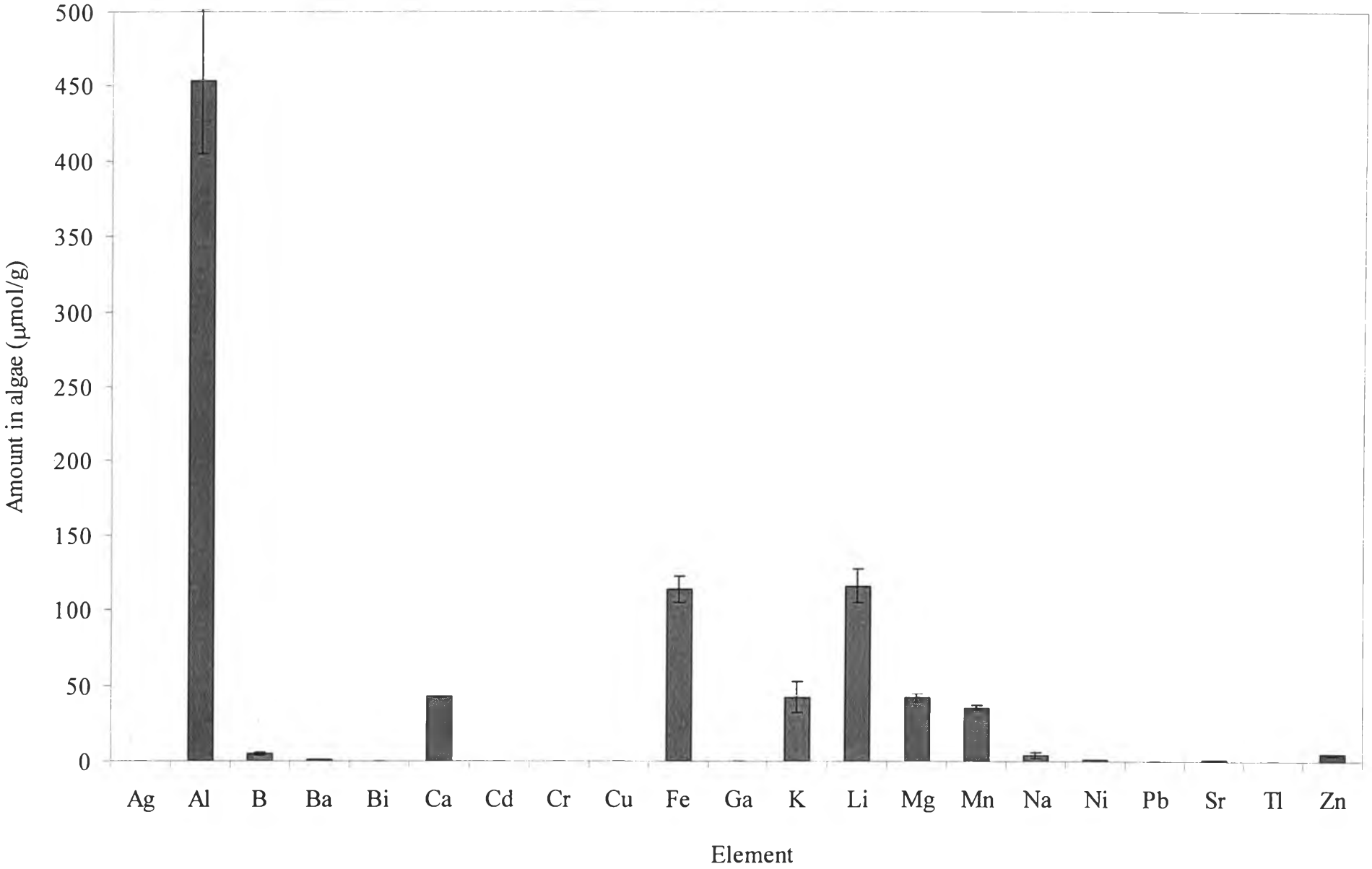

Figure 4.2 Metal composition in *Caulerpa lentillifera*

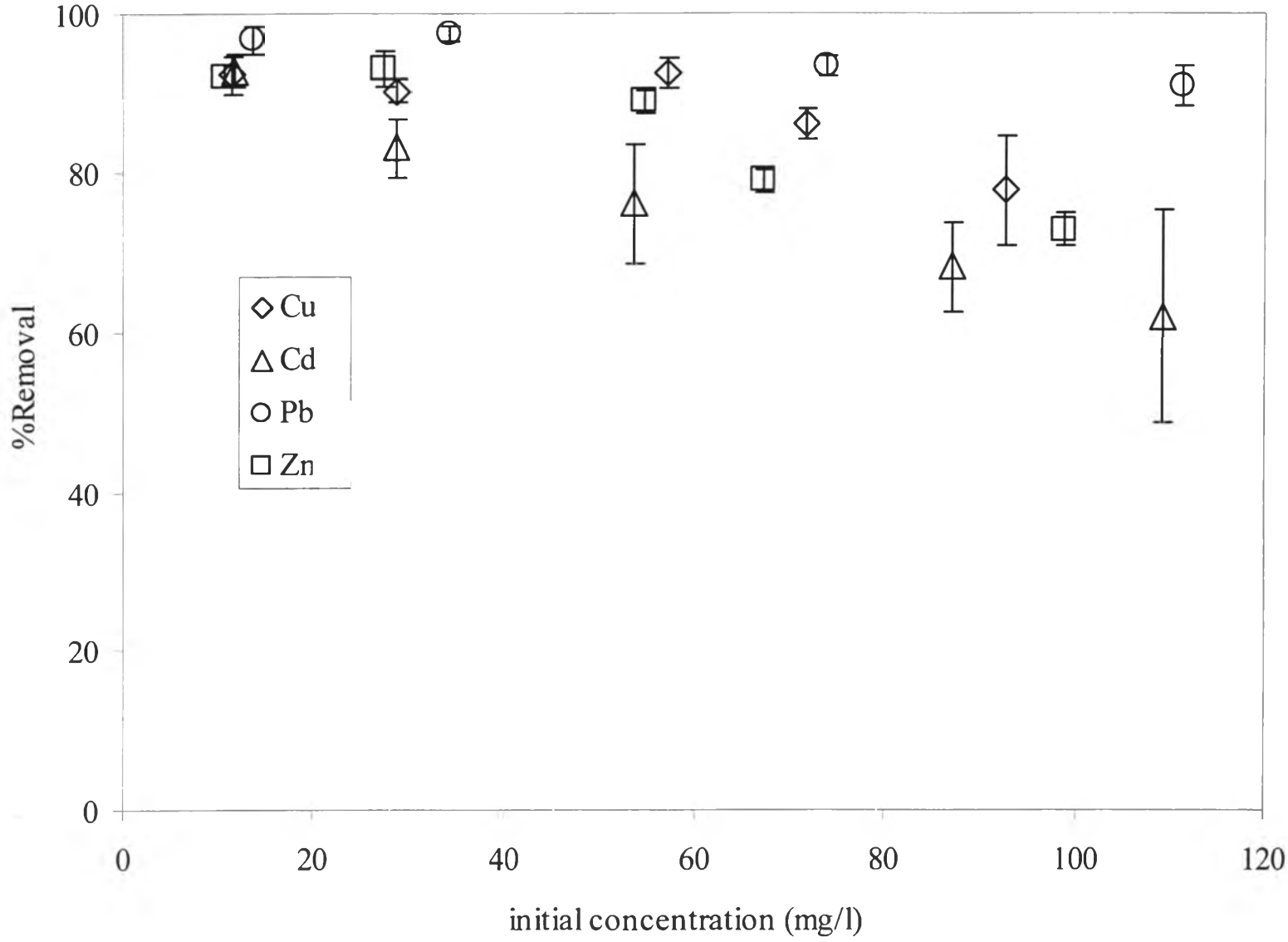

Figure 4.3.1 Removal percentage of heavy metals with mass unit concentration

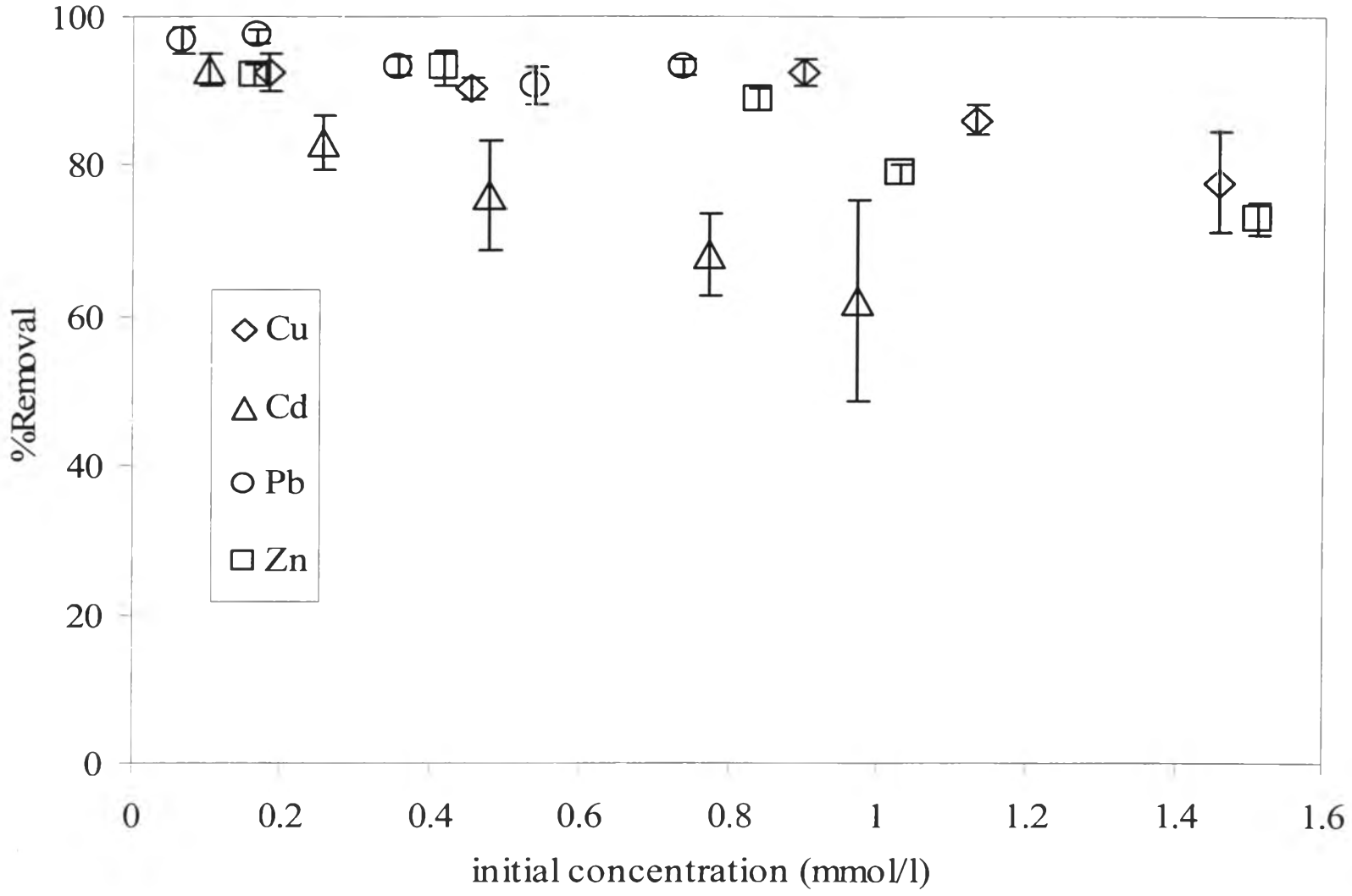

Figure 4.3.2 Removal percentage of heavy metals with molar unit concentration

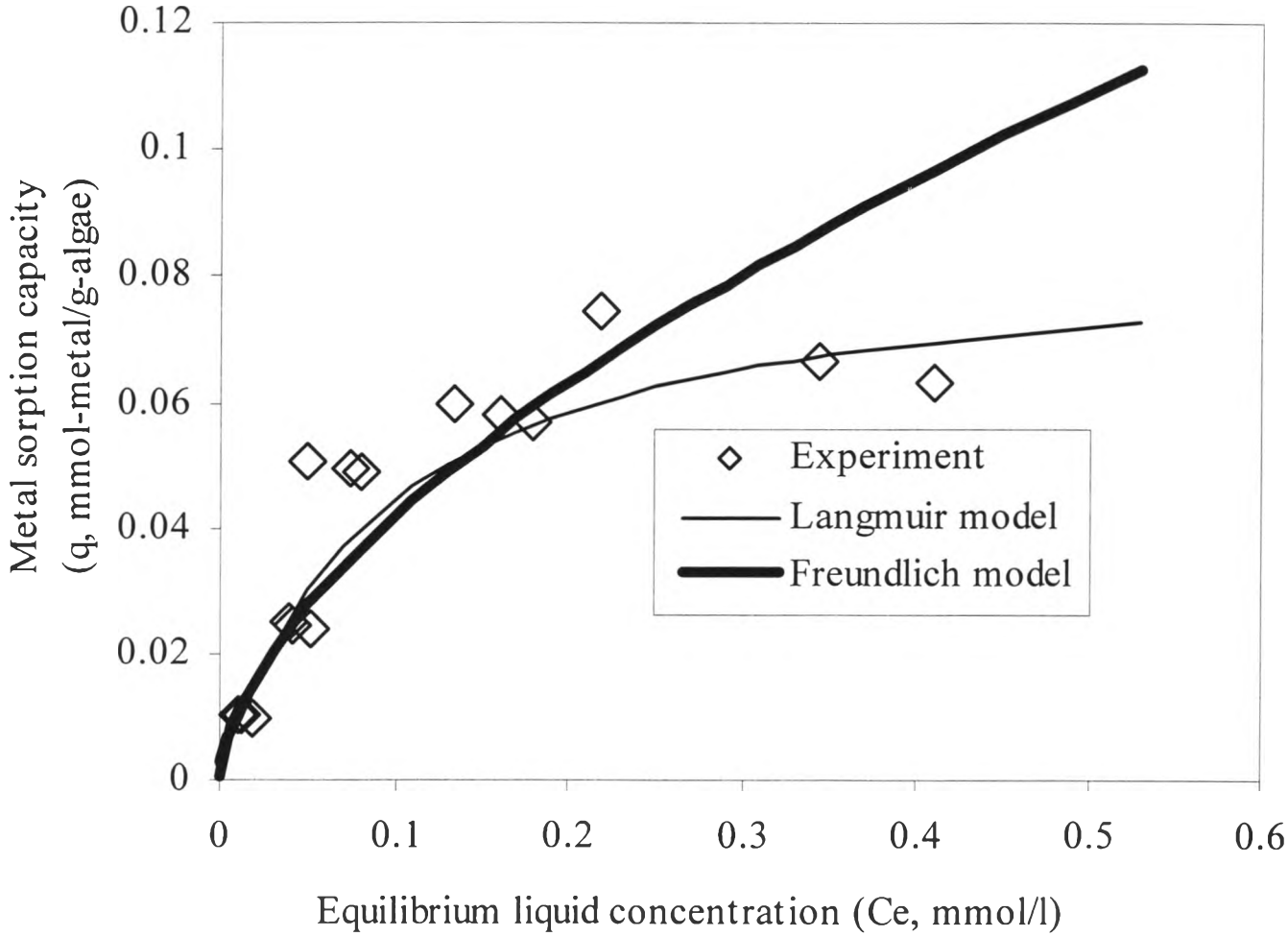

Figure 4.4.1 Isotherm of copper

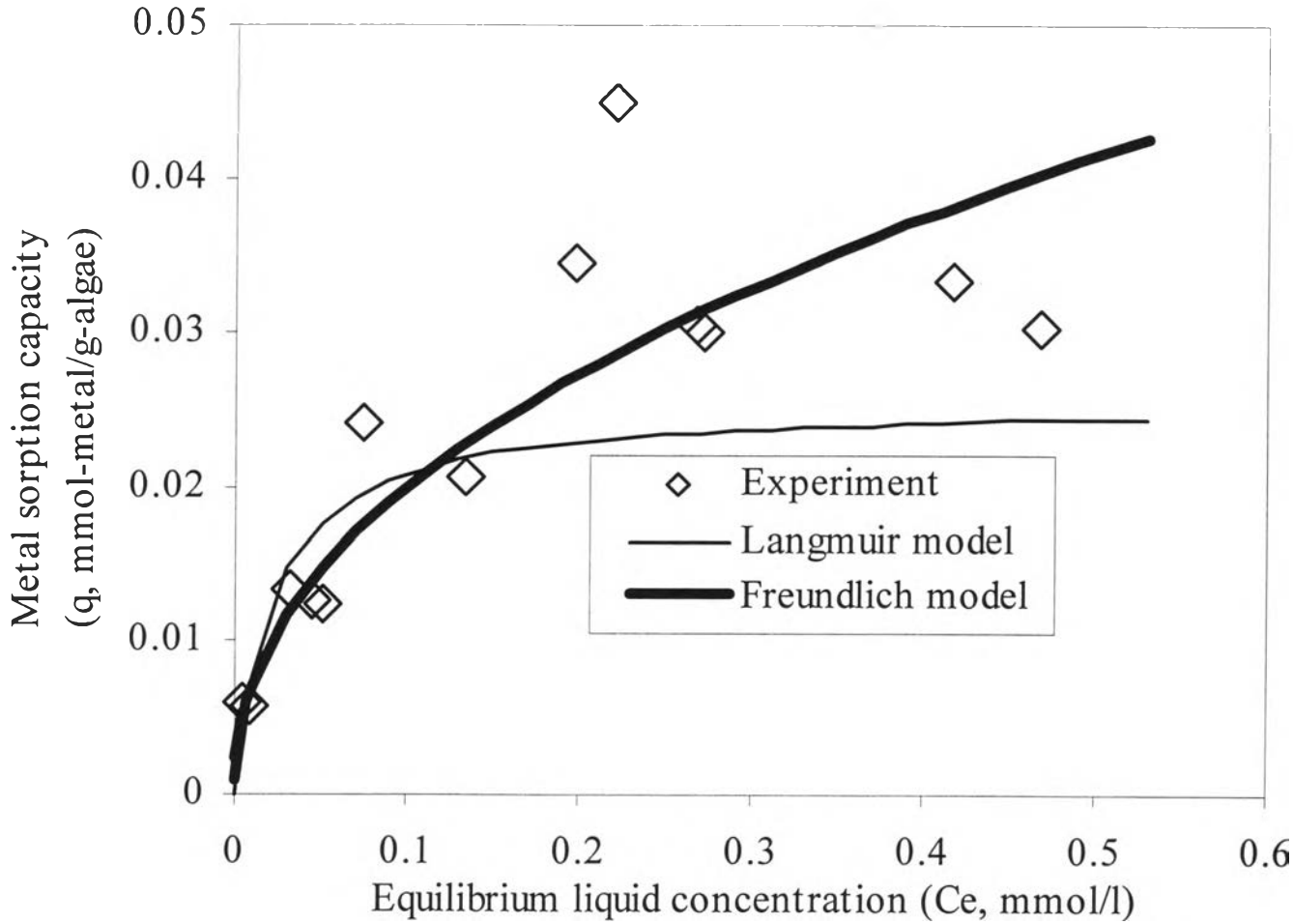

Figure 4.4.2 Isotherm of cadmium

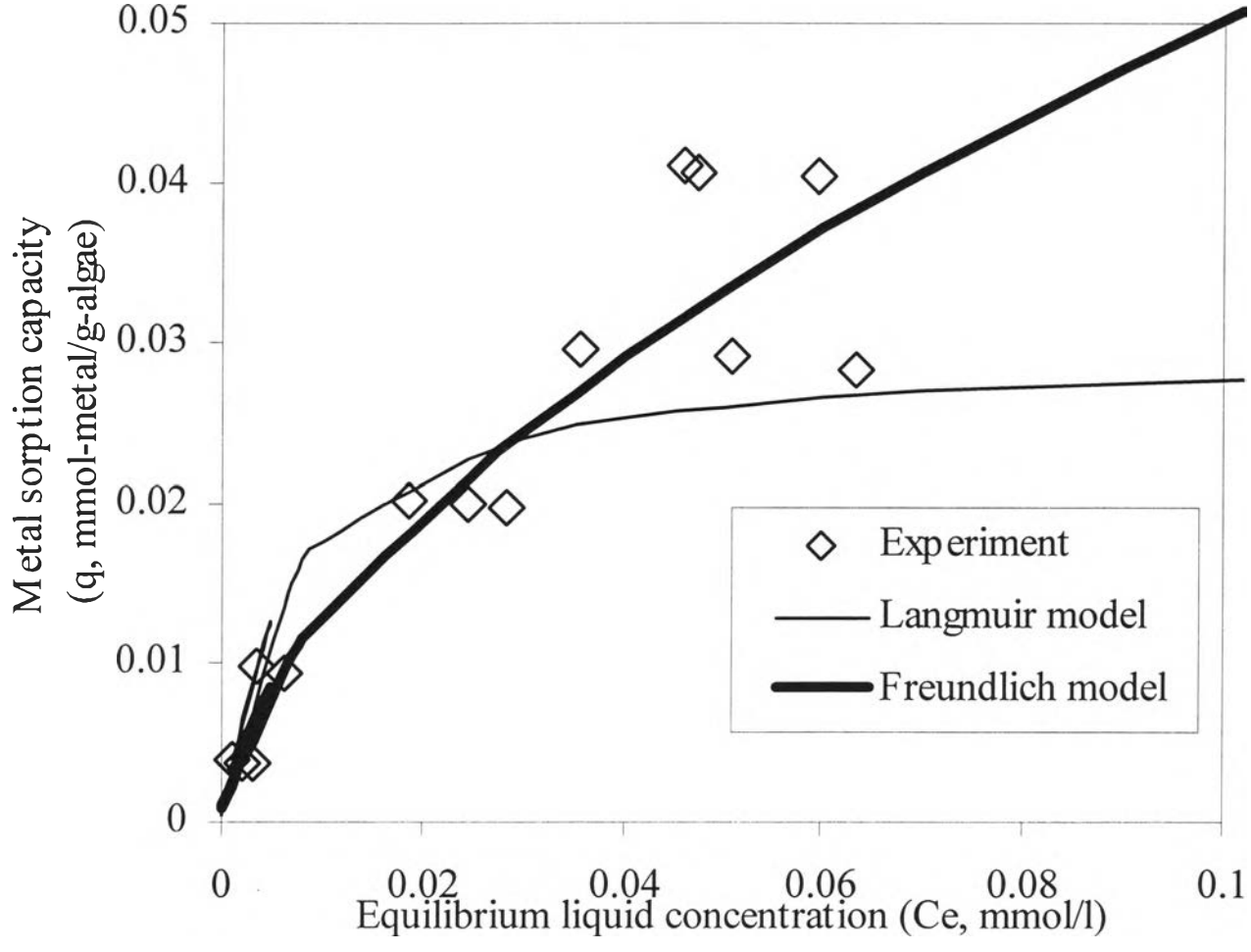

Figure 4.4.3 Isotherm of lead

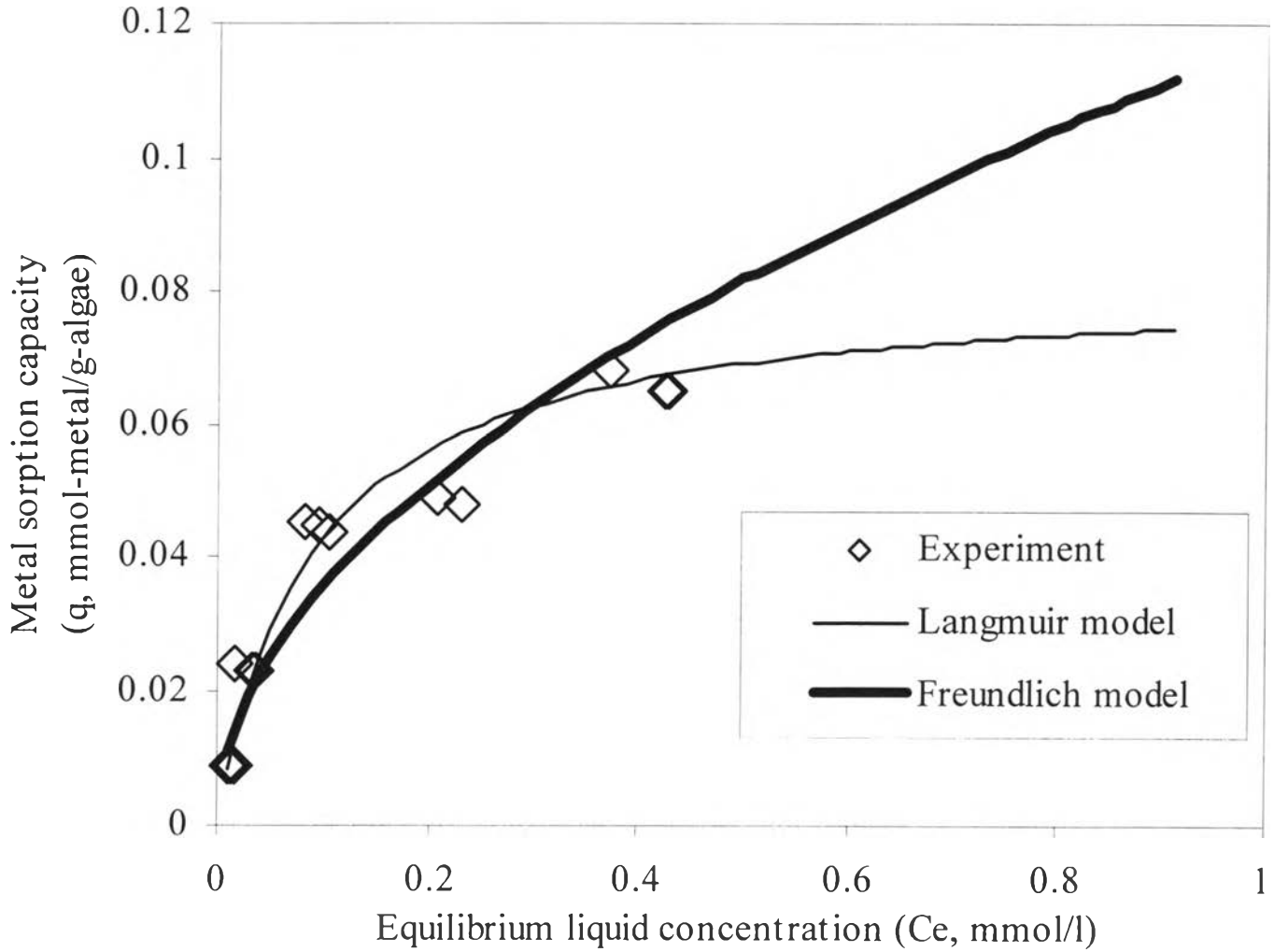

Figure 4.4.4 Isotherm of Zinc

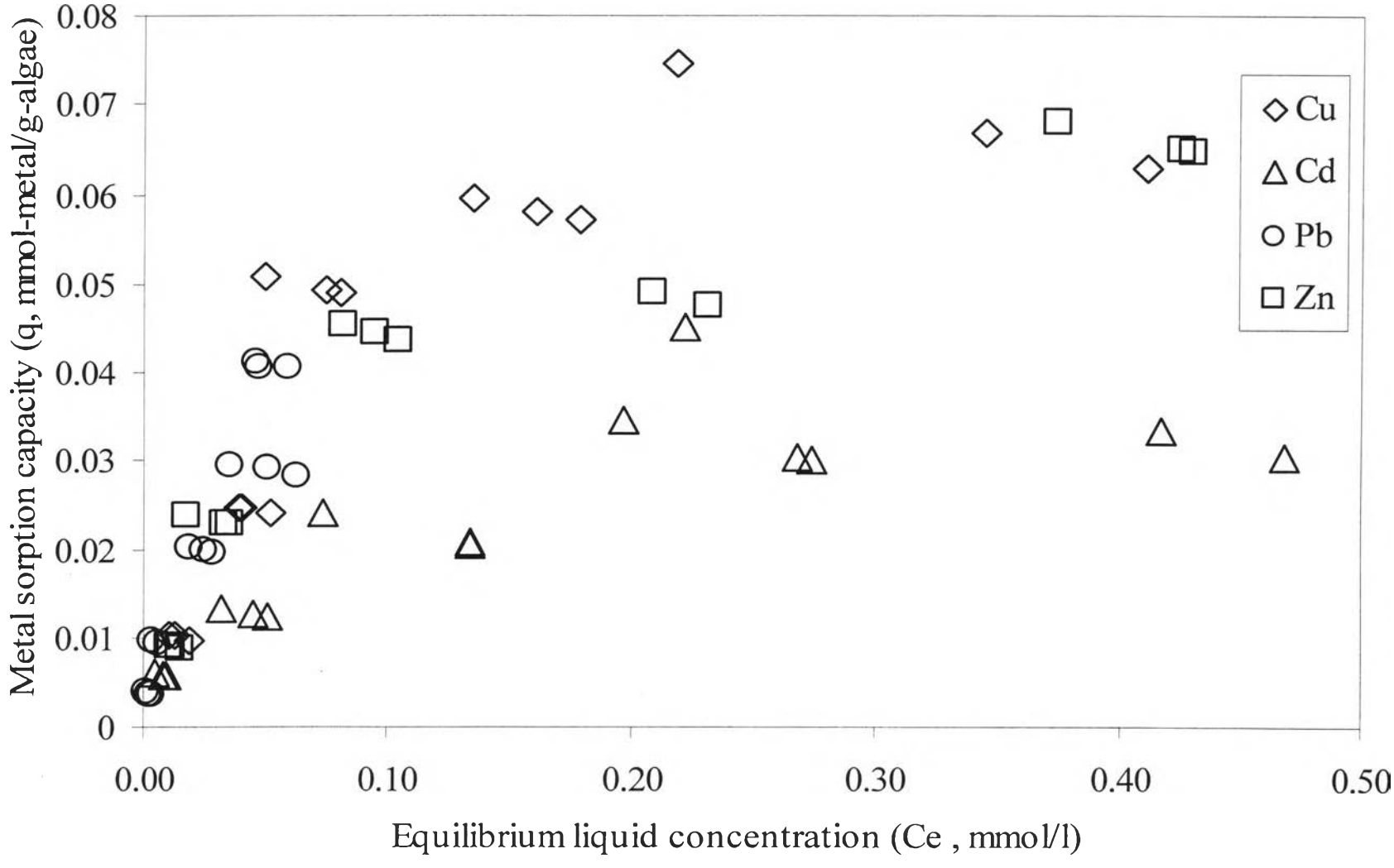

Figure 4.4.5 Isotherm of each metal (combinding Figures 4.4.1 – 4.4.4)

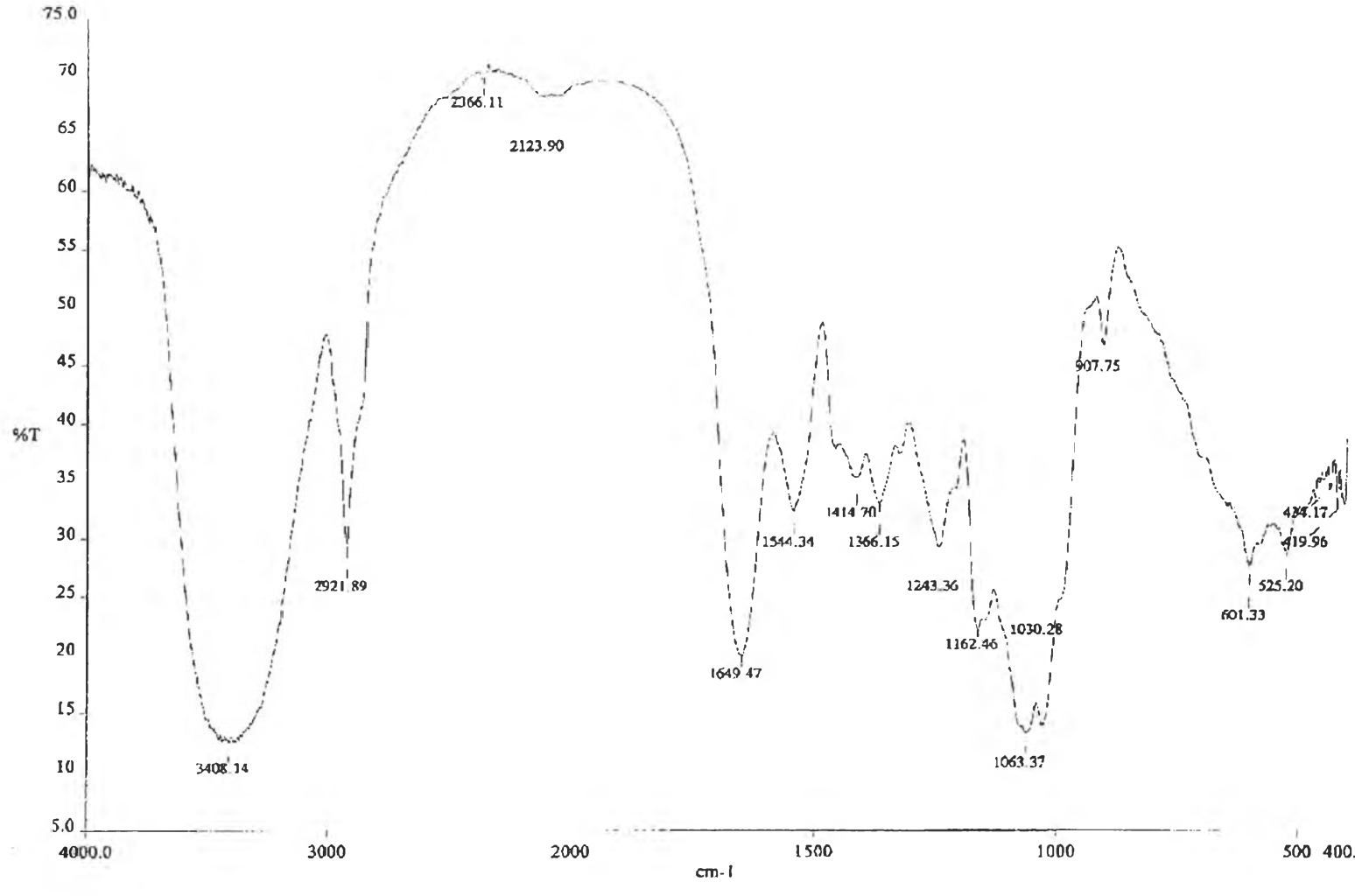

Figure 4.5.1 FTIR transmission spectra for pure algae

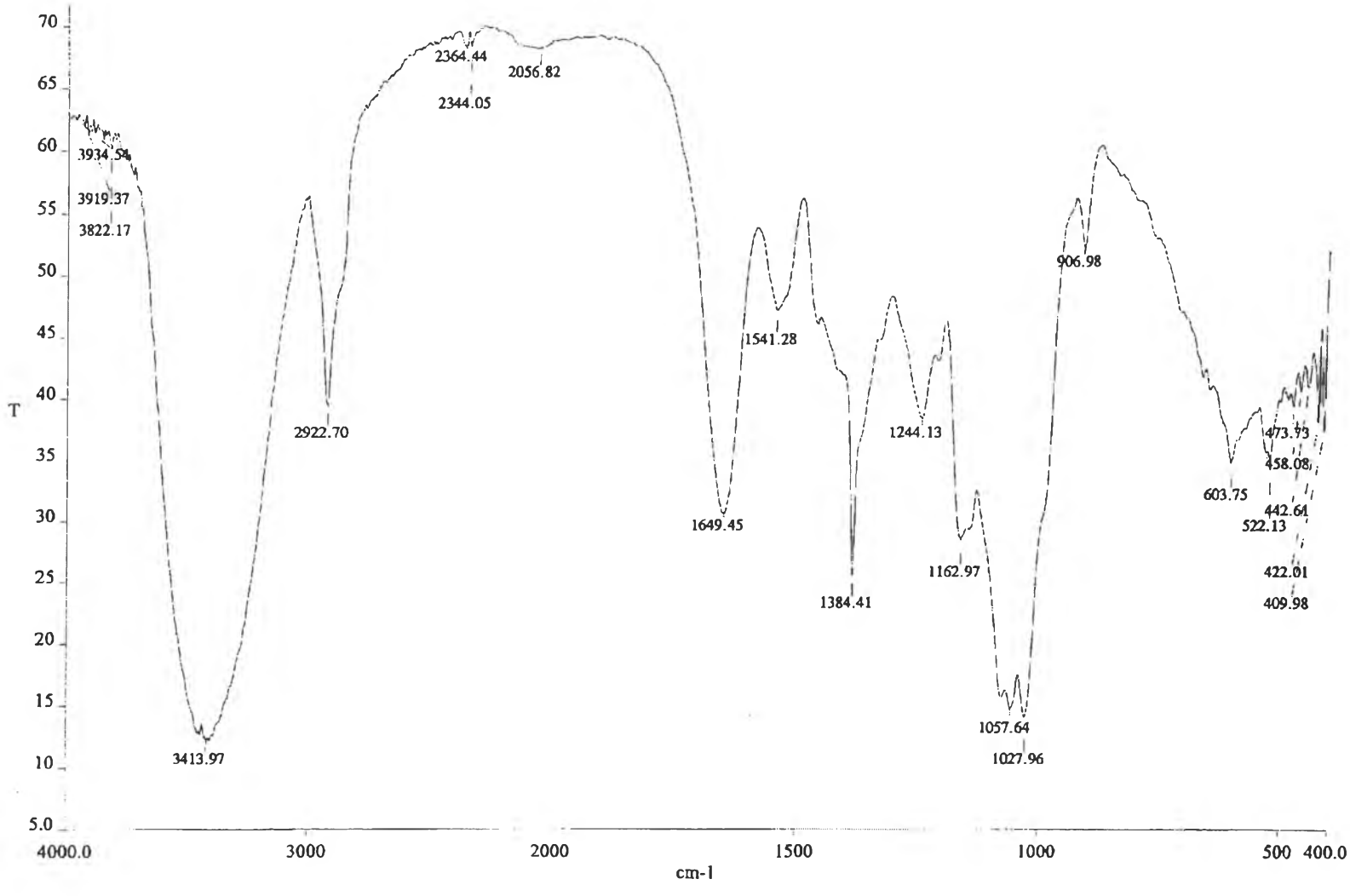

Figure 4.5.2 FTIR transmission spectra for spent algae with Cu

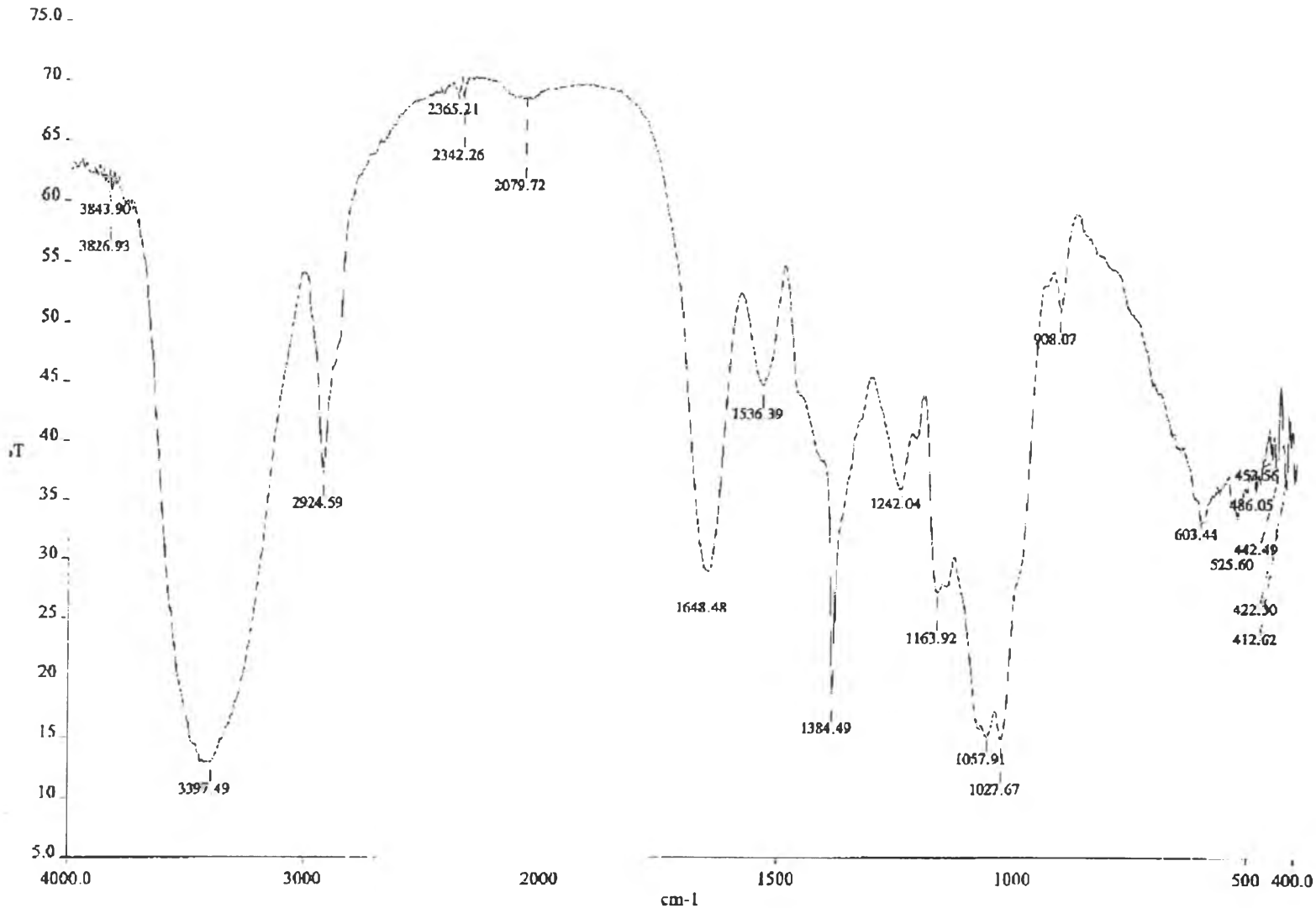

Figure 4.5.3 FTIR transmission spectra for spent algae with Cd

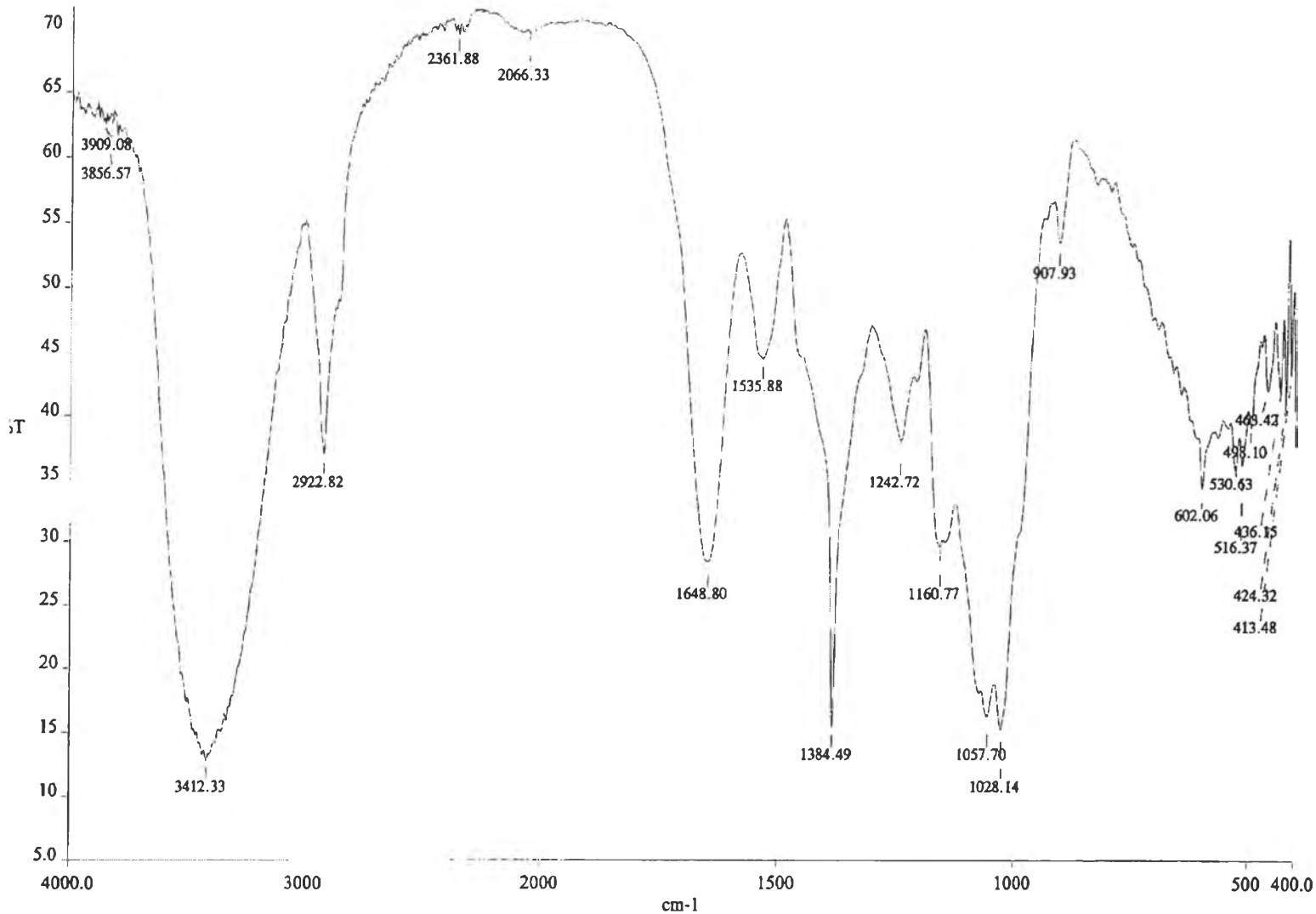

Figure 4.5.4 FTIR transmission spectra for spent algae with Pb

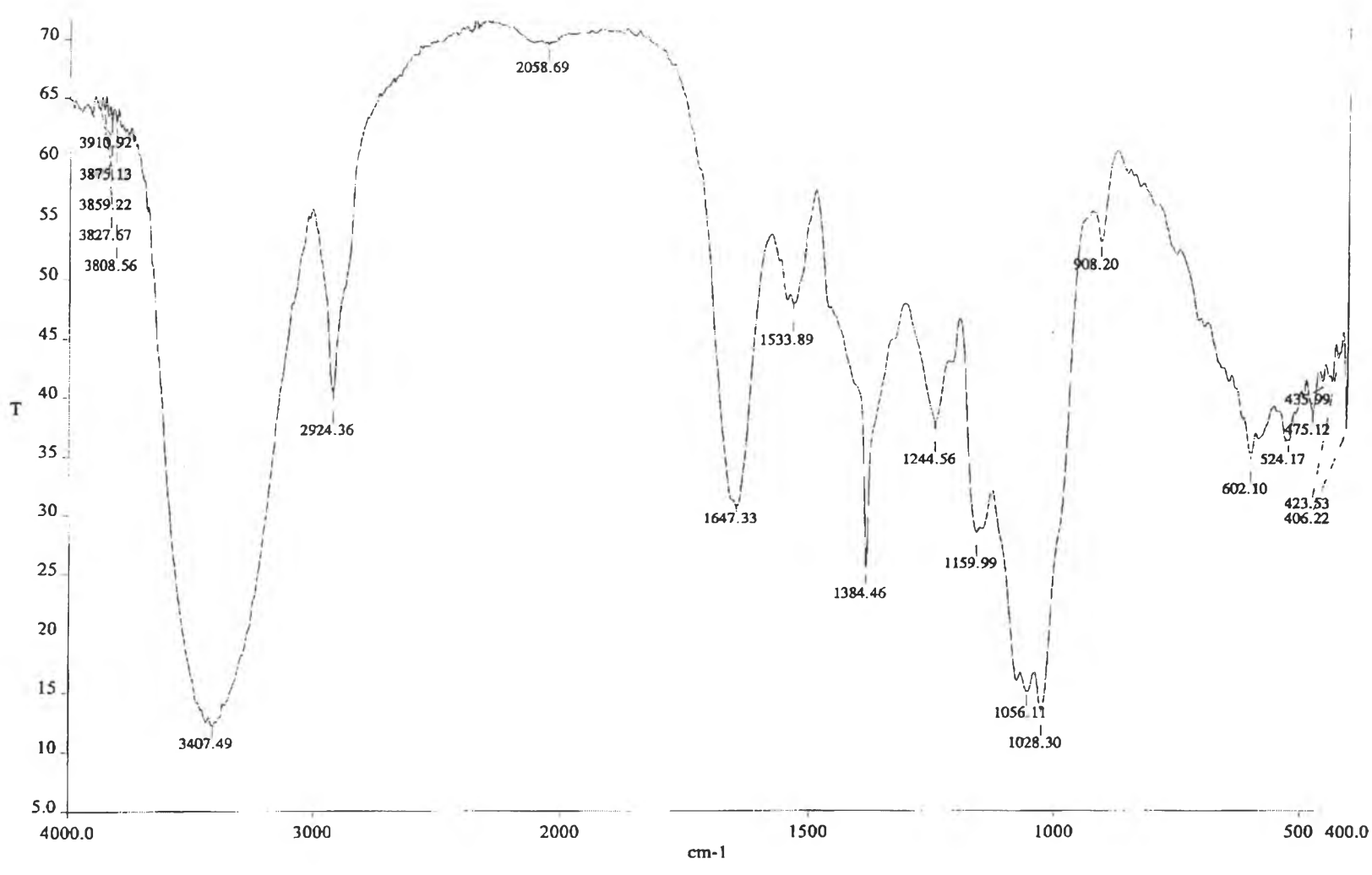

Figure 4.5.5 FTIR transmission spectra for spent algae with Zn

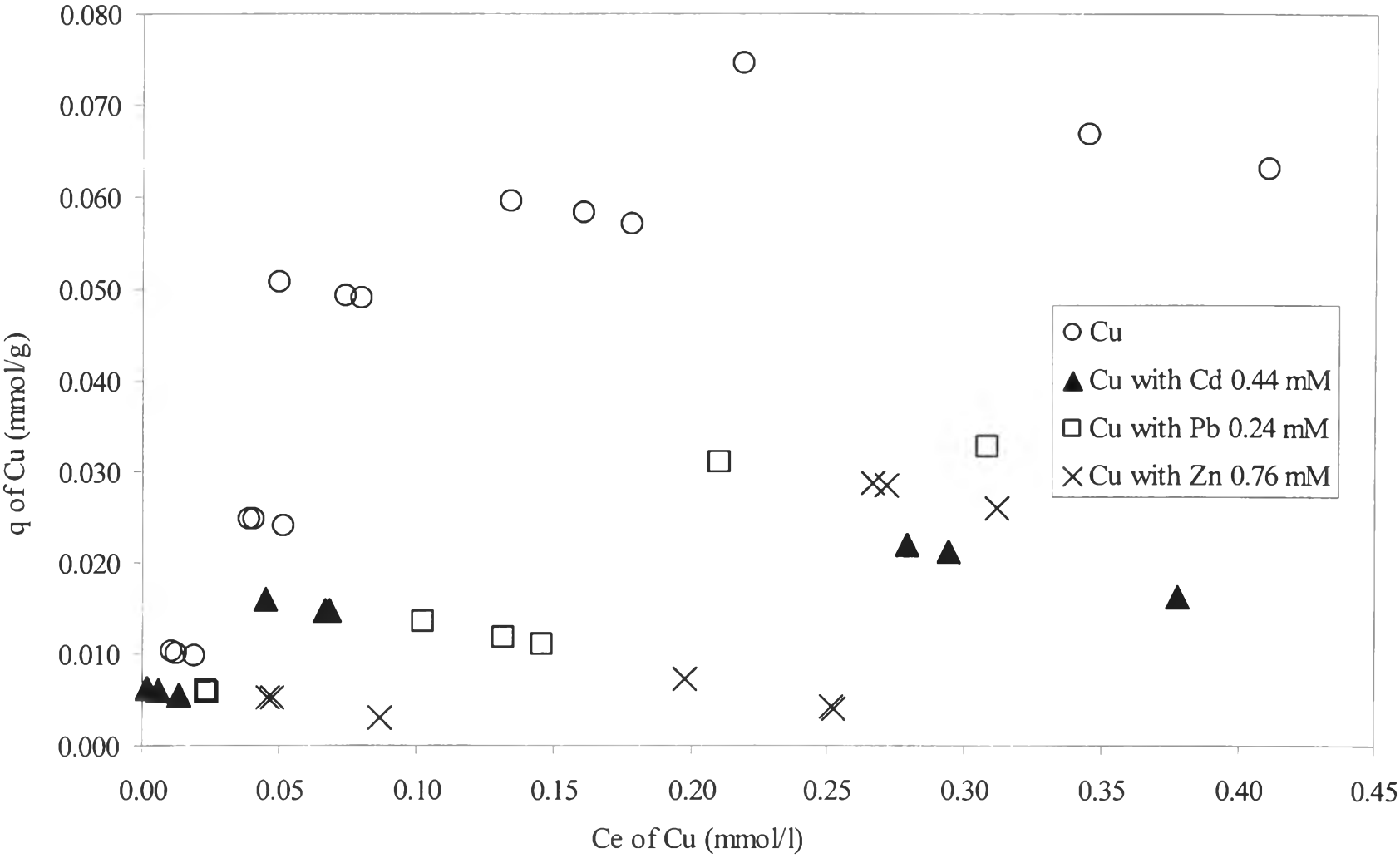

Figure 4.6 Isotherm of Cu in single and binary component system

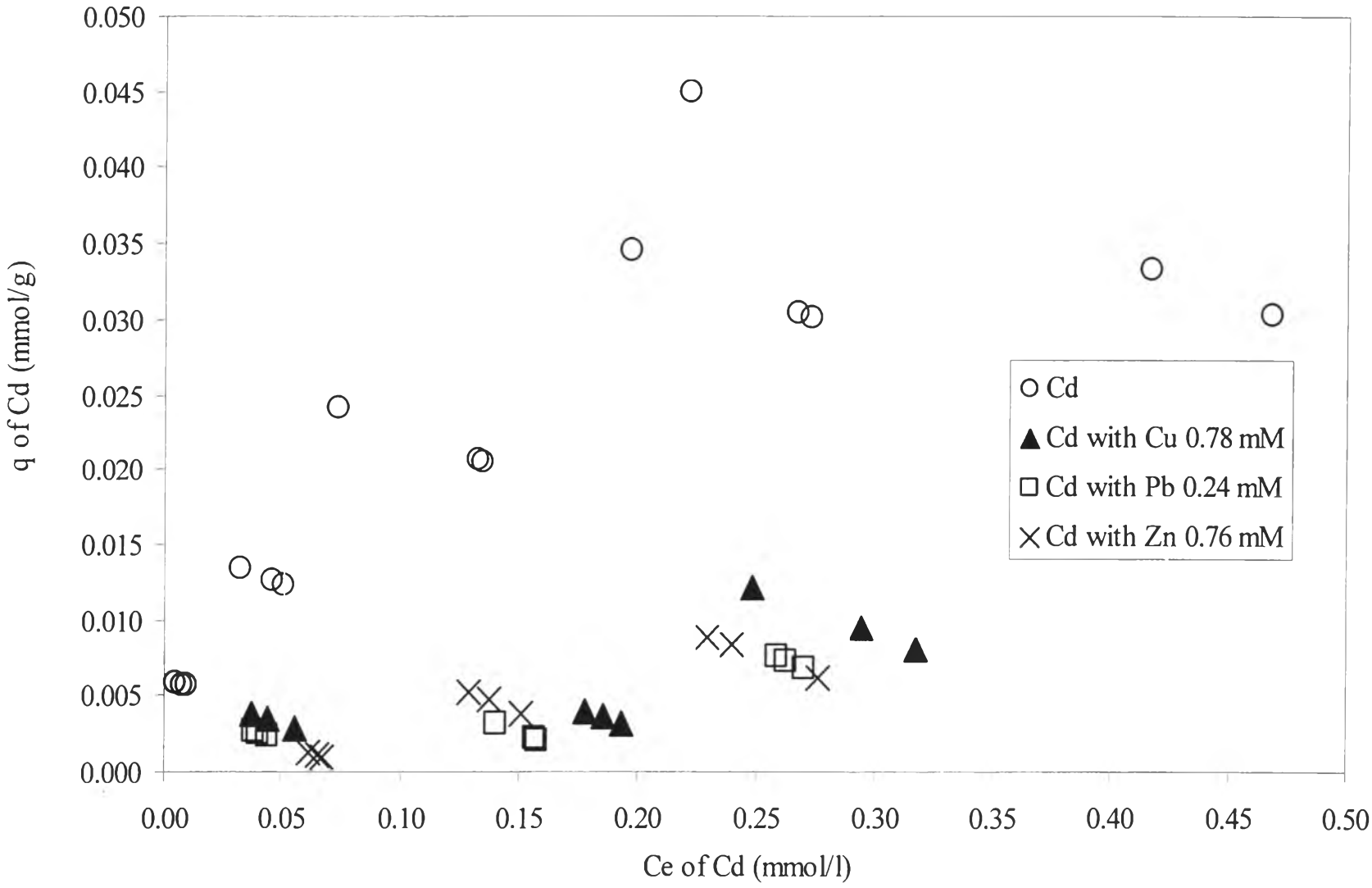

Figure 4.7 Isotherm of Cd in single and binary component system

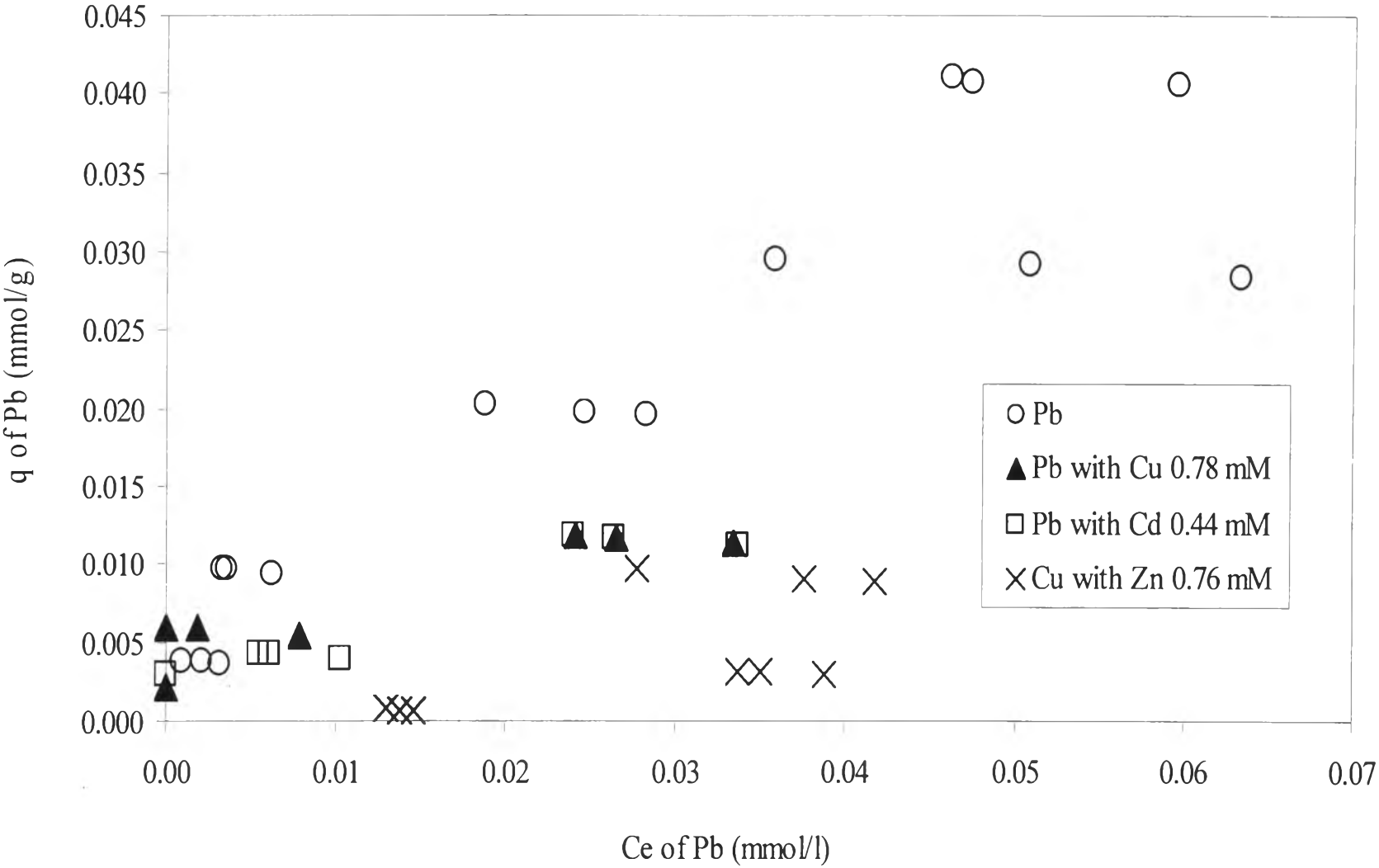

Figure 4.8 Isotherm of Pb in single and binary component system

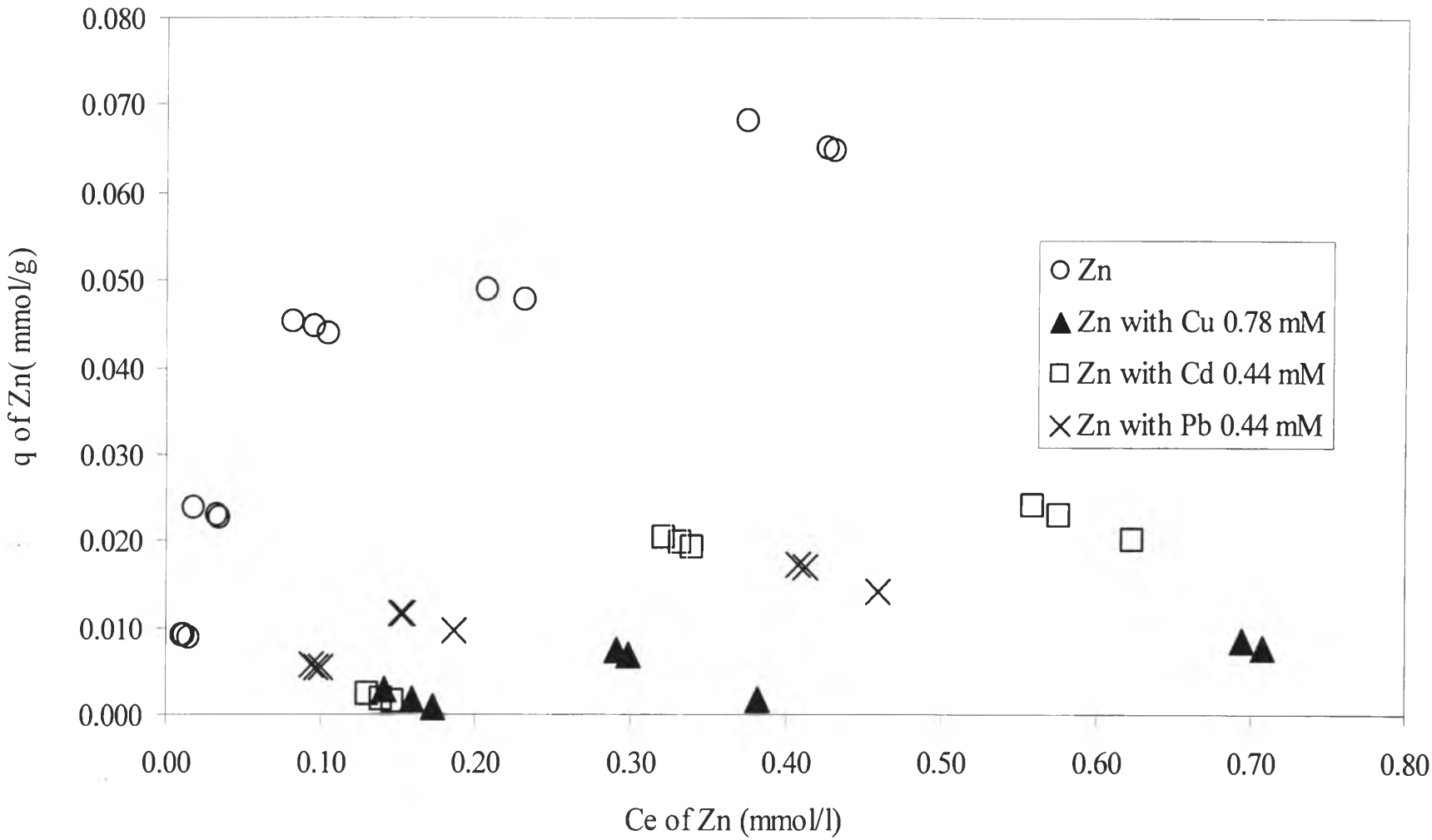

Figure 4.9 Isotherm of Zn in single and binary component system

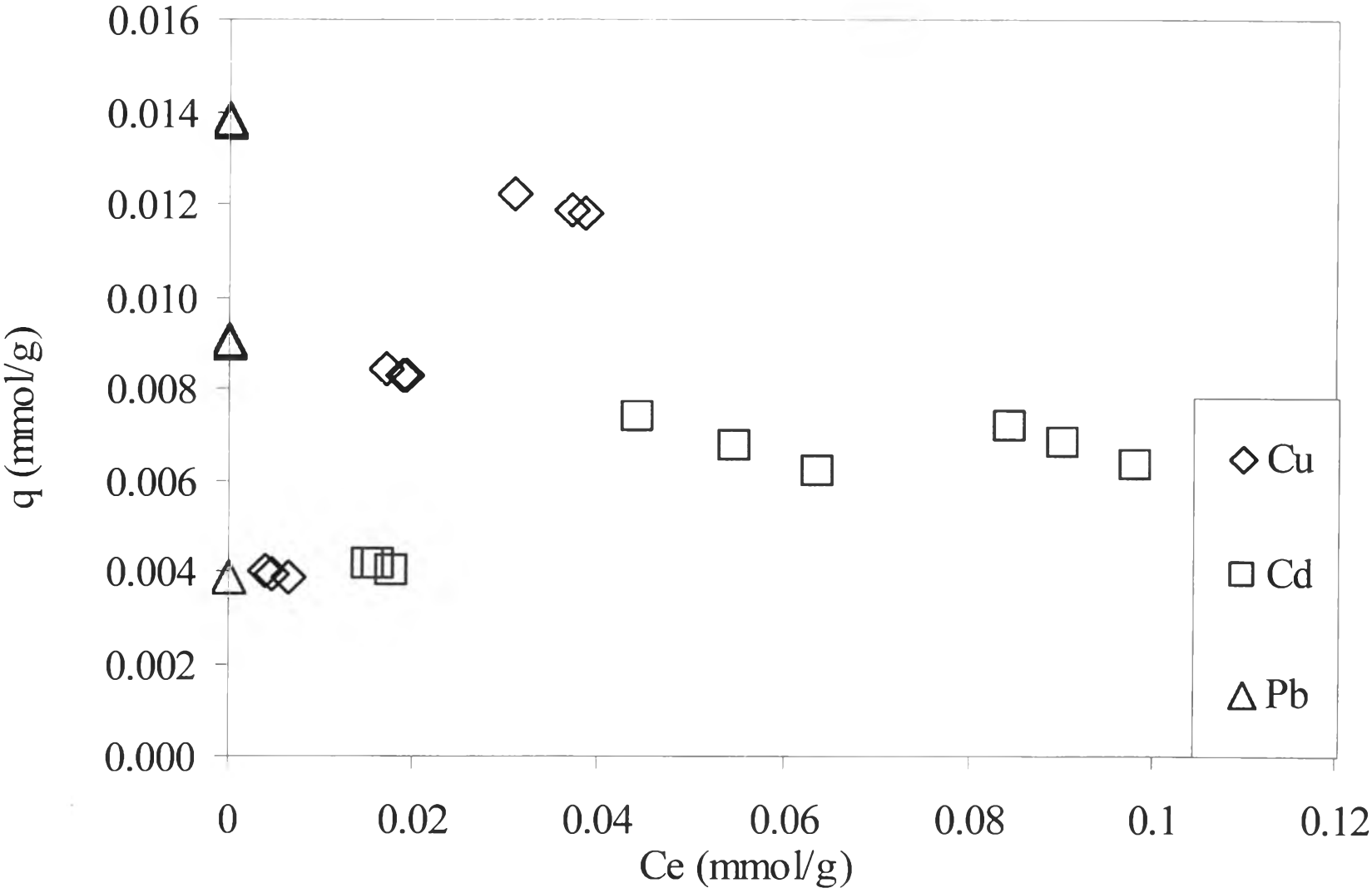

Figure 4.10.1 Isotherm of ternary component Cu, Cd, and Pb

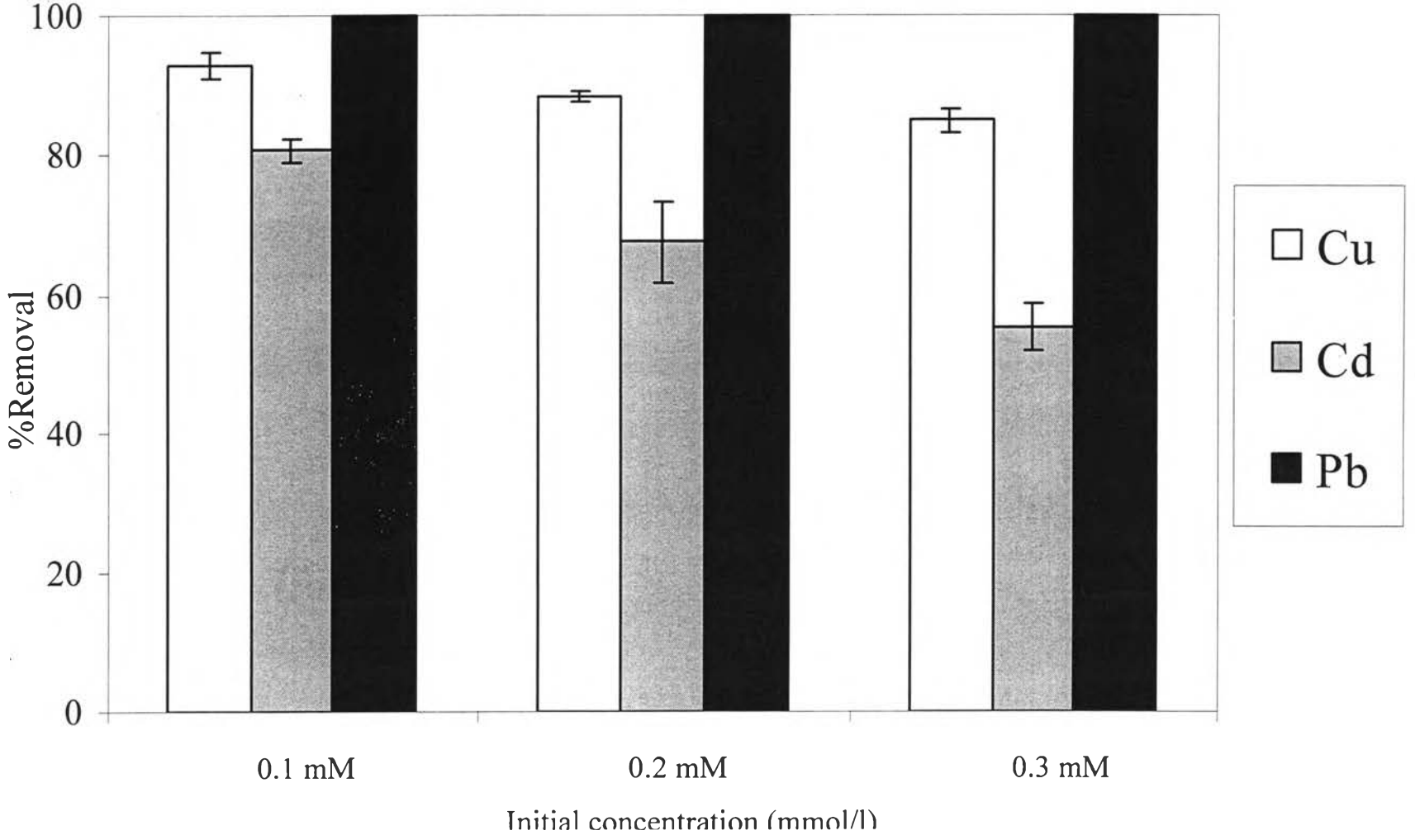

Figure 4.10.2 Removal of ternary component Cu, Cd, and Pb

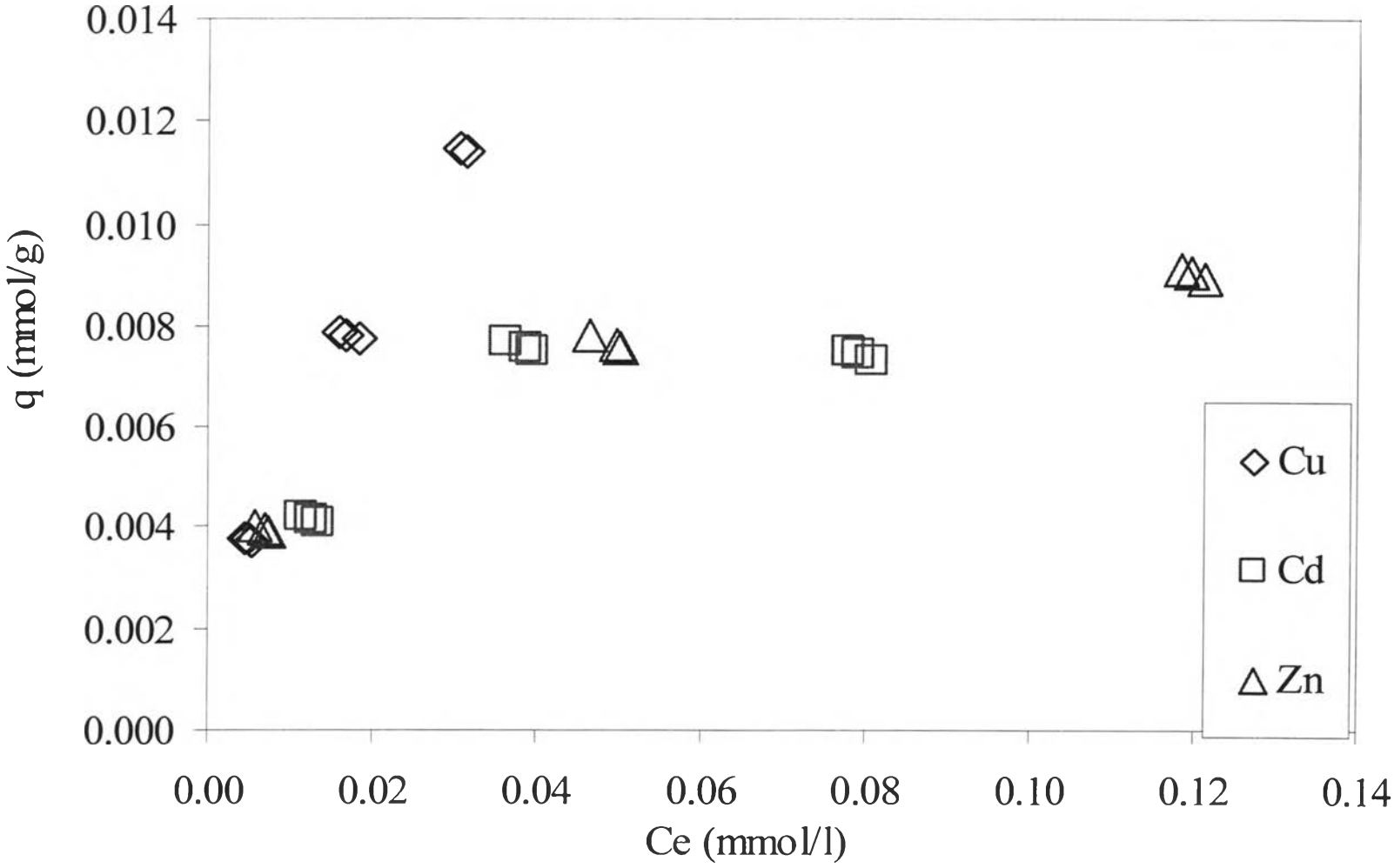

Figure 4.11.1 Isotherm of ternary component Cu, Cd, and Zn

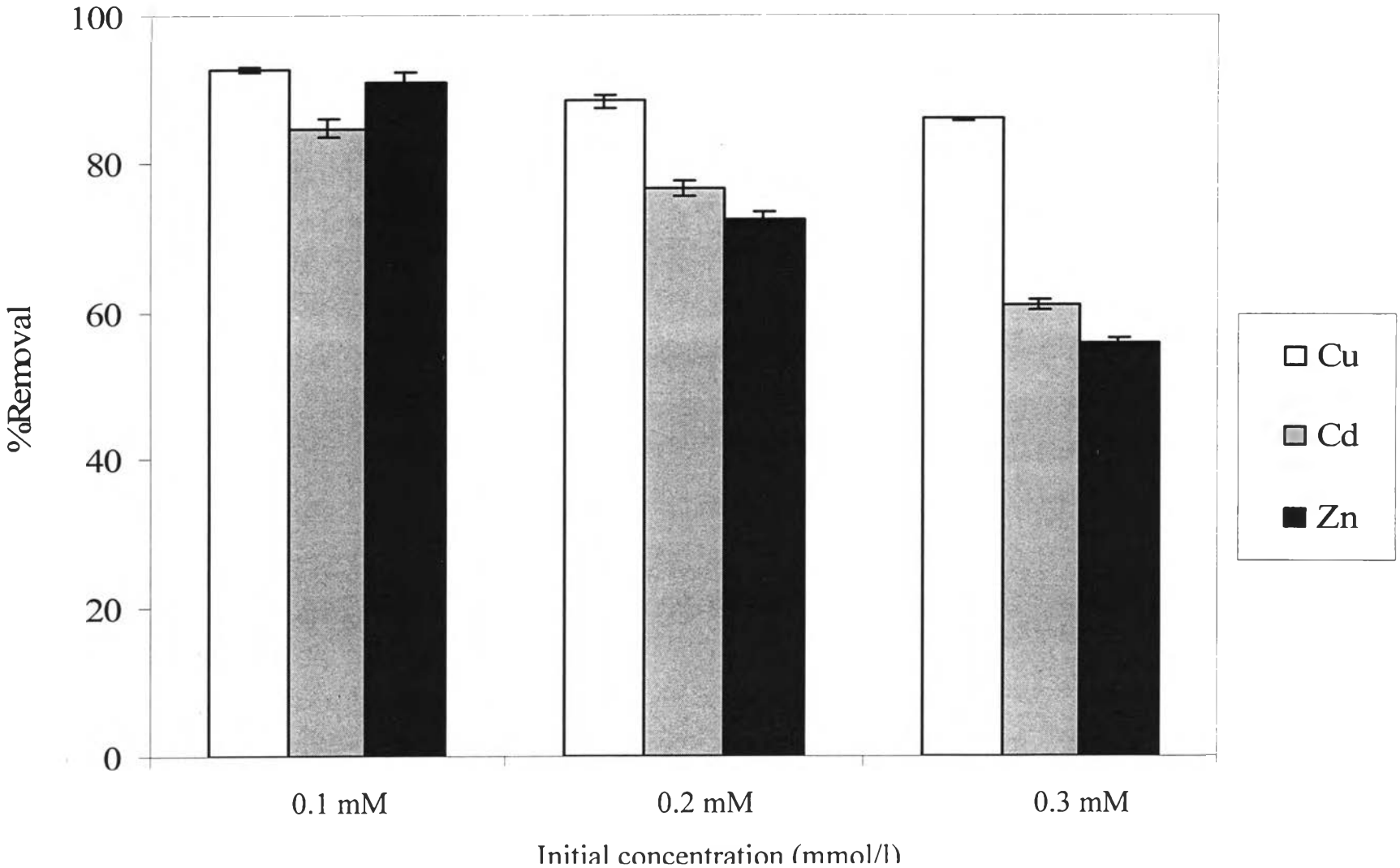

Figure 4.11.2 Removal of ternary component Cu, Cd, and Zn

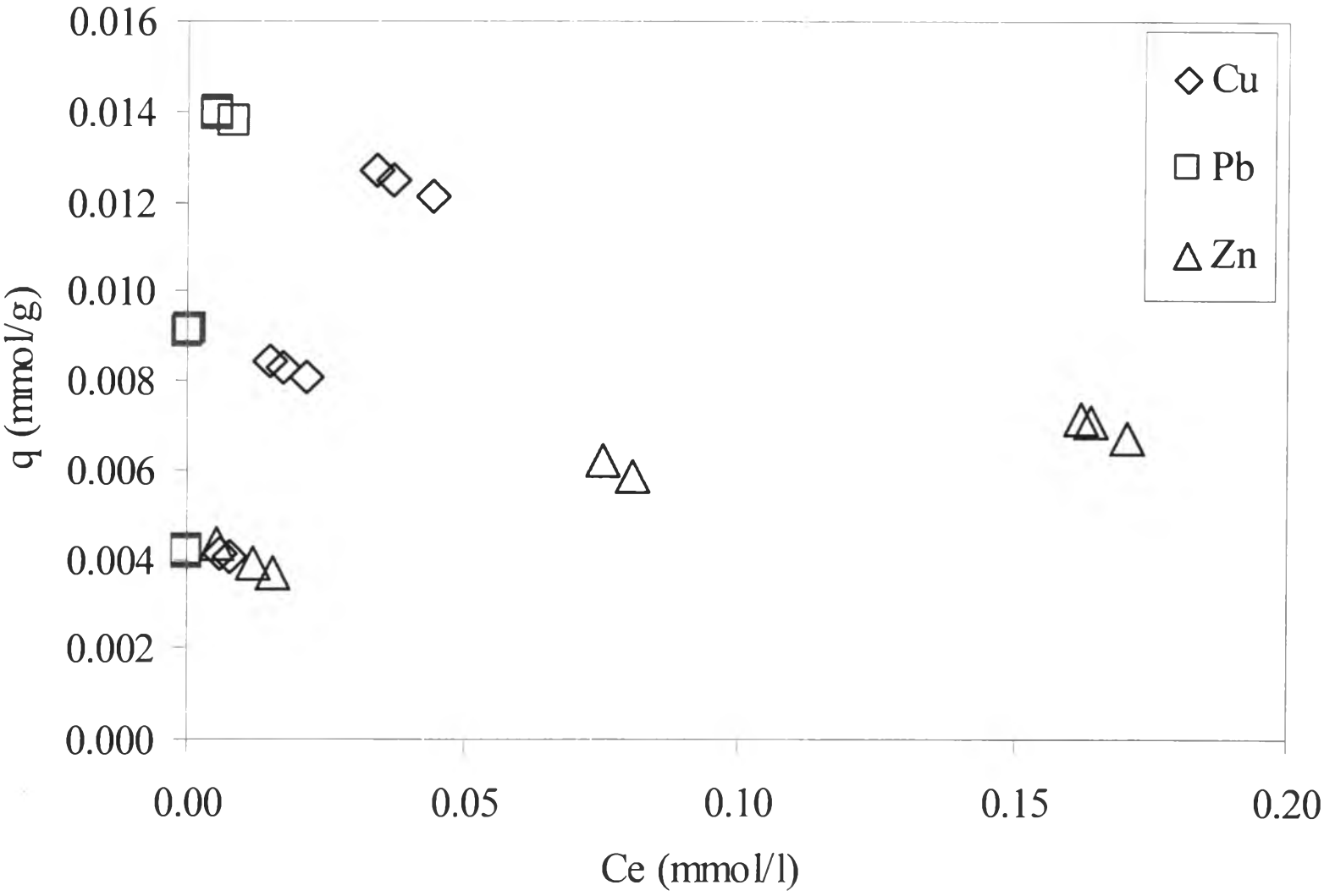

Figure 4.12.1 Isotherm of ternary component Cu, Pb, and Zn

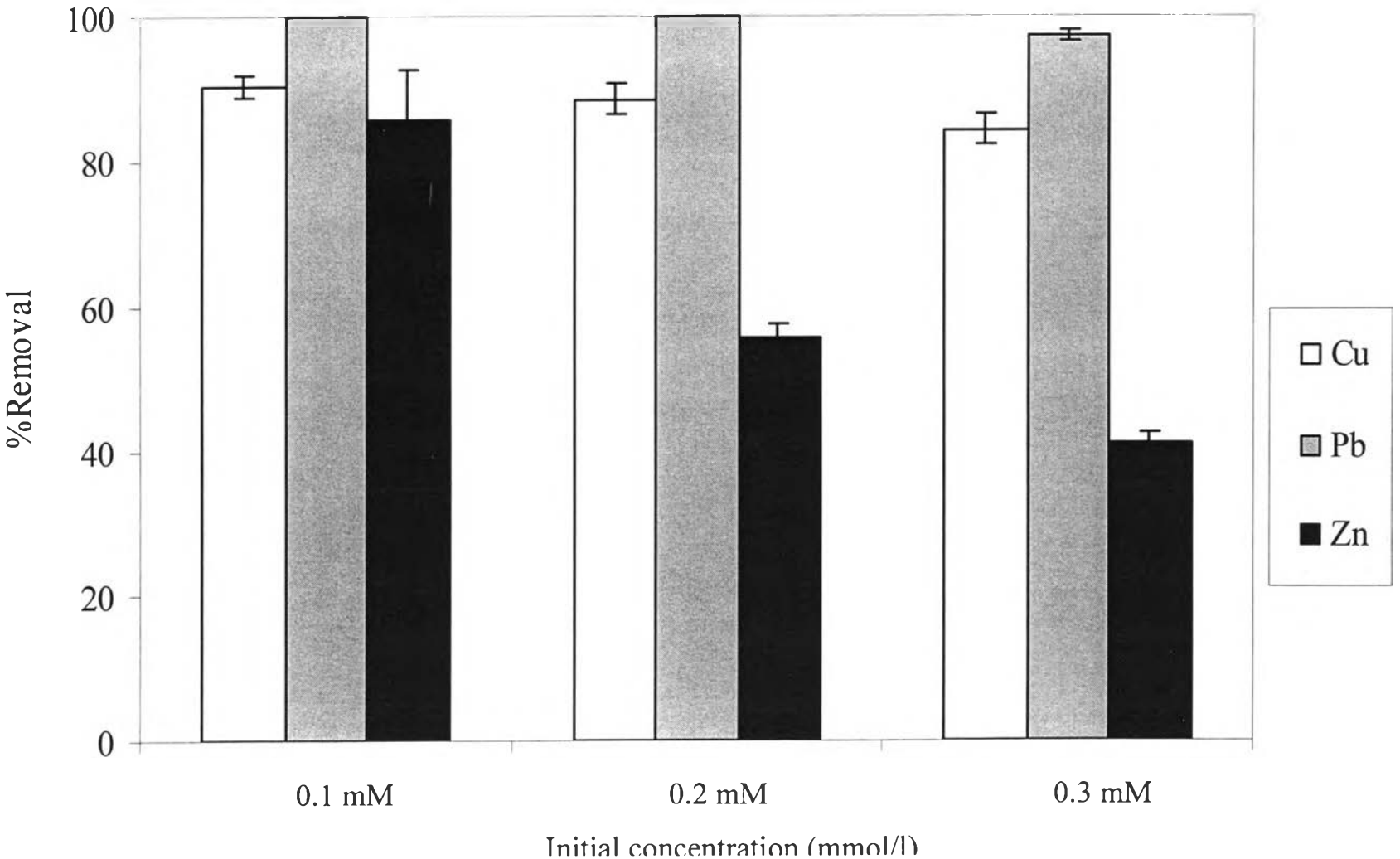

Figure 4.12.2 Removal of ternary component Cu, Pb, and Zn

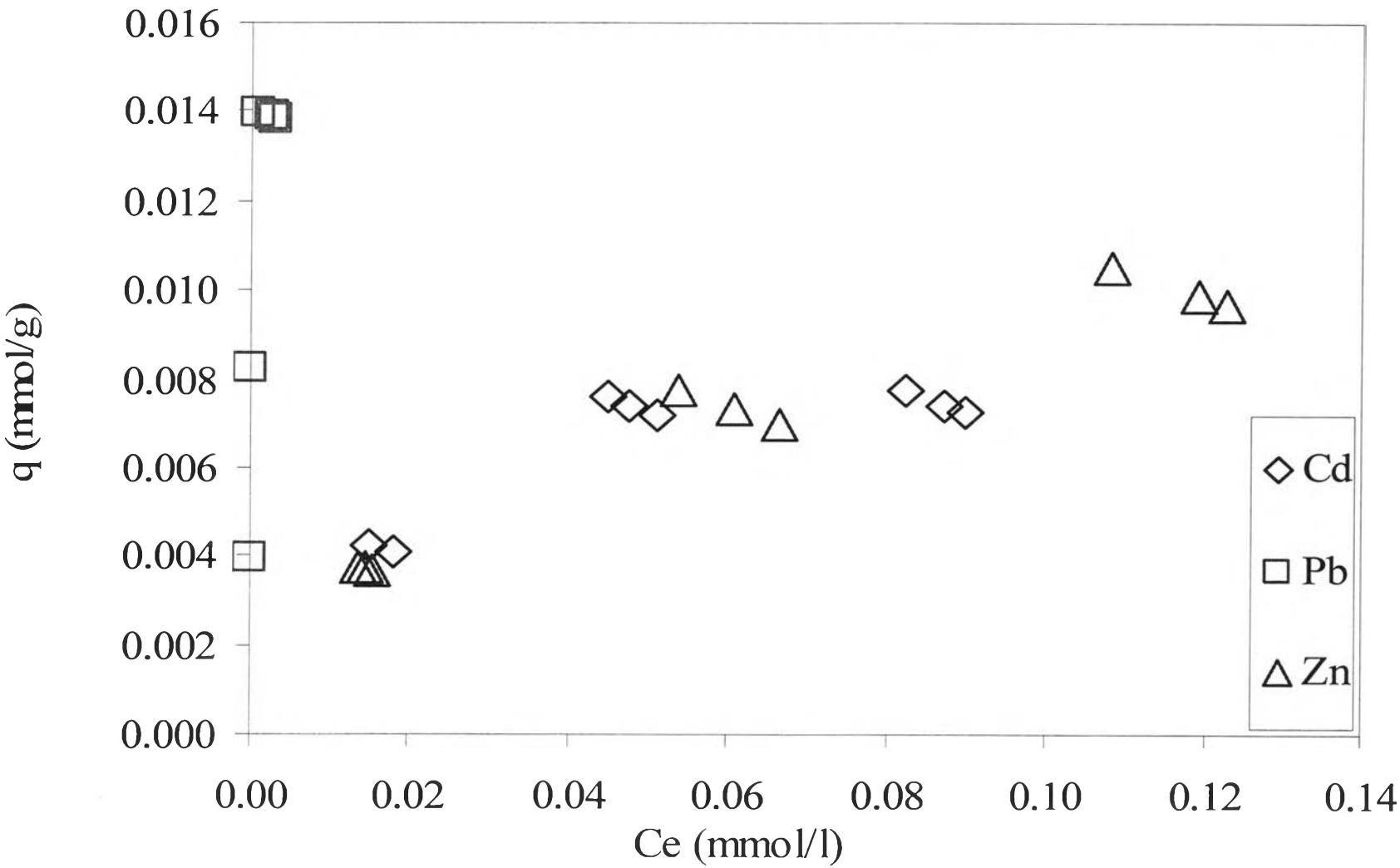

Figure 4.13.1 Isotherm of ternary component Cd, Pb, and Zn

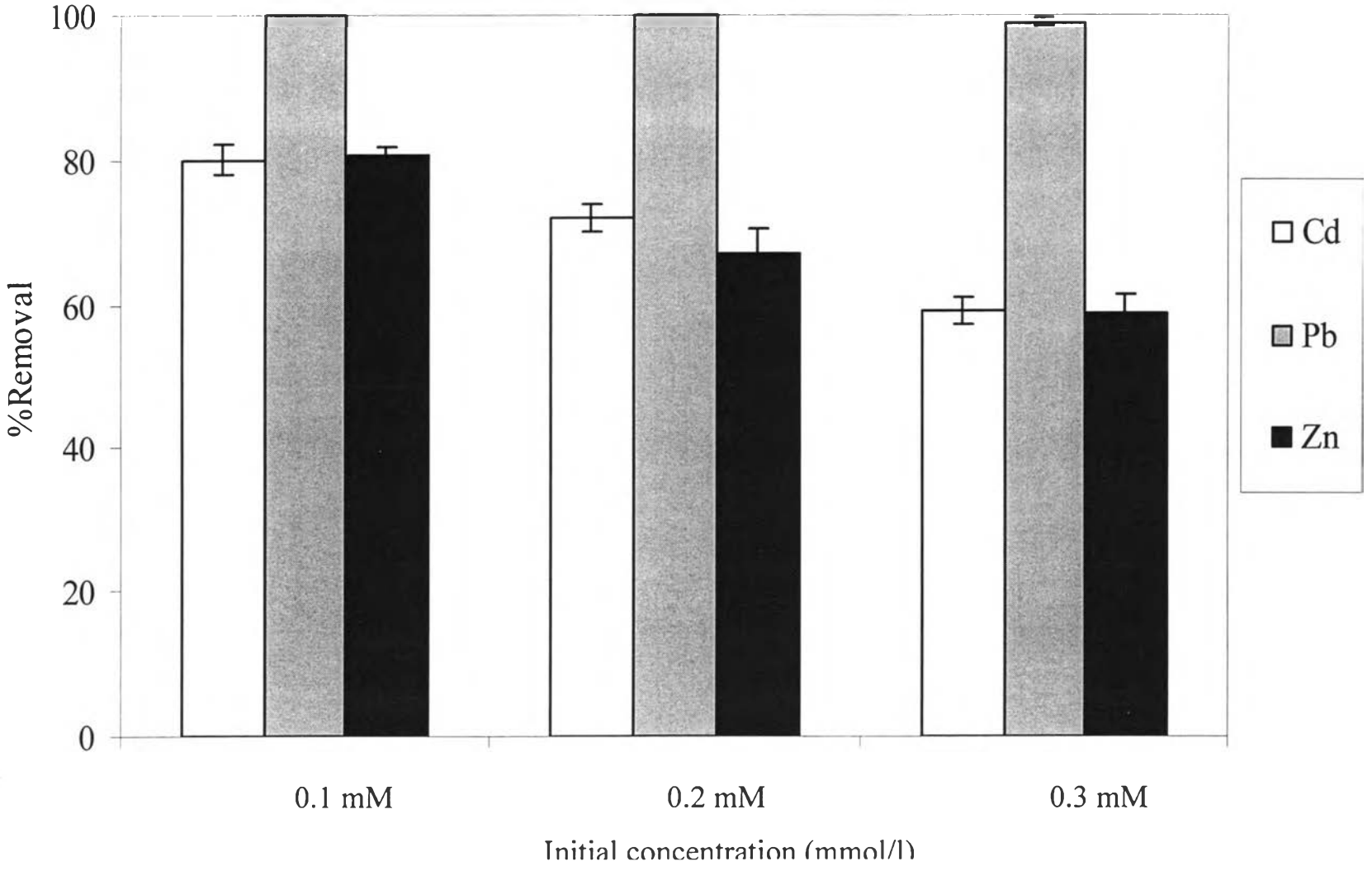

Figure 4.13.2 Removal of ternary component Cd, Pb, and Zn

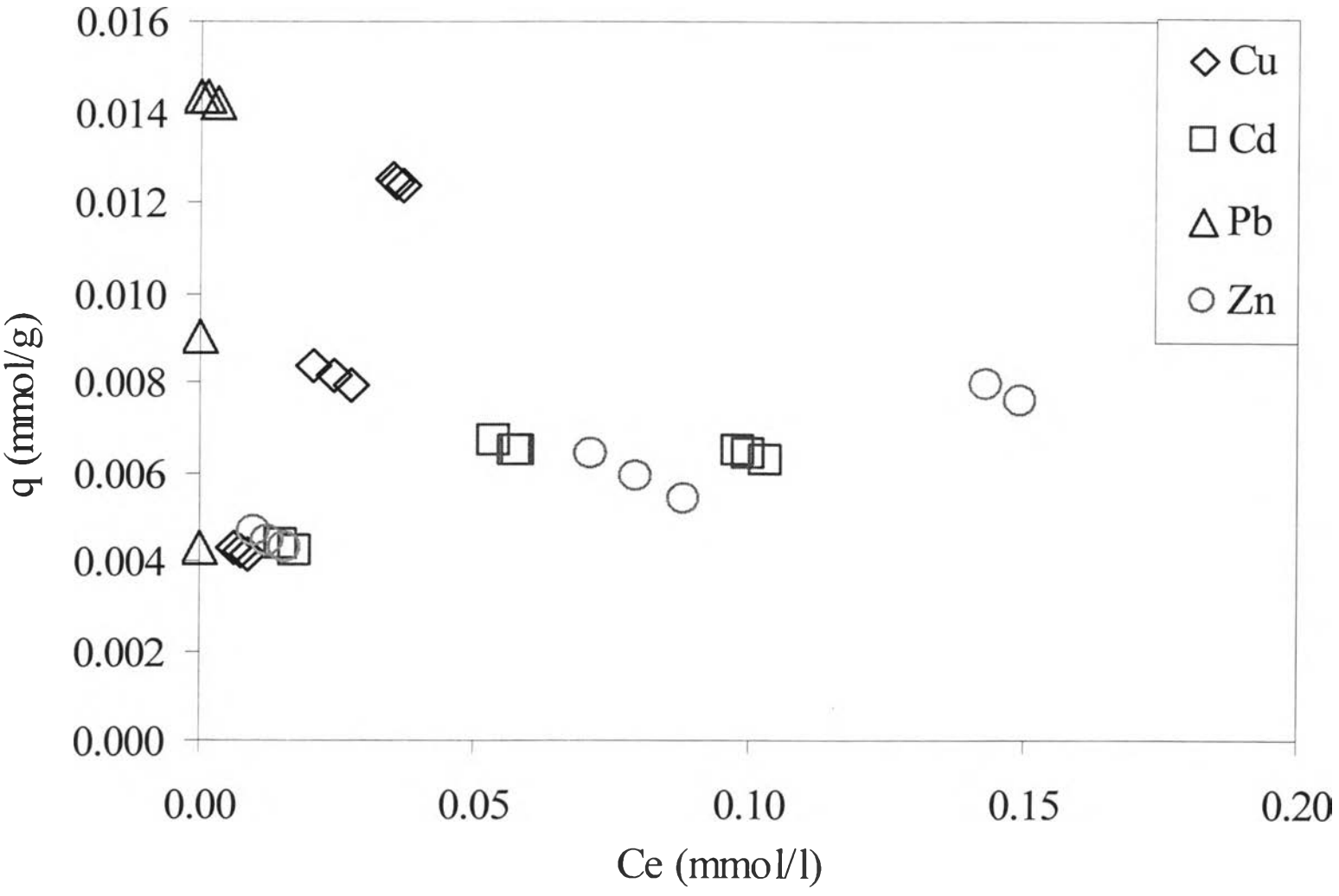

Figure 4.14.1 Isotherm of four component

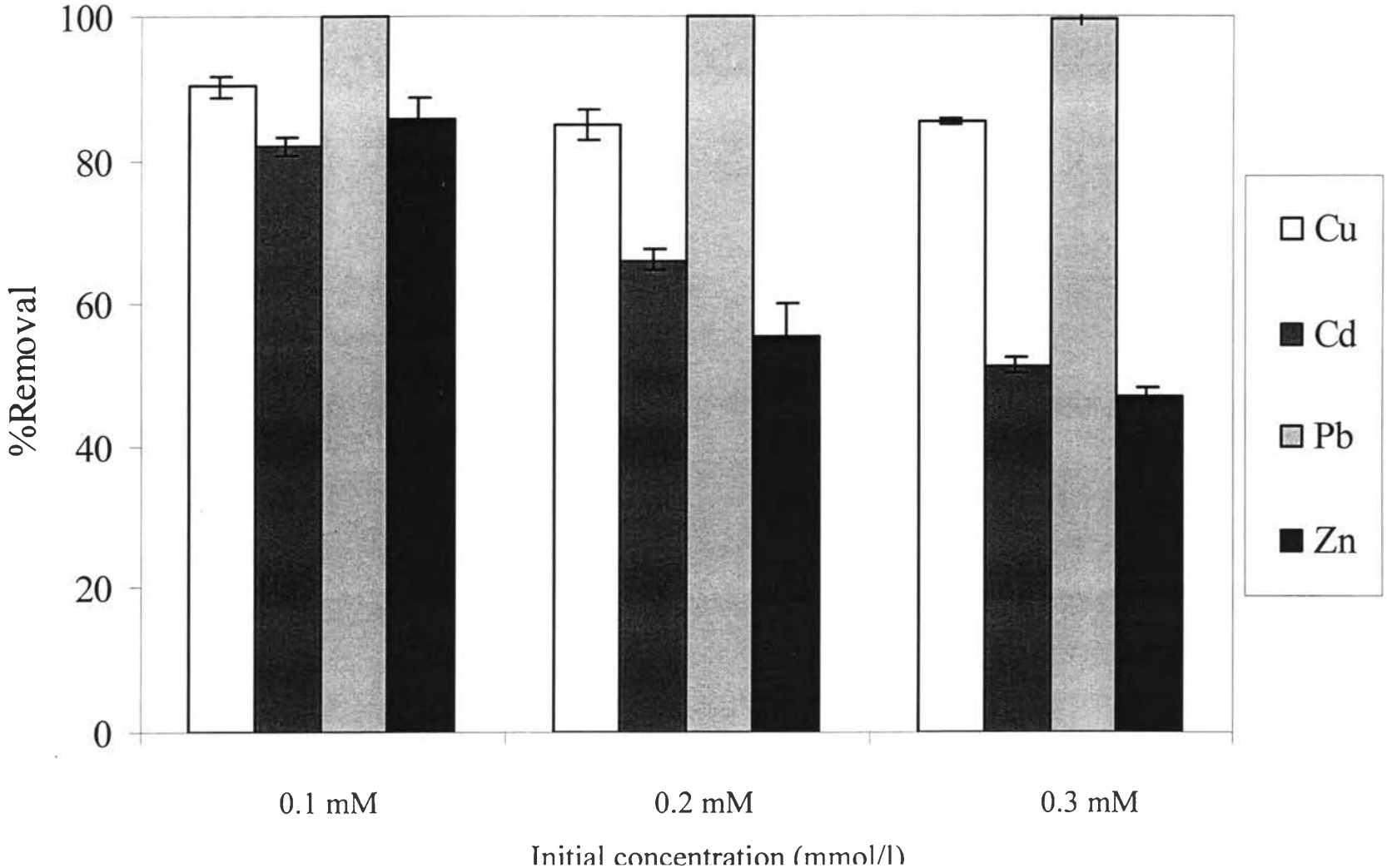

Figure 4.14.2 Removal of four component

Table 4.1: wave number of dominant peak obtained from transmission spectra.

	Pure algae	Algae with Cu	Algae with Cd	Algae with Pb	Algae with Zn
Carboxylic Acid					
O-H Stretching	2922	2922	2924	2922	2924
C=O Stretching	-	-	-	-	-
C-O Stretching	1244	1244	1242	1242	1244
O-H Bending	1414	-	-	-	-
Amine					
N-H Stretching	3408	3440	3398	3412	3408
N-H Bending	1650	1650	1648	1648	1648
C-N Stretching	1324	1076	-	-	1078
Amide					
N-H Stretching	3408	3414 3440	3398	3412 3330	3408
C-O Stretching	1650	1650	1648	1648	1648
Amino			-	-	
N-H Stretching	2922	2922	2924	2922	2924
C-O	1544 1414	1542 1458	1536 -	1536 -	1534 -
N-H Bending	1544	1542	1536	1536	1534
Sulfonyl					
S=O Stretching	-	1384	1384	1384	1384
Sulfonate					
S=O Stretching	1366	-	-	-	-
S-O Stretching	908	908	908	908	908
					800