BIOSORPTION OF HEAVY METALS BY RED ALGAE (*PALMARIA PALMATA*)

by
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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of
Master of Science
Agricultural and Biosystems Engineering
McGill University, Montreal

March 2001

0-612-70380-0

ABSTRACT

The research presented in this thesis entailed an investigation of heavy metal uptake by *Palmaria palmata*, a red marine alga. The alga was dry and organically certified. The heavy metals of interest were those commonly found in the wastewaters of the printed wiring board industry, namely $Cu^{2+}Pb^{2+}$, Cd^{2+}, Zn^{2+} and Ni^{2+}. The ultimate objective of the work was to determine whether or not the factors expected to influence the metal uptake to the greatest extent could be optimized within functional ranges, leading eventually to process design (beyond the scope of this thesis). These factors were pH, temperature, initial concentration of metal in aqueous solution, and contact time. A number of preliminary experiments were performed to establish a basis for the design of the optimization studies.

Data from sorption batch experiments were found to be best described by the BET and Langmuir models. Additionaly, lead and copper were sorbed more readily than the other metals, with respective maximum adsorption capacities of 15.17 and 6.65 mg/g (dry weight metal/dry weight biosorbent). These were therefore the metals used for the optimization phase. A Central Composite Design (CCD) was used to reduce the number of trials necessary given that four factors with various levels were involved. The factors were pH, temperature, initial concentration of metal in aqueous solution, and contact time. The first CCD was run on copper solutions with initial copper concentrations ranging from 0 to 20 mg/L. This range was extended to 0-500 mg/L, in a second CCD involving copper only. A third CCD was executed with lead as the target metal, with initial concentrations ranging from 0 to 200 mg/L. Finally, a CCD was executed with a binary solution involving copper and lead to permit evaluation of the presence of co-cation inhibition.

The analysis of the model describing the first results of copper uptake (0-20 mg/L initial concentration range) showed that the fit was adequate. The factors found to have the greatest influence on metal uptake were pH, initial metal concentration and temperature. The models describing the uptake of copper over the extended range, of lead in the mono metal system, and of lead in the binary system all suffered from lack of fit. In retrospect, it would appear that the ranges of initial concentrations used were too wide to permit a proper fit by a quadratic model. However, this is most probably due to the fact that the relationships between metal uptake and the other factors are usually S-shaped or logarithmic in character. There is also some indication from the raw data that there is a mutual and unequal inhibitory effect of the presence of one of the studied metals on the other (ie. copper and lead), and that these inhibitions are dependent on pH, temperature, and initial concentrations of metals. Furthermore, the inhibition of copper uptake by lead was greater, based on the results of the two mono-metal studies and the binary system study.

Although it was not possible to adequately define optimal regions of operation for the biosorption of heavy metals by *Palmaria palmata*, general trends were elucidated, and the limitations of the methodology used were clarified. It is therefore anticipated that future work could lead to a remediation process for PWB manufacturing wastewaters based on this relatively inexpensive and abundant biosorbent.

RÉSUMÉ

La recherche présentée dans cette thèse étudie l'extraction des métaux lourds par *Palmaria palmata*, une algue marine rouge. L'algue était séchée et certifiée biologique. Les métaux d'intérêt étaient ceux trouvés communément dans les eaux usées de l'industrie de fabrication des circuits imprimés, à savoir $Cu^{2+}Pb^{2+}$, Cd^{2+}, Zn^{2+} and Ni^{2+}. L'objectif final de ce travail était de déterminer si, oui ou non, les facteurs supposés avoir une influence remarquable sur l'extraction des métaux, pouvaient être optimisés, au sein d'intervalles fonctionnels, en vue d'une éventuelle application (en dehors du propos de cette thèse) Les facteurs étaient le pH, la température, la concentration initiale en métaux dans la solution aqueuse, et le temps de contact. Des expériences préliminaires ont été effectuées, pour établir le schéma directeur des études d'optimisation.

Les modèles qui convenaient le mieux à la description des données des expériences de sorption étaient les modèles BET et de Langmuir. Plus spécifiquement, le plomb et le cuivre ont été adsorbés plus facilement que les autres métaux, avec des capacités maximales d'adsorption respectives de 15.17 et 6.65 mg/g (poids sec de métal/ poids sec de biosorbant) Ce sont donc les métaux utilisés dans la phase d'optimisation. Un Central Composite Design (CCD) a été utilisé pour réduire le nombre d'expérience nécessaire, étant donné que quatre facteurs avec plusieurs niveaux étaient impliqués. Les facteurs étaient le pH, la température, la concentration initiale en métaux dans la solution aqueuse, et le temps de contact. Un premier CCD a été effectué sur des solutions de cuivre dont les concentrations initiales en cuivre variaient entre 0 et 20 mg/L. Cet intervalle fut étendu à 0-500 mg/L, dans un second CCD, impliquant le cuivre seulement. Un troisième CCD utilisa le plomb comme métal cible, avec des concentrations initiales s'établissant entre 0 et 200 mg/L. Finalement, un CCD a été appliqué à une solution binaire contenant le cuivre et le plomb, pour pouvoir évaluer l'inhibition causée par la présence d'un co-cation. L'analyse du modèle décrivant les premiers résultats de l'extraction du cuivre (intervalle de concentration initial 0-20 mg/L) a montré que le modèle était adéquat. Les facteurs ayant le plus d'influence sur l'extraction des métaux étaient le pH, la concentration initiale en métaux et la température. Les modèles décrivant l'extraction du cuivre dans l'intervalle élargi, du plomb dans le système mono-métallique et du plomb dans le système binaire souffraient tous d'une inadéquation par rapport au modèle. Rétrospectivement, il semblerait que les intervalles utilisés étaient trop larges pour permettre une adéquation convenable avec le modèle quadratique. Ceci est probablement dû au fait que la fonction reliant l'extraction du métal et les autres facteurs a une courbe en S ou est logarithmique. Les données expérimentales indiquaient aussi que la présence d'un métal étudié exerçait un effet inhibant, mutuel et inégal sur l'autre (ie. cuivre et plomb). Ces inhibitions dépendaient par ailleurs du pH, de la concentration initiale en métaux et de la température. De plus, l'inhibition de l'extraction du cuivre par le plomb était plus importante, en se basant sur les résultats des deux systèmes mono-métalliques et du système binaire.

Bien qu'il n'ait pas été possible de définir de manière adéquate les régions optimales d'opération pour la biosorption des métaux lourds par *Palmaria palmata*, des tendances générales ont été dégagées, et les limitations de la méthodologie utilisée ont été clarifiées. Il est donc possible de supposer que des futurs travaux pourraient conduire à un procédé de dépollution des eaux usées des entreprises de fabrication de circuits imprimés, basé sur ce biosorbant peu coûteux et abondant.

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ACKNOWLEDGMENTS

The skilled direction of my research supervisor, S. Prasher, Ph.D., McGill University Department of Agricultural and Biosystems Engineering, and co-supervisor J.Hawari, Ph.D., Biotechnology Research Institute Department of Environmental Analytical Chemistry, is greatly appreciated. The trust, respect, and flexibility extended by Dr. Prasher and Dr. Hawari made this an enjoyable and rewarding experience.

The SAS program to perform statistical analyses was patiently directed by P. Dutilleul, Ph.D., McGill University Department of Plant Science, who also provided valuable assistance in the application of the Central Composite Design modelling.

H. Lalande, McGill University Department of Natural Resources, generously provided training and assistance with the flame AAS maintenance. A. Corriveau, Biotechnology Research Institute Department of Environmental Analytical Chemistry helped me to develop a method to analyse heavy metals with the graphite furnace AAS.

D. Chan-Hum, as Departmental Secretary and Dr. Raghavan, as Chair of the Department provided prompt and reliable answers to every question. Natural Sciences and Engineering Research Council of Canada (NSERC) and Biotechnology Research Institute (BRI) supplied the funds and tools to conduct the present research project. Special thanks go to the Environmental Analytical Chemistry group of the Biotechnology Research Institute, for its support and for making the work environment pleasant and friendly.

I would also like to express thanks to P. Alvo for reading this manuscript and giving constructive comments.

Finally, I gratefully acknowledge my mother, Françoise, and all my family for their tremendous support and encouragement I have received from them over the course of my studies.

SYMBOLS AND ABBREVIATIONS

STATISTICAL SYMBOLS

X_k	Denotes an individual factor and its level k
Y	Response value
$\hat{y}$	Point estimate of y
$\bar{y}$	Denotes the mean of data set y
$s_{\bar{y}}$	Standard error of the mean
η	Response variable
ε	Experimental error
ϕ	Response surface, continuous function of the X_k
σ	Population standard deviation.
s	Sample standard deviation.
σ^2	Population variance. A measure of how far an individual measurement is from the population mean.
s^2	Sample variance. A measure of how far an individual measurement is from the sample mean
v	Degrees of freedom
β_i	Regression parameters of regression second-order equation
b_i	Estimate of the regression parameter
N	Number of experimental points
N_c	Number of cube points
N_a	Number of axial points
N_o	Number of center points
$F_{2,v,\alpha}$	F distribution
$t_{v,\alpha}$	Students t-distribution
α	Significance level corresponds to confidence level of 100(1-α) percent. Typically 0.05
n	Number of observations in a sample

SORPTION ISOTHERM SYMBOLS

c_i	Initial metal concentration (mg/L)
c_f	Final metal (or residual) concentration (mg/L)
c_s	Saturation concentration (or solubility limit) of the solute (mg/L)
M	Dry weight of biomass (g)
q_M	Uptake of metal M (mg/g)
q_{max}	Maximum metal uptake or maximum adsorption capacity (mg/g)
V	Volume of liquid (L)

CCD SYMBOLS

$INCONC_M$	Initial metal concentration of the metal M (mg/L)
PH	pH
TEMP	Temperature (°C)
TIME	Contact time (min)

ABBREVIATIONS

Al	Aluminum
ANOVA	Analysis of Variance
BET	Brunauer, Emmer and Teller model
CCD	Central Composite Design
Cd	Cadmium
Cu	Copper
Fe	Iron
h	Hour
kg	Kilogram (1000 g)
L	Litre
LOF	Lack of fit
mg	Milligram (0.001 g)
mg/g	Milligrams per gram (typical units for reporting heavy metal uptakes by biomass)
mg/L	Milligrams per litre (typical units for reporting heavy metal concentrations in aqueous liquid)

min	Minute
mL	Millilitre (0.001 litre)
mm	Millimetre (0.001 metre)
mmol	Millimole (0.001 mole)
MW	Molecular weight
Ni	Nickel
Pb	Lead
POTW	Publicly owned treatment works
ppb	Parts per billion
ppm	Parts per million
PWB	Printed Wiring Board
Sn	Tin
t	Ton (1,000,000 g)
US EPA	United States Environmental Protection Agency
Zn	Zinc

1. INTRODUCTION

1.1. Problem statement

Heavy metals can be defined as metallic elements with an atomic weight greater than that of iron (55.8 g/mol), or as elements with a density greater than 5g/cm^3 (Schüürman and Market, 1997). Concern about heavy metals is due primarily to their potential toxicity, persistence and tendency to become concentrated in food chains (bioaccumulation). Heavy metals, such as lead, mercury, zinc, copper, nickel, cadmium and chromium can be found in sewage, and in the environment in general, as a result of human activities. Manufacturers of printed wiring boards (PWB), an important sector of the electronics industry, use various heavy metals such as cadmium, zinc, nickel, copper and lead. Over 60% of the hazardous wastes produced by these manufacturers are found as soluble metals in their wastewaters (Judd et al., 1987). These are a serious environmental threat and must be dealt with accordingly. Increasingly stringent effluent discharge limits have made the removal and recovery of dissolved metals a major concern for this industry.

Conventional technologies to recover heavy metals from wastewaters include chemical precipitation, ion exchange, membrane technologies, and electrochemical treatment. However, these processes appear to be ineffective or extremely expensive, especially when the dissolved metals are at concentrations of 1 to 100 mg/L (Volesky, 1990). The conventional precipitation methods are still the main technology used by the printed board manufacturing facilities. Moreover, the total cost for the wastewater treatment of a small printed wiring board manufacturing process is about $ 18,000 (U.S currency) per year (Chang and McCoy, 1991). For these reasons, it is worthwhile developing alternate treatments that: a) cost no more than precipitation technology; b) are environmentally–friendly; and, c) can reduce the volume of and toxicity of the heavy metals.

A number of alternative technologies have been studied extensively in the last two decades. They are based on the metal-sequestering properties of certain natural biomasses, such as fungi, bacteria, and algae. Of the microorganisms studied, marine algae are gaining attention due to their availability, their capacity for high uptake of metals, and for their low cost. Although many studies have described the sorption characteristics of various algae, few have focused on the red algae species (*Rhodophyta*). Within the red algae group, *Palmaria palmata* has the highest solubility in water. In addition, *Palmaria palmata* algae are easily available and can be provided at minimum cost This thesis presents the results of the metal uptake by the red marine algae *Palmaria palmata* under various combined environmental conditions.

1.2. Objectives

The overall objective of this research project is to develop a statistical design (Central Composite Design or CCD) to evaluate the effect of changes in environmental factors and their mutual interactions on the biosorption in a mono and binary component system.

More specifically, the objectives of the present work are:

a) To characterize the sorption of various metals (Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Pb^{2+}) by red algae *Palmaria palmata.*
b) To experimentally quantify the metal uptake under varying physicochemical conditions, such as:
    - Initial metal concentration
    - pH
    - Contact time
    - Temperature

To satisfy these objectives, a comprehensive literature review was conducted that included form and fate of inorganic pollutant in the aquatic environment, biochemistry, remediation technologies and statistical methods.

Batchwise sorption experiments were conducted to quantify the metal binding. The data from the preliminary experiments were modelled mathematically. First a suitable theoretic isotherm model was formulated. Then, the metal binding constants were derived from the isotherm model. The influence of the above mentioned factors on metal sorption were modelled by a statistical design according to the following steps:

1. Theory: choice of an appropriate statistical experimental design, of the responses to be maximized, the factors to be varied, the factor levels and step size to apply in the experiments.
2. Experimental evaluation of the factors range.
3. Collection of the data according to the established statistical design.
4. Checking for the model fit.
5. 3-D graphical representation of the data to visualise the effect of two factors on the biosorption.
6. 2-D sections of the 3-D diagrams to estimate quantitatively the influence one physico-chemical parameter on the metal uptake in mono-metal and binary systems, and on the metal uptake inhibition due to the influence of the other metal in the binary system.

1.3. Thesis organization

The present thesis is organized into 8 sections. In the introduction, section 1, the overall project objectives and scope of work are defined. A comprehensive literature review, section 2, provides background material on a broad range of topics related to the development and application of heavy metal remediation. Section 3 describes experimental, analytical and statistical methods used in the experiments. Section 4 presents experimental results and discussions on the statistical analyses. A summary and conclusions are presented in section 5, followed by recommendations for future work in section 6. Finally, references are provided in section 7, and appendices are shown in section 8.

1.4. Scope of work

The scope of the present research project is to investigate metal uptake by red algae *Palmaria palmata,* in mono and binary sorption systems. The metals studied in the preliminary experiment are limited to those commonly found in the printed wiring board industry rinse waters and wastewaters, namely Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Pb^{2+}. The two metals for which *Palmaria palmata* exhibits the greatest affinity were selected for the following experiments. It is noted that the dissolved metal nitrate salts used in the bench-scale experiments are more homogeneous than industrial wastewater samples, as these latter may contain suspended solids, other metals or solvents which might interfere with the biosorption of the metals studied.

Although adsorption on the cell surfaces is the dominant mechanism in the uptake of metals by algae (Kuyucak and Volesky, 1989), biosorption (or sorption on biomass) will be used here to describe the removal of metals by algae through both surface adsorption and accumulation inside cells. Furthermore, a dead biomass is preferred to a viable one for several reasons: First of all, the biosorption process should deal with harsh environmental conditions in eventual industrial applications. Secondly, there is no requirement for maintenance and nutrition, and the biosorbents can be stored for a long time without having their performance altered (Young and Banks, 1998). Thirdly, there is no toxic effect of metals on microorganisms. Last but not least, in many cases sorption by dead biomass is more efficient than sorption by living biomass (May, 1984; Tsezos, 1990).

2. LITERATURE REVIEW

2.1. Introduction

This literature review provides background information related to the development of heavy metal removal processes. The broad scope of the literature review is to emphasise the interdisciplinary nature of remediation technologies. The fields of application include biology, chemistry, biochemistry, engineering, mathematics, and statistics.

The introduction section begins with a description of the heavy metal sources, followed by their chemical properties, their pollution in the aquatic environment, and their toxicity.

2.1.1. Heavy metal sources

Heavy metals enter the environment through natural and anthropogenic activities. For instance, the weathering of rocks naturally produces heavy metals. On the other hand, the burning of petroleum, and non-ferrous metalworking contribute to the emissions of heavy metals in the atmosphere. Nriagu and Pacyna (1988) estimate that an average of 332,350 t lead, 35,370 t copper, 55,650 t nickel and 131,880 t zinc are emitted in the atmosphere each year. Furthermore, the main causes of water pollution are household waste, iron and steel production, metal smelters, metal plating or finishing operations, mining and fertilizer applications. The anthropogenic median annual inputs of lead, copper, nickel and zinc into aquatic systems were reported to be 138,000 t, 112,000 t, 113,000 t and 226,000 t, respectively (Nriagu and Pacyna, 1988). The two principal sources of heavy metals in soils are the disposal of ash residues from coal combustion and general disposal of commercial products on land. Nriagu and Pacyna (1988) estimated the average annual worldwide emissions into the soils to be 796,000 t for lead; 954,000 t for copper; 325,000 t for nickel and 1,372,000 t for zinc). As far as the Canadian situation is concerned, the third National Pollutant Release Inventory (NPRI) was performed in 1995 (CEPA, 1995) for 1,758 Canadian facilities. Copper, zinc and lead were among the 10 pollutants released on site to water in the greatest quantities. Heavy metal releases are summarized in Table 1.

Pollutant	air	water	Underground	Land	Total releases
Copper	485	12,418	0	1,353	14,262
Zinc	647	2,021	1	3,764	6,443
Lead	663	81	0.04	822	1,572

Table 1. Heavy metals (and their compounds) released on site to different environmental compartments (tonnes), from NPRI (CEPA, 1995).

2.1.2. Forms and fate of heavy metals in the aquatic environment

Many processes can change the physico-chemical forms (or speciation) of the heavy metals and can have a great influence on the stability of metals in solutions. The processes involved are:

(1) oxidation / reduction,
(2) adsorption / desorption,
(3) precipitation / dissolution,
(4) aggregation / disaggregation.

➢ Cadmium

Since cadmium is present in the + 2 oxidation state in oxygenated surface waters, redox reactions are not common for this element (EPA, 1985). Cadmium can form complexes with hydroxide, carbonate, chloride, sulfate and humic material. However, uncomplexed cadmium is the dominant species below pH = 6.9 (EPA, 1985).

➢ Copper

Copper occurs in four oxidation states: 0, +1, +2 and +3, but most commonly as +1 (cuprous), and +2 (cupric). Cuprous copper (Cu (I)) is unstable in aerated aqueous solutions, and is normally oxidized to cupric copper (Cu (II)) (Leckie and Davis, 1979). Divalent copper salts are very soluble in water. Copper is generally more soluble in acidic waters, and precipitates as $Cu(OH)_2$ at pH values above 6.5. In the presence of excess cupric ion in alkaline waters, carbonates, hydroxides, oxides and sulphides form precipitates. In the presence of soluble organic matter, sorption of copper may be relatively ineffective.

- Lead

Lead exists in several oxidation states: 0, +1, +2 and +4. However, the divalent form Pb^{2+} is the stable ionic species in most of natural environments (US EPA, 1979). Precipitation and sorption are the principal mechanisms controlling the distribution of lead in the aquatic environment. In the presence of inorganic ligands, lead can precipitate as a number of compounds including $PbSO_4$, $PbCO_3$, $Pb(OH)_2$, PbS, and $Pb_3(PO_4)_2$. At $5 \leq pH \leq 7$, most of the lead is precipitated and sorbed as sparingly soluble hydroxides (CCME, 1994). At $7 \leq pH \leq 9$, $PbCO_3$ is the major species (EPA, 1985). Below about pH 5-6, the formation of cationic species prevents the formation of hydroxides (CCME, 1994).

- Nickel

Nickel forms can be found in the oxidation states: 0, +1, +2, +3 and +4. But the most important valence state is +2. Nickel can precipitate as hydroxide and carbonate in natural rivers. Although nickel can be adsorbed by iron, manganese oxides and organic substances, it is relatively more mobile in the aquatic environment than other heavy metals (EPA, 1985). At pH=7, in the presence of organics, practically all the nickel is present as free divalent nickel (Vuceta and Morgan, 1978).

- Zinc

The chemistry of zinc is quite similar to that of cadmium. In aqueous solutions, zinc exists in the +2 oxidation state. Sorption of zinc by hydrous metal oxides, clay minerals and organic materials, is common in aquatic systems. In the presence of dissolved solids, zinc is transported in solution as hydrated cations or complex species. In the presence of suspended solids, sorption of zinc may occur (EPA, 1985). However, at pH 6.2-8.0, the predominant species is the free cation (Vuceta and Morgan, 1978).

2.1.3. Toxicity

- Lead

Lead is a metal that is toxic to humans, aquatic fauna and livestock. It has no known physiological function. Acute or classical lead poisoning in humans is manifested by anaemia, alimentary symptoms, wrist and foot drop, renal damage, and sometimes encephalopathy. Concentrations of lead greater than 0.8 mg/L in the blood are unequivocal evidence of clinical lead poisoning (Lester, 1987). Furthermore, lead has been associated with behavioural effects and intellectual impairment in children. Lead is also embryotoxic and tetratogenic in humans, and causes spontaneous abortions.

➢ Cadmium

Cadmium has been established to be a very toxic heavy metal. The "Itai-Itai" disease was reported in Japan, and is believed to be due to cadmium poisoning, resulting in osteomalacia and fractures (CEPA, 1994). In addition, cadmium may cause renal tubular dysfunction, and formation of kidney stones (CEPA, 1998 (a)). Inorganic cadmium compounds have been classified as "probably carcinogenic to humans", on the basis of inhalation studies in animal species (CEPA, 1998(a)).

➢ Copper

Copper is an essential element used in the process of blood formation and iron utilization. The human daily requirement is 0.03 mg/kg for adults (Lester, 1987). Symptoms of acute toxicity are gastric ulcers, hemolysis, jaundice, hepatic necrosis, and renal damage. For instance, the ingestion of copper-contaminated water at concentrations of 0.8 mg/L was reported to cause pink disease in infants (National Academy of Science, 1977). Furthermore, copper has been shown to be carcinogenic to animals but not conclusively so to humans (Kurzel and Cetrulo, 1981).

➢ Nickel

Nickel is rather similar to copper in that it is a metal that does not have serious effects on humans but has an appreciable phytotoxicity when in solution. While nickel has been shown to be an essential element for normal growth in some animals, its role as an essential element for man has not yet been confirmed. The most toxic form of nickel known to man is nickel carbonyl. When inhaled, nickel carbonyl can cause severe lung damage and produces symptoms of headaches, vertigo, nausea, vomiting, and insomnia. Nickel is also capable of causing mutagenic effects by binding to phosphates and heterocyclic bases of nucleic acids in the place of other elements such as magnesium (Lester, 1987). Moreover, Doll et al. (1990) concluded that exposure to high concentrations of soluble, oxidic or sulphidic forms of nickel resulted in increases in mortality due to lung and nasal cancer. However there was no evidence that elevated mortality due to cancer was associated with exposure to metallic nickel.

- Zinc

Zinc is an essential element, with a human daily requirement of 10 to 20 mg. (Lester, 1987). Zinc tends to be less toxic than other heavy metals. However, some symptoms of zinc toxicity are vomiting, dehydratation, electrolyte imbalance, stomach pain, nausea, lethargy, dizziness, and muscular incoordination. Last, the role of zinc as a carcinogen or carcinogen is unclear.

To summarize, lead, cadmium, copper, nickel and zinc are metals of interest due to their toxicity to humans, animals and plants. They are all listed on the Canadian National Pollutant Release Inventory substances (NPRI) (CEPA, 1995). Furthermore, cadmium and nickel are considered as priority substances under the Canadian Environmental Protection act (CEPA, 1994). Last, the International Agency for research on the Cancer identified the nickel as carcinogenic and cadmium as probably carcinogenic (CEPA, 1995).

2.2. Heavy metal pollution by printed wiring board manufacturing industry

Section 2.2 described the behaviour of heavy metals in the aquatic environment. Since more than 60% (by weight) of waste from the printed wiring board (PWB) production line results in aqueous metallic forms (Chang and McCoy, 1991), removal of heavy metals from PWB manufacturing waste is the major concern of the present study. This section will highlight the importance, the sources and the American regulation of heavy metal pollution in the PWB industry.

2.2.1. Overview of the PWB manufacturing industry

Printed wiring boards are physical structures on which electronic components such as semiconductors and capacitors are mounted. Printed wiring boards are mainly used in electronic toys, guided-missiles, other airborne electronic equipment, electrical wiring in cars, biotechnology and medical devices, digital imaging technology, industrial control equipment, radios, television sets and computers. Generally, small companies produce up to 3,000 to 5,000 ft^2 board/ month (279 to 465 m^2 board/ month) whereas large companies process 30,000 to 50,000 ft^2 board/ month (2,790 to 4,650 m^2 board/ month) (EPA, 1990). Average daily wastewater flow ranges from 7,000 gpd to 11,000 gpd (26.5 m^3/ day to 41.6 m^3/ day). The heavy metal concentrations are 190.5, 105.9, 4.4, and 285.7 mg/L for copper compounds, lead compounds, nickel, and zinc compounds, respectively (EPA, 1990 (c)).

2.2.2. Wastewater generation and discharge

The production processes of the PWB manufacturing industry are comparable to those of the electroplating and metal finishing industries. Among the numerous steps required to process a PWB, the major source of inorganic pollution results from the disposal of the spent plating baths and rinses following the plating process. Another main source of metallic contaminants is the chemicals used as part of the plating preclean operations or in the removal of excess surface metal. Last etch rinses may also include high metal concentrations. Table 2 summarizes the inorganic chemicals used in the most metallic polluting steps of a PWB process.

Process steps	Definition	Chemical used containing heavy metal
Electroless plating • With copper	Chemical deposition of a uniform conducting layer of metal on the board surface	• Palladium chloride • Metallic tin pellets • Copper sulfate
• With tin-lead		• Tin chloride
Etching	Removal of rust inhibitor or other metals (etching of copper after the stencil printing process)	• Cupric chloride • Lead, nickel, tin • Nickel chloride • Nickel sulfamate
Electroplating • With copper	Electrochemical deposition of a uniform conducting layer of metal on the board surface	• Copper sulfate • Copper pyrophosphate • Copper acids
• With tin-lead		• Tin-lead

Table 2. Industry Profile and Description of chemical use for the Printed Wiring Board Industry (EPA, 1995 (b)).

Furthermore, a summary of typical concentrations of raw waste for PWB manufacturing is shown in Table 3.

Constituent	Range (mg/L)
Total suspended solids	0.998-408.7
Cyanide, total	0.002-5.3
Cyanide, free	0.005-4.6
Copper	1.582-535.7
Nickel	0.027-8.4
Lead	0.044-9.7
Chromium, hexavalent	0.004-3.5
Chromium, total	0.005-38.5
Fluorides	0.648-680.0
Phosphorus	0.075-33.8
Silver	0.036-0.2
Palladium	0.008-0.10
Gold	0.007-0.19

Table 3. Raw waste data-printed wiring board manufacturing (EPA, 1987).

2.2.3. Laws and regulations affecting PWB manufacturing wastewater

Electronic industry wastewaters are regulated by increasingly strict effluent discharge limits. This section will focus on the environmental laws and regulations of PWB wastewaters in Canada and the United States.

In Canada, liquid effluents coming from PWB manufacturing are regulated at the federal level by the "Metal finishing liquid effluent guidelines" issued by Fisheries and Environment Canada in 1977, and by the Transportation of Dangerous Goods Act (TGDA), promulgated by transport Canada in 1985. Provincial environment ministries also establish their own guidelines. In some provinces, the regulatory requirements may be controlled by agencies such as the provincial ministry of agriculture or the ministry of health (EPS, 1987). For example, the provincial Environmental Quality Act of Quebec regulates discharges to sanitary sewers and storm sewers (water courses). Some municipalities may have ambient concentration requirements and can have a permit system for industries connecting to the sanitary sewer line. For instance, Montreal Urban

Community authorities force the plating industries of Montreal to install special pretreatment systems (EPS, 1987).

The most important U.S. federal regulations regarding pollution prevention and recycling are: the Pollution Prevention Act of 1990 (which formalizes a national policy and commitment to waste reduction); the Emergency Planning and Community Right-to-know Act (EPCRA, also known as SARA title III, which requires companies to report the amounts of listed toxic chemicals entering the environment); the Resource Conservation and Recovery act (RCRA which includes regulations of hazardous wastes); and the Clean Water act (CWA, which covers regulation of wastewater discharges). More specifically, wastewater discharges from PWB manufacturing processes are regulated by two categorical regulations found in the CWA: 40 CFR 413 (Electroplating Regulation) and 40 CFR 433 (Metal Finishing Regulation). Part 413 is applied to PWB manufacturing facilities that have been operating since July 15, 1983. All the other companies are regulated by part 433, which is more stringent than part 413.

In addition to these federal regulations, some states such as Arizona, California, Minnesota and Texas passed state pollution laws that include source reduction and waste minimization plan. The Clean Water act allows some qualified Publicly owned Treatment Works (POTW) to regulate industrial discharges. The local requirements imposed by these POTW that can be even more stringent than federal guidelines.

Canadian federal metal finishing and United States electroplating heavy metal discharge limits are summarized in Table 4.

	Maximum total concentration (mg/L)	
	Canada (EPS, 1977)	USA (EPA, 1990 (a))
Cadmium	1.5	0.26
Chromium	1.0	1.71
Cyanide (total)	3.0	0.65
Copper	1.0	2.07
Lead	1.5	0.43
Nickel	2.0	2.38
Zinc	2.0	1.48

Table 4. Canadian finishing liquid effluent guidelines for direct discharges and US pretreatment standards for the metal finishing category (40 CFR 433).

2.3. Conventional treatments of metal contaminated PWB manufacturing wastewater

Due to increasing costs for chemicals, energy, treatment and disposal, some PWB manufacturing firms may prefer to use process solution maintenance and chemical recovery technologies rather than waste treatment. For example, in a bleed-and-feed process, fresh chemicals can be supplied to the process solutions to maintain the process bath within operable chemical range, with the bleed stream going to wastewater. Alternatively, if the bath exceeds tolerable limits, reusable components can be removed by recovery technologies such as ion exchange, electrowinning (section 2.3.3) or evaporation. However, implementing pollution prevention alternatives requires accurate technical and economical information. Therefore, end-of pipe treatment often competes with bath maintenance, or recovery technologies. The primary purpose of the wastewater treatment systems in the PWB manufacturing pollution control is the removal of dissolved metals. The following section is therefore consecrated to examples of the most common remediation methods to remove and/or recover metal ions from contaminated wastewaters.

2.3.1. Chemical precipitation

Precipitation is used as the treatment scheme to extract heavy metals from solutions by almost 75% of the plating companies (Cushnie, 1985). Precipitation of metals from contaminated water involves the conversion of soluble heavy metal salts to insoluble salts that will precipitate. Physical methods such as clarification (settling) and/or filtration will then remove the precipitate from the treated water. This process requires adjustment of pH, addition of a chemical precipitant, and flocculation.

The most common precipitation methods in industries are hydroxide (or lime) precipitation, sulphide precipitation and sodium borohydride precipitation. The advantages and disadvantages of each precipitation method are summarized in Table 5.

Type of precipitation	Advantages	Disadvantages
Hydroxide precipitation	• Automatic control • Well accepted in industries	• Presence of chelating agents having adverse effect on metal removal • Not effective in the case of mixed metal waste • Generation of large volume of sludge
Sulphide precipitation	• Effective process due to low solubilities of metal sulphides • Broad optimal operating pH range	• Potential formation of toxic H_2S gas • High costs
Sodium borohydride precipitation	• Production of low sludge volume • Treatment of lower effluent concentration	• High cost of the sodium borohydride reagent

Table 5. Advantages and disadvantages of the most common precipitation methods.

Several studies have demonstrated the effectiveness of precipitation methods for the removal of heavy metals from electroplating wastewater. For instance, Abrego (1997) evaluated precipitation and flotation for removing a variety of metals. This technology was reported to be successful over a wide pH range (3-12) in wastewater obtained from the electroplating industry. The metal ions Pb^{2+}, Ni^{2+}, Sn^{2+}, Fe^{2+}, and Al^{3+} were effectively stripped from the contaminated samples to below levels required by discharge regulations, but the method was not effective for Cu^{2+}. In addition, Kongsricharoern and Polprasert (1996) reported chromium removal by a bipolar electrochemical precipitation process (ECP). The electroplating wastewater used for this laboratory-scale research contained chromium concentrations in the range of 570-2100 mg/L. The chromium removal efficiencies were higher than 99% and the chromium concentrations in the treated effluent were less than 0.5 mg/L.

The total costs of precipitation systems consist of the capital costs, which depend mainly on the design flow rate, and the operating costs. Operating costs include chemical costs and sludge disposal. For example, capital costs for 75- and 250-litres-per-minute (20-gpm and 65-gpm) packaged metals precipitation systems are approximately $85,000 and $115,000 (US Currency), respectively. Operating costs (excluding sludge disposal) range from $0.08 to $0.18 per 1,000 litres ($0.30 to $0.70 per 1,000 gallons) of water containing up to 100 mg/L of metals (US Currency). Sludge disposal represents approximately $0.13 per 1,000 litres ($0.50 per 1,000 gallons) of water treated (US Currency). Actual sludge disposal costs (including fixation and transportation) have been estimated at approximately $330 per metric ton ($300 per ton) of sludge (US Currency).

In addition, sludge disposal may be estimated to increase operating costs by approximately $0.13 per 1,000 litres ($0.50 per 1,000 gallons) of water treated (US Currency). Actual sludge disposal costs (including fixation and transportation) have been estimated at approximately $330 per metric ton ($300 per ton) of sludge (US Currency).

Although the costs are low for the treatment of high volume, and this reliable process is well suited to osmotic control, precipitation technology presents many disadvantages:

- The presence of multiple metal species may lead to removal difficulties as a result of amphoteric natures of different compounds.
- As discharge standards become more stringent, further treatment may be required.
- Reagent addition must be carefully controlled to preclude unacceptable concentrations in treatment effluent.
- Efficacy of the system relies on adequate solids separation techniques (e.g., clarification, flocculation, and/or filtration).
- Process may generate toxic sludge requiring proper disposal.

- Process can be costly; depending on reagents used, required system controls, and required operator involvement in system operation.
- Treated water will often require pH adjustment.
- Metals held in solution by complexing agents (e.g., cyanide or EDTA) are difficult to precipitate.

For these reasons, precipitation appears to be a complex technology. It is not considered as environmentally friendly because of the problem of sludge disposal and the recovery of spent chemical products.

2.3.2. Ion exchange

Ion exchange technologies have been successfully applied by metal finishing industries for several decades. Ion exchange is more widespread in the PWB industry than in the plating industry because of the limited number of regulated ionic species present in PWB wastewater. Indeed, copper, lead and nickel are the only metal ions present in significant concentrations, and all are amenable to ion exchange (or more specifically cation exchange).

The system that is most commonly used involves cation exchange resins to remove metal ions from a waste stream. Ion-exchange resins are insoluble polymers that have active ionogenic groups that are either permanently ionized, or capable of ionization or acceptance of protons to form the charged site. The resin interacts with mobile ions of opposite charge from the external solution. Ion exchange resins are capable of exchanging an H^+ ion for a cation in the waste stream, or in the case of anion resins, an OH^- ion for an anion in the waste stream. The resin is regenerated by an acid (cation resin), or a base (anion resin), when the exchangeable ions have been depleted.

Few studies reported the characteristics of resins (such as operating conditions, and efficiency of regeneration) used to treat wastewaters from printed wiring board manufacturing activities. However, Gold et al. (1987) compared different chelate resins for the treatment of contaminated electroless and electroplating baths. The results demonstrated that the Amberlite IRC-718 resin is more selective to copper (94%) than nickel, and is relatively selective to zinc and lead (50%). On the other hand, the Duolite ES-467 resin is more efficient for lead and zinc (89% removed) than for copper (75% removed but with a leakage of 40% at end-of-pipe). Duolite CS-346 (a macroporous chelating resin), Amberlite IR-124, Amberlite IRC-718 (synthetic aluminosilicates) and Valfor 200 (zeolite) cost $11.00/kg, $6.61/kg, $11/kg and $0.551/kg respectively (Czupyrna, 1989). The total cost for a typical ion exchange system ranges from $ 0.08 to $ 0.21 per 1,000 litres ($0.30 to $0.80 per 1,000 gallons, US Currency) treated (EPA, 1990 (b)). This cost approximation includes preatrement and discharge costs, resin utilization and regeneration costs.

The main advantages of ion exchange resins are their effectiveness, their relatively insensitivity to flow variations and the large variety of specific resins available. Moreover, most ion exchange resins can be completely regenerated, and can be used for numerous cycles without being replaced. The following disadvantages should be noted. The accumulation of deposits may shorten the useful life of resins. This technology is not usually feasible at high levels of total dissolved metals. The costs involved for large-scale heavy metal removal are usually prohibitive.

2.3.3. Electrowinning

Ion exchange systems are generally used in conjunction with electrowinning in PWB facilities. More specifically, electrowinning is used to remove metallic ions from concentrated rinse water, spent process solutions and ion exchange regenerant.

An electrowinning design consists of a rectifier and a reaction chamber containing the electrolyte in which are set the cathodes and anodes. Metal ions from solutions are reduced on the cathode at a rate that depends on the metal concentration in the electrolyte, the current and cathode area, and the species of metal being recovered.

Metals that are most commonly recovered by electrowinning are copper, gold and silver. As an illustration, an electrowinning efficiency of copper recovery from a microetching process was reported to range from 80 to 90%. The metal content was reduced to 1 mg/L in the waste stream (EPA (a), 1990). The total cost (including equipment and installation costs) for the electrowinning of high copper concentration baths was about $7,200 per year (US Currency).

Although electrowinning may be very efficient for a wide variety of chemical solutions in the PWB industry and there is no metal sludge generation, this technology suffers from many restrictions. First, conventional electrowinning is only efficient for removing metal from solutions containing a moderate to high concentration of metal ions (> 3,000 mg/L). Secondly, electrowinning is not applicable to solutions containing hydrochloric acid, because electrolysis of solutions containing chlorine ions can result in the formation of chlorine gas. Last, the recovered metal is usually not pure enough to be used as anode material in plating processes.

2.3.4. Membrane technologies

Of the many membrane separation processes available for the removal of inorganic contaminants from wastewater, only two (electrodialysis and reverse osmosis) are commonly used in the metal finishing industry. These are electrodialysis and reverse osmosis. Both processes include semipermeable membranes to separate salts from solution and are described below.

2.3.4.1. *Reverse osmosis*

Reverse osmosis is an ex-situ separation process most commonly used in the desalination of the water. However, in the past decades a particular effort has been made for the application of reverse osmosis in the metal-finishing industry, with recovery of concentrated solutions of metal salts and reuse of the water in cleaning.

Reverse osmosis is aimed at separating water from ionic solutes (metal salts for example) and macromolecules. Reverse osmosis is a pressure driven reversal of the natural process of osmosis. In the osmosis process, water is transferred through a semipermeable membrane from the waterside of the membrane to the dilute solution side until an osmotic equilibrium is reached. In the reverse osmosis process, a hydraulic pressure (typically from 200 to 1200 psi) is applied to the salt solution side. This arrests or reverses the flow of water through the membrane depending on whether the pressure equals or exceeds the osmotic pressure. Three types of semipermeable membrane materials can be used in the reverse osmosis units: cellulose acetate, hollow fibber polyamides, and polyether / amide on polysulfone membranes (thin film composite). Up to a few years ago, reverse osmosis membranes were made almost exclusively of cellulose acetate. But new thin-film composite has gained more attention recently. The performance of reverse osmosis depends on membrane composition and configuration, pressure, temperature and concentration of the feed water, the ionic charge and size of the specific treated ions. The larger the ion and the higher its valence, the better the rejection of the metal ion. Thus, divalent ions are more highly rejected than monovalent ions. This is of particular interest since the most common regulated species found in the PWB manufacturing wastewaters are divalent (copper, lead, nickel, cadnium and zinc).

Although very few studies discuss the use of reverse osmosis technology to the copper plating line, an example of reverse osmosis application to recover and recycle copper cyanide is given by Holland et al. (1988). The two-stage rinsing/recycling process could reject 98% of copper cyanide, a compound that can be directly reused.

Typical costs for filtration methods are in the range $0.36 to $1.20 (US Currency) per 1,000 litres ($1.38 to $4.56 per 1,000 gallons) treated (EPA, 1990 (b)). Reverse osmosis has been used for many years to treat industrial and domestic wastewaters. The advantages of reverse osmosis are its ability to efficiently remove efficiently almost all the contaminants contained in the effluent. However, currently available reverse osmosis membranes are sensitive to chlorine, pH and oxidants, and are liable to fouling by submicron particulates entering with the feed or by precipitation of salts at the surface of the membrane, as the feed stream is concentrated. Furthermore, the extensive feed pretreatment and the high transmembrane pressure required result in high-energy costs. This technology, which has proved successful in the desalination of brackish water and seawater, might therefore not be economically competitive for metal recovery.

2.3.4.2. *Electrodialysis*

Electrodialysis is a mass separation in which electrically charged membranes and an electrical potential difference are used to separate ionic species from an aqueous solution and other uncharged components. More specifically, ionic materials are selectively transported within a stack of closely spaced ion exchange membranes. The driving force is provided by voltage from a rectifier and is imposed on electrodes at the two ends of the stack.

Electrodialysis is used successfully for recovering gold, silver, nickel, and tin from electroplating rinsewaters (EPA, 1987). In addition, Automatic Plating of Bridgeport (APB), in conjunction with the Connecticut Hazardous Waste Management Service, tested the feasibility of using electrodialysis to recover nickel from spent rinsewater from a nickel plating line. Estimates (EPA, 1995(a)) indicated that the system can recover 26,964 pounds of nickel and 1.07 million gallons of water from the waste treatment system. Capital costs for the system including installation were approximately $110,000 (US Currency). At current nickel prices of about $2.80 US per pound, capital cost recovery is less than 3 years, interest included. Compared to the other membrane methods, such as reverse osmosis, electrodialysis requires minimal amounts of energy. In addition, due to more thermally and chemically stable membranes, electrodialysis processes can be run at elevated temperatures and in solutions of very low or high pH. The disadvantage of electrodialysis is that the membrane stack requires careful handling and periodic maintenance.

2.3.5. Evaporation

Evaporation is the use of an energy source to vaporize a liquid form from a solution, slurry, or sludge. In electroplating, nonvolatile metal salts are concentrated in the evaporating water and can be reused. Atmospheric evaporators operate at atmospheric pressure and release the moisture to the environment. Vacuum evaporators are also used and vaporize water at lower temperatures. The Cart marker process (EPA, 1987) is an example of an atmospheric evaporator. Chromic acid additions were reduced by about 95% and the waste treatment by sodium bisulphate was eliminated. On the other hand, the cadmium platter process is an example of the vacuum evaporator and is used to recover cadmium salts from a cadmium cyanide plating system (EPA, 1987).

Operating costs includes electrical power for the blower and pump equipment, and heat for evaporation (usually 626 watts per litre or 3.371 watts per gallon). Evaporation is an easy, maintenance-free, reliable and commonly applicable process. The main disadvantages are high-energy consumption and undesirable constituents in the recycled bath.

2.3.6. Carbon adsorption

Carbon adsorption is a separation technology used to remove and recover certain inorganic compounds from single--phase fluid streams. Granular activated carbon (GAC) is used as the adsorbent. Activated carbons consist of amorphous forms of carbon that have been treated to increase the surface area/volume ratio of the carbon. The most widely used activated carbons are F-400 activated carbon from Calgon, which is made from bituminous material, and rice-hull activated carbon (RHAC). Some batch experiments have compared the heavy metal removal efficiency of the two types of GAC (Kim and Choi, 1998). The activated carbon F-400 was reported to effectively remove chromium and lead but did not remove cadmium, while the rice-hull successfully removed cadmium and lead but did not remove chromium. This phenomenon is linked to the interaction between the various surface charges of heavy metals and the distinct surface functional group SiO_2 remaining on the surface of RHAC.

Although activated carbon has a high adsorption capacity, GAC efficiency may be limited by the following factors:

- Costs are high, especially at high contaminant concentration levels. Costs associated with GAC depend on numerous parameters, such as wastewater flow rates, contaminant concentration, and mass loading. In general, at flow rates of 0.4 million litres per day (0.1 gpd), costs are about $1.70 per 1,000 litres (or $6.30 per 1,000 gallons) (US Currency) (EPA, 1190(b)). In addition, at each regeneration step, the activated carbon loses about 10% of its weight and 15% of its uptake capacity. Carbon losses add some extra cost (about 1.5 cents/ lb, US Currency) to the costs of the system and prevent activated carbon to be used for large-scale removal of heavy metals from industrial effluents.

Other factors may limit the efficiency of the GAC process:

- The presence of multiple contaminants requires bench tests to estimate GAC usage for mixtures.
- Streams with high suspended solids (> 50 mg/L) may cause fouling of the carbon and may require a pretreatment.
- Water-soluble compounds and small molecules are not adsorbed well.
- Spent carbon should be disposed of properly.

Carbon will provide effective purification at low cost when it is employed under certain conditions to do certain tasks, but it is usually advised to use carbon with other cooperating methods of separation. For example, comprehensive biofilms of bacteria bound together in an excreted polysaccharide matrix and attached to granular activated carbon (GAC) have been used for the biosorption of cadmium, copper, zinc and nickel (Scott et al., 1995). Another study shows that yeast immobilized in alginate acid, and alginate itself, have higher metal removal capacity than GAC and *Saccharomyces cerevisiae* yeast immobilized in GAC (Wilkins and Yang, 1996).

2.4. An alternative to the conventional treatments: the biosorption technology

As previously seen, potential wastewater treatment include chemical precipitation, ion exchange, membrane technologies, and electrochemical treatment. These processes appear to be ineffective or extremely expensive, especially when the dissolved metal is at a concentration of 1 to 100 mg/L (Volesky, 1990). In addition, they often create secondary problems since they give rise to metal-bearing sludges. Efforts are presently being made to develop novel technologies that:

(1) cost no more than precipitation technology
(2) are environmentally–friendly, and
(3) can efficiently reduce the volume and toxicity of the heavy metals.

Alternative technologies termed biosorption have been extensively studied in the last two decades and are based on the metal-sequestering properties of certain natural biomasses, such as fungi, bacteria, and algae. This section will give a more precise definition of biosorption, some examples of commonly used biomaterials and some current processes and commercial applications. Finally, the reasons why biosorption technology promises to fulfil the above requirements will be discussed.

2.4.1. Definition

Biosorption can be defined as the physico-chemical binding of metal/radionucleide species in solution to cellular components of biomass (Gadd, 1988). More specifically, the metal binding in biosorption may be due to a combination of several sequestering mechanisms such as complexing, co-ordination, chelation, adsorption, ion exchange or micro-precipitation (as metal or metal salts) (Volesky, 1990). However, ion exchange is believed to be the most important mechanism in the sorption of heavy metals by algal biomass. (Kuyucak and Volesky, 1989; Shiewer, 1996).

2.4.2. Biosorbent types

Various types of microbial biomass have been used for their biosorptive potential, including bacteria (Beveridge and Koval, 1981; Wallberg et al., 1991; Kelafa et al., 1999), fungi (De Rome and Gadd, 1987; Prakasham et al., 1999), marine and freshwater algae (Ferguson and Bubela, 1974; Schiewer, 1996; Kratchovil and Volesky, 1997; Zhou et al., 1998). Studies using the mixed microbial biomass found in activated sludge have also been reported (Tien and Huang, 1991). Some microbial derivatives such as excreted metabolites, polysaccharides or cell wall constituents have also been used as biosorbents. For instance, chitosan, a fungal cell wall component, was shown to have good metal-sequestering properties. Unusual biomass types, such as spent animal bones (Al-Asheh, 1999(a)) and canola meal (Al-Asheh, 1999(b)) have been found to efficiently remove heavy metals from aqueous solutions. It should be stressed here that both living and dead

cells are capable of uptake and accumulation of heavy metals. However a dead biomass is preferred to a viable one for the following reasons:
(1) The biosorption process is often executed in harsh environmental conditions.
(2) There is no requirement for maintenance and nutrition, and the biosorbents can be stored for a long time without affecting their performance (Young and Banks, 1998).
(3) There is no toxic effect of metals on dead microorganisms, so their sorptive capacity is not affected.
(4) In many cases, sorption by dead biomass is more efficient than sorption by living biomass (May, 1984; Tsezos, 1990).

Moreover, of the numerous microorganisms studied, marine algae are gaining attention due to their availability, their high metal uptake and low costs. For instance, marine brown algae showed similar or better sorption capacity than ion-exchange resin (Holan et al., 1993; Prasetyo, 1992).

2.4.3. Biosortion applications

2.4.3.1. Process design

The process of metal recovery using biosorbents is basically a solid-liquid contact process consisting of the metal uptake (sequestering) cycle and the metal desorption (elution) cycle. The metal-laden solution can contact the solid sorbent phase in many different types of process configurations such as upflow and downflow reactors, rotating biological contactors, trickle filters, fixed packed-bed contactor, and fluidized-bed contactor. Downflow contactors should theoretically be the most cost-effective process, since they do not require power other than from gravity to transfer water. However, in this type of process, the operator does not have a total control over the effluent retention times within the reactor. Another disadvantage of this system is the possible compaction of the bed with subsequent backflow of the waste stream. On the other hand, optimization of the contact process is possible by arranging the contact beds, columns or mixing vessels in series or parallel (Volesky, 1990).

2.4.3.2. Regeneration of the biomass

The metal laden biosorbent can be incinerated and/or stored in landfill, reducing the volume of the initial heavy metal contaminated matrix (in comparison with wastewater). But the active agent is preferably regenerated through a desorbing cycle using relatively inexpensive acid such as sulphuric acid (Tsezos, 1984). Thus, the purpose of desorption is not only to re-solubilize biomass-bound metals in a more concentrated form, but also to recover metals if they are economically significant.

2.4.3.3. Current commercial applications

Two main commercial biosorbents have been available since the early 1990's: AMT-BIOCLAIM™ and AlgaSORB™ (Kratchovil and Volesky, 1998).

Bacillus biomass has been used to elaborate a granulated metal sorption agent, AMT-BIOCLAIM™ (MRA), useful for wastewater treatment and metal recovery companies. AMT-BIOCLAIM™ can accumulate metal cations (2.90 mmol of lead per gram; 2.39 mmol of copper per gram; 2.09 mmol of zinc per gram; 1.90 mmol of cadnium per gram; 0.8 mmol of silver per gram) with efficient removal (>99%) from dilute solution (Brierley, 1986). In addition, the system is not affected by concentrations of elements such as calcium or magnesium.

Chlorella vulgaris-based biosorbent AlgaSORB™ is produced by Bio-recovery Systems Inc. (Las Cruces, New Mexico) and efficiently removes metal from concentrations of 100 mg/L to levels below 1mg/L. The medium consists of dead algal cells immobilized in a silica gel polymer and functions as a biological ion exchange resin to bind both metallic cations (such as mercury Hg^{2+}) and metallic oxoanions (such as selenate SeO_4^{2-}). AlgaSORB™ can be regenerated for more than 100 biosorption-desorption cycle. This technology can remove heavy metal ions from groundwater or surface leachates that contain high levels of dissolved solids. For example, the AlgaSORB™ sorption process was tested on mercury-contaminated groundwater at a hazardous waste site in Oakland, California. It can also treat rinse waters from electroplating, metal finishing, and printed circuit board manufacturing operations. Metals removed by this technology include aluminium, cadmium, chromium, cobalt, copper, gold, iron, lead, manganese, mercury, molybdenum, nickel, platinum, selenium, silver, uranium, vanadium, and zinc. This technology was accepted into the emerging technology program in 1988 in the United States.

2.4.3.4. Costs

The total cost of the bacteria-based AMT-BIOCLAIM™ was shown to result in a 50% saving over alkaline precipitation and a 28% saving over ion exchange (Wase and Forster, 1997). Whereas AlgaSORB™ requires immobilization methods that are simple and inexpensive. The immobilization cost of 1 lb. (=0.45 kg) of *Chlorella* biomass is about $0.25.

2.4.4. Conclusion : suitability of the biosorption technology for an industrial use

Compared to conventional systems such as ion exchange, activated carbon or membrane technologies, the main advantages of biosorption are:

- The process is rapid (most of the sorption described in the literature is achieved within one hour).
- The removal of metals is selective and efficient, even at low concentration. For instance, biosorbents and ion exchange resins (Duolite GT-73 and IRC-718) were compared for the removal of heavy metals from plating factory wastewater (Brower et al., 1997). NCP (*Neurospora crassa* gene expressed in the periplasmic space) was most efficient in removing the heavy metals at near neutral pH and when metal concentrations were very low concentrations (ppb levels).
- The system can operate over a broad pH range (3 to 9), and temperature range (4 to 90 C°) (Volesky, 1990).
- The technology offers low capital investment and operation costs (using cheap raw biomass, and including waste products from other industries, such as fermentation or pharmaceutical by-product).
- The volume of chemical sludge to be disposed of is eliminated or minimized
- The material has particle size, shape and mechanical strength suitable for use under continuous flow conditions.
- The regeneration of the biosorbent is metal - selective and economically feasible.

Biosorption is therefore an economically and technically efficient technology to remove and recover metals from wastewater streams.

2.5. Properties of *Palmaria palmata*

2.5.1. Occurrence

The marine alga *Palmaria palmata* (or *Rhodymenia palmata* or popularly known as Dulse in Great Britain and North America) is a red alga (*Rhodophyceae* family). *Palmaria palmata* belongs to the *Florideophyceae* class and to the *Palmariales* order. *Palmaria palmata* is found in the lower littoral and upper sublittoral zones of rocky coasts (Van Den Hoek et al., 1995). More specifically, this species is distributed throughout cool waters of the North Atlantic and North Pacific (from the Hudson bay to Arctic Canada, from Greenland to New Jersey, along Iceland coasts, from Norway to Portugal and in Japan). Epiphytic *Palmaria palmata* can grow attached to other algae, especially to the *Laminaria. sp.* stipes. *Palmaria palmata* can also be found fixed to rock, wood, and other solid substrata (Bird and MacLachlan, 1992).

2.5.2. Structure

The *Palmaria palmata* algae are about 50 cm to 1m tall, with a short stipe (< 5 mm) (Bird and MacLachlan, 1992) This heteromorphic species is usually composed of initially simple fronds, dividing dichotomously or trichotomously to form expanded palmate lamina. The lobes are generally 2-7 cm wide (Bird and MacLachlan, 1992), often with numerous leafy lateral proliferations. The colour of *Palmaria palmata* varies from light to deep wine-red, according to the red accessory pigment phycoerythrine / blue pigment phycocymine ratio. The algae tissue consists of a medulla of one or two layers of large spherical cells and a cortex with small cells.

2.5.3. Main chemical constituents of red algae

It is believed that the heavy metal sequestering capabilities of algae are due to the interaction between chemical groups on the surface of algae and metals. The chemical composition of algae is therefore crucial in the heavy metal removal mechanism. More precisely, algae contain polysaccharides and proteins including functional groups such as carboxyl, sulphate, carbonyl, amino, and amido groups, from where the protons can be dissociated, leaving a negative net charge on these polymers (Lobban and Harrison, 1994). Consequently, these macromolecules can act as cationic exchangers in the metal sorption mechanism.

2.5.3.1. Polyssacharides

The main organic constituents of algae cell mass are polysaccharides. For instance, the carbohydrate content of *Palmaria palmata* ranges from 38 to 74% (on a dry weight basis) (Morgan et al., 1980). The most common *Rhodophyceae* polysaccharides can be found in the cell wall matrix (cellulose), in the cuticle (mannan), and in mucilage (xylan, galactan) or as a food reserve (floridean starch). The content of these biomolecules depends not only on the species, but also on the season, depth of immersion, localization on the plant and state of development of the algae cells (Percival and McDowell, 1967). *Rhodophyceae* are characterized by polysaccharides containing.D- or L-galactose residues in which the galactan units are alternately ß-(1,3) and ß-(1,4) linked. The most common polysaccharides involved in the chemical composition of algae are:

- Cellulose

Cellulose consists of ß-(1,4) linked glucose residue (Percival and McDowell, 1967).it occurs mainly in the cell wall. Since cellulose is fibrillar and uncharged, its role in the cation sorption process may not be as essential as the other polysaccharides. The cellulose content in red algae ranges from 1% for *Endocladia muricata* to 9% for *Gelilidium cartilagineum* (Percival and McDowell, 1967). The cellulose content of *Rhodymania palmata* was reported to be 2.4% (Percival and McDowell, 1967).

- Mannan

Mannan is a structural polysaccharide composed of linear chains of ß-(1,4) linked D-mannose units. The insolubility of mannan is likely to be due to the close alignment of the molecules and the formation of possible aggregates tightened by hydrogen bonding (Percival and McDowell, 1967).

- Xylan

Xylan the major polysaccharide of *Palmaria* is water-soluble. Xylan is composed of ß-(1,3) and ß-(1,4) linked D-xylose units which contain no sulfate ester or methoxyl group (Percival and McDowell, 1967; Morgan et al., 1980).

- Storage polysaccharides

The major storage polysaccharide in red algae is floridean starch. Floridean starch is a polymer of α–1,4 and α–1,6 linked glucose and has a structure comparable to amylopectin (Percival and McDowell, 1967). Another common reserve product of the *Rhodophyceae* is floridoside. The floridoside content of *Palmaria palmata* was found to be has high as 25% of the dry weight (Chaumont, 1978).

- Sulfated polysaccharides

Some polysaccharides can be esterified or methoxylated with organic sulfate. The most commonly sulfated polysaccharides found in *Rhodophyceae* are galactans (bearing trivial names agar and carrageenan), porphyran, and furcellaran. However, this section will only focus on the galactan family, which is the most abundant cell wall constituent in red algae and contributes to gel formation.

The composition of agar and carageenan is based on repetition of galactose and 3-6, anhydrogalactose residues, linked ß-(1,4) and α–(1,3) respectively. Carageenans are more sulfated than agar, due to the presence of D-galactose and anhydro-D-galactose in the former and D-galactose, L-galactose and anhydro-L-galactose in the latter (Craigie, 1990). Moreover, negatively charged sulfated groups are likely to bind cations (Lobban and Harrison, 1994). Therefore, the metal binding capacity of the sulfated polysaccharides may depend on the degree of sulfatation. This correlation has been observed by Veroy et al (1980). The sorption of Pb^{2+} and Cd^{2+} to sulfated algal polysaccharides was believed to be mainly due to electrostatic attraction between sulfate ester groups and the metal cation. Hydroxyl groups were assumed to be essential donor groups (Veroy et al., 1980).

In addition, galactans of the *Rhodophyceae* are characterized by their high viscosity and their ability to form gels (Percival and McDowell, 1967). For instance, kappa and iota carrageenans are believed to form double helices in cold water, giving viscous solutions. Specific ions (e.g. K^+, Rb^+, Cs^+, NH_4^+, or Ca^{2+}) may promote side-by-side aggregation of double helices, forming a three-dimensional gel (Craigie, 1990). This reaction is thermoreversible and depends on the nature of cations combined with ester sulphate groups of the carrageenans and on the other salts present in the solution (Percival and McDowell, 1967). A nested, cation-specific single helix model, the Smidsrod and Grasdalen model, has also been proposed. This model was initially developed to explain the mechanism of binding of calcium by the alginates of the family *Phaephycae* (brown algae). In *Rhodophyceae*, the cation possibly promotes intermolecular aggregation of single kappa carrageenan helices. Since adjacent carrageenan chains have a zigzag conformation, the cation fits into the space according to an egg box structure. As far as the sorption mechanism is concerned, the niches formed between the two adjacent bands of the egg box model are believed to be a favourable molecular conformation for the binding of divalent ions (Lobban and Harrison, 1994). Additionally, the carrageenans of red algae (and the alginates of brown algae respectively) occur naturally as salts of metal cations such as Na^+, K^+, Ca^{2+} and Mg^{2+} (Percival and McDowell, 1967). The binding of metal ions is often described as an ion exchange (Smidsrod and Haug, 1968), whereby the uptake of one bivalent metal cation leads to the release of the polysaccharide counter ions according to the equation:

$$2NaPol. + Me^{2+} \rightarrow Me(Pol.)^2 + 2\,Na^+ \quad \text{(Volesky, 1990)}$$

Where *Pol.* is the algae polysaccharide and *Me* the bivalent metal ion.

2.5.3.2. Proteins and glycoproteins

Rhodophyceae proteins are soluble when associated with the mucilage. The cuticular proteins are highly insoluble in most solvents (Craigie, 1990). The total nitrogen content of *Palmaria palmata* is high in comparison to other algae species (MacPherson and Young, 1949). Similarly, *Palmaria palmata* has the highest crude protein content among the *Rhodophyceae* species. More specifically, the protein content can be as high as 35%

in *Palmaria palmata* (Morgan et al., 1980), 5% in *Porphyridium aerungineum* (Percival and Foyle, 1979) and 16% in *Rhodella maculata* (Evans et al., 1974).

Aspartic and glutamic acids are the preponderant amino acids in *Palmaria palmata.* Arginine, glycine, alanine, leucine and valine are also present at relatively high concentrations (Morgan et al., 1980). It should be mentioned here that the prevalence of amino acids and low concentration of basic amino acids is characteristic of most other *Rhodophyceae*. Therefore the large amounts of amino and amido acidic groups in *Rhodophyceae* are likely to be deprotonated and be responsible for metal sorption.

Proteins of *Rhodophyceae* are sometimes reported to form protein-polysaccharide conjugates. For example, serine was found to form O-glycosidic linkages in a protein from *Furcellaria lunbricalis* (Krasil'nikova and Medvedeva, 1975).

2.5.4. Conclusion : Justification of the *Palmaria palmata* choice

Many studies have investigated the biology and heavy metal uptake of brown or green algae, whereas red algae present still unsolved problems. As Dixon stressed (1973), "It is not generally appreciated how little is actually known about most marine and fresh water algae and the *Rhodophyta* is probably the worst known of any algal group". In addition *Palmaria palmata* has one of the highest (50%) water-soluble fraction in comparison with the other red algae (Cronshaw et al., 1958).

2.6. Influence of physico-chemical factors on the biosorption mechanism

As discussed in the previous section, the sorption performance can be correlated to the number of binding sites, which is related to the chemical composition of the chosen biosorbent. However, the amount of metal that can be bound depends also on the metal species, its initial concentration, as well as other physico-chemical parameters such as pH, temperature and contact time. The influence of these particular factors on the sorption mechanism will be described in the following section.

2.6.1. pH

It is generally agreed that the sorption of metal cations increases with a rise in pH (Darnall et al., 1986; Fourest and Roux, 1992; Holan et al., 1993; Ricou et al., 1999; Tanigushi, 2000). The pH dependence of metal cation binding can be explained by:

- The availability of the active sites. At low pH, the binding sites on the biosorbent are protonated and metal cations compete with protons for the same sorption sites (Tobin et al., 1984; Volesky, 1990). At very low pH, desorption of the bound metal is possible (Aldor et al., 1995).
- Formation of hydroxide species may occur at high pH, especially for high valence and small size ions (Baes and Mesmer, 1976) and sorption may become more efficient. For instance, thorium uptake is low at pH ≤ 2 because solubility is high and the main species is Th^{4+}. As pH increases, hydrolysis products come out, especially $Th(OH)_2^{2+}$, which are taken up more efficiently than Th^{4+} (Tzesos, 1981). It should be mentioned that hydroxide (or oxide) formation becomes significant for Cd, Pb, Cu, and Zn at pH 7, 6.5, 5, and 5.5, respectively and their precipitation starts at pH 9–10, 7, 6.5 and 8.5, respectively (Baes and Mesmer, 1976).

2.6.2. Co-ions

The presence of other ions (co-ions) can affect the sorption of metal ions (primary ion) to the biomass. This interaction can be synergistic, antagonistic or non-interactive, and cannot be predicted on the basis of single metal studies (Ting and Teo, 1994).

During biosorption on algae, metal ions often compete with each other for surface binding sites (Crist et al., 1981). For example, mutual inhibition was observed during the sorption of Cd^{2+}, Cu^{2+} and Zn^{2+} on brown algae *Ascophyllum nodosum*, Zn^{2+} being the weakest bound ion and Cu the most strongly bound ion (Chong and Volesky, 1995). Similarly, Pb^{2+}, Cd^{2+} and Cu^{2+} sorption on the fungus *Aspergillus niger* was found to be lower when the metal ions were present in a mixture than when they were present individually in the solution (Kapoor et al., 1999). In addition, Puranik and Paknikar (1999) studied the influence of Cd^{2+}, Cu^{2+}, Co^{2+} and Ni^{2+} on lead and zinc sorption by

Streptovercillium cinnamoneum and *Penicillium chrysogenum*. Mutual inhibition was observed in binary systems containing Zn^{2+}, whereas systems including lead displayed unequal inhibition. Furthermore recent studies have shown that light metals such as Na^+, Ca^{2+}, Mg^{2+}, and K^+ competed with heavy metals for ionic sorption sites and diminish heavy metal sorption. (Matheickal and Yu, 1999; Tanigushi et al., 2000). However the inhibition of the metal ion binding to microbial biomass by Na^+, and K^+ was shown to be lower than the uranium or radium binding inhibition by heavy metals such as Zn^{2+}, $Cu^{2+}$and Fe^{2+} (Tzesos, 1990).

The binding strength to the biomass depends on metal chemistry (including parameters such as the charge, the ionic radius, the Pauling electronegativity) affinity for binding sites, and the type of metal binding (electrostatic or covalent).

2.6.3. Initial metal concentration

The initial metal concentration strongly influences the metal uptake. As long as the binding sites are not saturated, the higher the initial concentration of the metal ion, the larger the metal uptake. However, it is apparent that increasing the amount of the available metal results in decreasing the fraction of metal bound (Blanco et al., 1999). The relationship between variable initial metal concentrations and metal uptake, at a fixed temperature and fixed biomass concentration is commonly represented by sorption isotherms. The method to obtain such sorption isotherms and the calculation of the biosorption constants of the model are described in section 3.4.

2.6.4. Biomass concentration

It is commonly reported in the literature that the amount of metal bound per biomass decreases as the biomass concentration increases. For instance, this trend has been observed for the biosorption of Ni, Cd and Zn on mycelial wastes of *Rhizopus arrhizus* (Fourest and Roux, 1992), the biosorption of Cu^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} on immobilized *Phormidium laminosum* (Blanco et al., 1999) biomass, the biosorption of Cu^{2+}, $Ni^{2+}$and Cr^{6+}on dried algae (Dönmez et al., 1999). Contributing factors that have been suggested for such biomass concentration dependency include electrostatic interactions between cells (De Rome and Gadd, 1987; Young and Banks, 1998), interference between binding sites, and reduced mixing (Gadd et al., 1988) at high cell densities.

2.6.5. Temperature

Temperature was reported to influence the sorption mechanism. For instance, the binding of Co^{2+} to brown algae *Ascopyllum nodosum* was increased by 50 to 70% when the temperature was increased from 4 °C to 23 °C (Kuyucak and Volesky, 1989). However, only small further increases were observed between 40 °C and 60 °C. In addition, for temperatures of 60 °C or more, the biosorbent texture was denatured and the uptake capacity decreased.

Generally, sorption mechanisms are exothermic, that is to say equilibrium constant decreases with increasing temperature. However, the binding of Cu and release of light metals were believed to be endothermic (Haug and Smidsrod, 1970). The positive entropy change was assumed to result from a larger ordering effect of Cu^{2+} (than Ca^{2+}) upon the water in the hydration sphere.

Overall, the temperature is believed to have a smaller effect on the biosorption than the other factors (Tsezos, 1990). As an illustration, the effect of temperature over the range 4°C to 23°C on copper adsorption by filamentous fungi was not significant (De Rome and Gadd, 1987).

2.6.6. Contact time

As reported in the literature, the metal sorption is often rapid, and usually complete in less than one hour. For metabolism independent uptake by algae, sorption is generally achieved in 5-10 minutes (Gadd, 1988). Zhou et al. (1998) observed that within 6 minutes of contact, 60% of the total Cu^{2+} uptake by the microalgae *Spirulina platensis* and that equilibrium was reached in 8 hours. Binding of metal ions to aquatic particulates is considered to be a fast chemical reaction, with an equilibrium time depending only on the mass transfer resistance (Stumm et al., 1994).

2.7. Statistical methods

2.7.1. Statistical optimization methods

The application of appropriate statistical experimental design in industry contributes to improve productivity, and to reduce process variability, development time and overall costs. When the problem involves data that are subject to experimental error, statistical optimization methods which are based on factorial experimental design are considered as the only objective approach of analysis (Montgomery, 1984). Statistical optimization methods indeed provide means of reaching optimum operating conditions by changing more than one factor at a time. The independent experimental variables are usually referred to as «factors», and, hence, the term «factorial» has been used. Multifactorial design has two main advantages:

(1) It can measure the effects of changes in operating variables and their mutual interactions on the process performance, whereas in the traditional «one-factor-at-a-time» experiments, each parameter is considered to be insensitive to the to the other process variables.

(2) It allows one to find the optimum with a smaller number of experimental runs than by the one-factor-at-a-time approach.

Of the numerous optimization methods, response surface methodology (RSM) is commonly used for fermentation processes (Karthikeyan et al., 1996; Caillièret- Ethuin et al., 1998; Manimekalai and Swaminathan, 1999), in engineering and industrial chemistry (Hendrix, 1980), and in analytical chemistry (McMinn et al., 1984).

After the experimental data are collected according to the appropriate experimental design, their predicted values may be represented by a hypersurface. The shape of this three-dimensional response surface can be visualized graphically in a contour plot.

The aim of the following section is first to summarize the terminology, then to justify the use of a specific response surface methodology: the Central Composite Design (CCD), and finally to give some theoretical background on the CCD.

2.7.1.1. Terminology

Factors: The factors are processing conditions or input variables whose values or settings can be controlled by the experimenter. In a regression analysis, factors can be qualitative (e.g. catalyst type, supplier of a material) or quantitative (temperature, concentration, etc.). The specific factors whose levels will be studied in detail are those that are quantitative in nature. Their levels (or settings) are assumed to be fixed or controlled (without error) by the experimenter. Factors and their levels will be denoted by $X_1, X_2, \ldots, X_k$, respectively.

Response: The response variable is the measured quantity whose value is assumed to be affected by changing the levels of factors. The true value of the response corresponding to any particular combination of the factor levels and in absence of experimental error is denoted by η. However, because experimental error is present in all experiments involving measurements, the response value that is actually observed from η. We may now write the observed value of the response Y as:

$$Y = \eta + \varepsilon$$

where ε is the random error component.

Response function: The response function is the relationship that exists between a response η and the factors $X_1, X_2, \ldots, X_k$, which can be written as:

$$\eta = \phi(X_1, X_2, \ldots X_k)$$

The function ϕ is called the true response function and is assumed to be a continuous function of the X_k.

Predicted Response function: The form of the relationship ϕ between the response and the independent variables is generally unknown. Thus, it is needed to find a suitable approximation for the true functional relationship between Y and the set of independent variable. Usually, a low-order polynomial or some other type of empirical model equation is used (Khuri and Cornell, 1987). However, the most commonly response function equation is the general second-degree polynomial (Mead, 1988).

Response surface: It is often convenient to view the problem geometrically. This is the reason why the relationship $\eta = \phi\ (X_1, X_2, \ldots, X_k)$ between η and the levels of k factors may be represented by surface referred to as the *response surface* (Box and Hunter, 1957), or as hypersurface. Thus, the response surface of k factors is a subset of (k+1)-dimensional Euclidean space (Khuri and Cornell, 1987).

Contour representation of a response surface: The plotting of the response surface contours is a commonly used technique to help visualize the shape of a three-dimensional response surface. More specifically, contour plots are lines of equal response for different levels of the factor, and could be compared to isobars on a weather map. For example, a two-factor response surface requires normally three axes, but the use of a contour plot will reduce the response surface plot by one dimension (Bayne and Rubin, 1986).

2.7.1.2. Fitting a second-order response surface

The most commonly used response surface equation is the second-degree polynomial: (Mead, 1988):

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where Y is the response, X_i (i=1...k) the levels of the k factors, β_o is the off-set term called the intercept, β_i (i=1...k) the unknown coefficients (or regression coefficients) of the fitted second-order polynomial, and ε denotes the residual.

The regression parameters β_i (i=1...k) of equation (1) can be estimated using the method of least squares. If these estimates are denoted by b_i (i=1...k), the predicted response "Y hat" can be calculated from the prediction equation (2):

$$\hat{Y} = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} b_{ij} X_i X_j \quad \textbf{(2)}$$

The quadratic response-surface model of equation (2) can be checked for adequacy of fit with the analysis of variance (ANOVA) (section 2.7.3).

2.7.1.3. Determining the coordinates of the stationary point

If the model is found to be adequate, it can be used to determinate the coordinates of the optimum point (also called stationary point). The stationary point is at which the levels X_i (i=1...k) maximize the predicted response, such that the partial derivatives $\frac{\partial \hat{Y}}{\partial X_1} = \frac{\partial \hat{Y}}{\partial X_2} = \cdots = \frac{\partial \hat{Y}}{\partial X_k} = 0$.

A general solution for the stationary point can be obtained under a matrix form for sake of simplicity. Thus writing the second-order equation (2) in matrix notation, we have:

$$\hat{Y} = b_0 + X'b + X'BX \quad \textbf{(3)}$$

Where: $X = \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_3 \end{bmatrix}$ $\quad b = \begin{bmatrix} b_1 \\ b_2 \\ \vdots \\ b_3 \end{bmatrix}$ and $B = \begin{bmatrix} b_{11} & \frac{b_{12}}{2} & \cdots & \frac{b_{1k}}{2} \\ & b_{22} & \cdots & \frac{b_{2k}}{2} \\ & & \ddots & \vdots \\ \text{Symmetric} & & & b_{kk} \end{bmatrix}$

The partial derivatives of $\hat{Y}$ with respect to the vector X equated to 0 are:

$$\frac{\partial \hat{Y}}{\partial X} = b + 2BX = 0 \quad \textbf{(4)}$$

The solution to equation (3) is $X_o = -\frac{1}{2} B^{-1} b$ **(5)**

By substituting equation (5) in equation (3) the predicted response at stationery point can be obtained:

$$\hat{Y}_0 = b_0 + \frac{1}{2} X'_0 b$$

However, to describe the nature of the response in the neighbourhood of the stationary point, it is useful to transform the fitted model into a new coordinate system, in which the origin is the stationary point X_0. The result of this transformation is called the canonical form of the model and can be expressed as:

$$\hat{Y} = \hat{Y}_0 + \lambda_1 W_1^2 + \lambda_2 W_2^2 + \cdots \lambda_k W_k^2$$

The $\{\lambda_i\}$ are the eigenvalues (or characteristic roots of the matrix B) and the $\{W_i\}$ are the transformed independent variables of the canonical form. The nature of the response surface can be determined from the sign and the amplitude of the $\{\lambda_i\}$.

For instance if all the $\{\lambda_i\}$ are positive, then X_0 is a point of minimum response. If all the $\{\lambda_i\}$ are negative, then at X_0 the surface is a minimum. Last, if the $\{\lambda_i\}$ are of different sign, X_0 is a saddle point (or minimax point).

2.7.1.4. Comparison of full factorial design and CCD

A response surface cannot be efficiently fit without a proper choice of experimental design. Two principal multifactorial designs are used to estimate the coefficients of a second order response surface equation: the factorial design and the Central Composite Design. Before considering specific requirements of the Central Composite Design, the two experimental designs are compared in the following section.

The two main factorial structures for response surface investigation are the 2^k and 3^k factorial design, where k represents the number of factors. However the 2^k structure does not allow estimation of quadratic terms in the second-degree polynomial, which makes this structure impractical for the determination of the optimum point (Mead, 1988). Thus the 3^k structure appears to be more appropriate since it gives all the parameter estimators of the full quadratic model (equation (1)).

However, the 3^k structure suffers severely from the greater number of combinations of factor levels compared to the number of parameters, and is quite inefficient for k=3. For four independent variables for instance, the factorial design would require $3^4 = 81$ combinations to estimate the 15 parameters of the second order polynomial (1) (Mead, 1988).

Box (1954) suggested the CCD as a feasible alternative to the factorial design. The CCD indeed is more efficient in generating a response surface than a complete factorial design.

For example, to estimate the coefficients of the second-order response surface structure, the CCD with ($k = 4$) will only require 31 treatment combinations (Box and Hunter, 1957) rather than 81 for the full factorial design. Panneton et al. (1999) compared the effectiveness of the CCD with that of a 3^3 factorial design, based on a theoretical and empirical approach. The model fitting ability and the capability of identifying optimum operating conditions were found to be equal for both designs. However, the CCD tended to be more conservative.

The CCD includes replication of the center points to allow a test for lack-of-fit sum of squares. A significant lack-of-fit term indicates that higher-order terms are present in the data (Panneton et al., 1999). The reasons why a CCD is recommended rather than a full fractional design are summarized:

(1) CCD provides estimates for all coefficients in the approximating model with the advantage of requiring fewer treatment combinations, with the benefit of less work time and operating costs.

(2) CCD is more efficient in representing the data and defining how the response surface behaves in the region of optimum conditions.

(3) CCD provides a test for lack of fit and allows the experiment to be performed in block.

2.7.1.5. Orthogonal and uniform-precision rotatable Central Composite Designs

An experimental design for fitting a second-order model, such as the Central Composite Design should have a minimum of 3 levels for the factors (Montgomery, 1984). In addition, the desirable properties of the CCD are: rotatability, uniform precision and orthogonality (Panneton et al., 1999). Specific arrangements into a standard orientation can be defined so that a set of experimental points should meet the requirements of an orthogonal and uniform-precision rotatable CCD.

- Classical CCD

Classical CCD described by Box and Hunter (1957) can be derived from the 2^k factorial design. The general form of a central composite Design is composed of:

$N_c = 2^k$ cube points (factorial points) whose coded coordinates are $(\pm1, \pm1, \ldots \pm1)$

$N_a = 2k$ axial points $(\pm\alpha), 0,0, \ldots, 0), (0, \pm \alpha), 0, \ldots, 0), \ldots, (0,0,0, \ldots, \pm \alpha)$

and N_0 center points $(0,0,0, \ldots 0)$

for a total of $N = N_c + N_a + N_0$ experimental units.

- Rotatibility

A Central Composite Design is said to be rotatable when the variance of the estimated response $\hat{Y}$ at a distance α from the design center is a function only of the distance of the point from the design center, and not a function of the direction (Panneton et al., 1999). The term rotatable comes from the fact that, in a rotatable design, the variance of $\hat{Y}$ is unchanged when the design is rotated about the center (0,0,0,…, 0) (Montgomery, 1984). Actually, the value of α for rotability depends on the number of points in the factorial portion of the design. More precisely, the CCD is made rotatable by choosing a value of $\alpha = (N_c)^{1/4}$ (Box and Hunter, 1957).

- Uniform-precision

In a uniform-precision design, the variance of $\hat{Y}$ at the origin is equal to the variance of $\hat{Y}$ unit distance from the origin (Montgomery, 1984). The uniform-precision property allows more protection against bias in the regression factors due to the presence of third-order or higher terms in the true surface. Actually, a uniform precision can be obtained by replicating the center point to increase the precision at that point (Panneton et al., 1999).

- Orthogonality

Orthogonality means that all the estimated coefficients are independent. Or in other words, non-significant coefficients can be removed without affecting the other coefficients. Orthogonality requires that the distance of axial points and the number of replications at the center of the cube be properly chosen (Panneton et al., 1999).

- Summary of design paramètres

Table 6 provides the design parameters both orthogonal and uniform-precision rotatable central composite design for k = 4 factors.

N_c **cube points**	N_a **axial points**	N_0 **center points**		N **experimental units**		$\alpha = (N_c)^{1/4}$
		(up)	(orth)	(up)	(orth)	
16	8	7	12	31	36	2

Table 6. Values of design parameters for orthogonal (orth) and uniform-precision (up) rotatable central composite design with k= 4 factors (Box and Hunter, 1957)

Finally, it should be mentioned that the three properties (rotatibility, uniform precision and orthogonality), cannot be achieved simultaneously (Panneton et al., 1999). A uniform-precision rotatable central composite design is thus selected for the present study.

2.7.2. Model evaluation

The adequacy of the response surface model can be evaluated by several criteria. First and foremost is the test for lack of fit (LOF). The test for lack of fit is based on the ratio between the LOF mean square and the residual mean square, where the LOF mean square is the difference between the residual sum of squares (RSS) and the pure error sum of squares (ESS), divided by the LOF degrees of freedom (df) (difference between residual df and pure error df) (Khuri and Cornell, 1987).

If LOF exists, the model should not be used and another model should be sought (Draper and Smith, 1981). Lack of fit is an indication that the presumed model does not describe the true shape of the surface, whether or not it explains a high percentage of the overall variability in the data (as represented by the coefficient of determination, or R^2). It may happen that one obtains a high R^2 when lack of fit exists. This is usually due to the fact that the model contains a large number of terms compared to the number of data available (over-fitting) (Box, 1987). One must plan to provide data for estimation of pure error by ensuring that there are repeat runs at one or more sets of identical settings of the factors involved in the experimental design (as is the case for the center point in the CCD).

If there is no lack of fit, the adequacy of the model is then based on the F-test, which compares the mean square attributable to the model terms, to the residual mean square. If the model can be considered significant, then the further adequacy of the model can be estimated on the basis of the coefficient of determination (R^2) or on the ratio of R^2 to the maximum R^2 (R^2_{max}) when there are repeat runs at one or more points (Draper and Smith, 1981).

The coefficient of determination is commonly used to estimate the adequacy of fit with a regression model and can be expressed as (Montgomery, 1984):

$$R^2 = \frac{SS_R}{S_{yy}} = \frac{\sum_{j=1}^{n}(\hat{y}_j - \bar{y})^2}{\sum_{j=1}^{n}(y_j - \bar{y})^2}$$

where j is the level of the response variable y, $\bar{y} = \frac{1}{n}\sum_{j=1}^{n} y_j$ and $\hat{y}$ is the estimated value of y after fitting with the linear regression model. SS_R is defined as the regression sum of squares of the linear regression model, and S_{yy} is the total corrected sum of squares for y.

R^2 is a measure of the percentage of variability in the data that is explained by the terms in the model, including variability due to pure error. For example, $R^2 = 0.9567$ means that 95.67% of the variability in the data is accounted for by the model Since R^2_{max} is the variability that is explained by the model, other than that due to pure error, R^2 should be compared to R^2_{max} when there are repeat runs (Draper and Smith, 1981).

2.7.3. Analysis of variance

Analysis of variance (ANOVA) is an efficient method to compare the means of different statistical populations and to test the significance and adequacy of a model. More precisely, the sum of squares can be used as a measure of the overall variability in the data. The mean squares are defined by the ratio of the sum of squares of the model and error variances to their respective degrees of freedom. If treatment means are equal, then the mean squares are an estimate of the variance δ^2 The Fisher variance ratio (F value) can be estimated by dividing the mean square due to model variance by that due to error variance. An F-ratio different from 1 would mean that the factors describe adequately the variation in the data and that the estimated factor effects are significant. The Student t distribution and their corresponding p values are used to check the significance of the coefficients that explain the mutual interactions between the test factor. The smaller the value, the more significant the corresponding factor. The ANOVA results can be calculated by hand, or by commercial computer programs such as EXCEL or SAS. Guidance for interpretation of program outputs is given in many statistical textbooks (Mead, 1988; Montgomery, 1984) or guides provided by SAS institute Inc.

2.7.4. Assessing standard error from difference of two averages

A t-test is usually used to compare the average of two samples.

Assuming that two independently distributed random variables y_a and y_b have, respectively, averages $\bar{y}_a$ and $\bar{y}_b$, and variance δ^2_a and δ^2_b, the variance of the difference is (Berthouex et al., 1994):

$$V(\bar{y}_a - \bar{y}_b) = \frac{\delta_a^2}{n_a} + \frac{\delta_b^2}{n_b}$$

where n_a and n_b are the sample sizes.

The variances δ^2_a and δ^2_b.may be estimated from the sample data by their estimate standard deviation $S_a{}^2$ and $S_b{}^2$:

$$S_a^2 = \frac{\sum(y_a - \bar{y}_b)^2}{n_a - 1} \qquad \text{and } S_b^2 = \frac{\sum(y_b - \bar{y}_b)^2}{n_b - 1}$$

Then the population variances are of equal amplitude, the pooled estimate of the variance S^2_{pool} is:

$$S^2_{pool} = \frac{(n_a - 1)S_a^2 + (n_b - 1)S_b^2}{n_a + n_b - 2}$$

And the standard error of $\bar{y}_a - \bar{y}_b$ is $S_{\bar{y}_a - \bar{y}_b} = S_{pool}\left(\frac{1}{n_a} + \frac{1}{n_b}\right)^{1/2}$

The confidence interval can be then calculated by a Student's t distribution, as following

$$(\bar{y}_a - \bar{y}_b) \pm t_{v,\alpha/2}\left(S_{\bar{y}_a - \bar{y}_b}\right)$$

Where $v = n_a + n_b - 2$ is the degree of freedom and α is the level of the confidence interval. For example, for a 95% confidence interval, $\alpha = 0.05/2 = 0.025$.

2.8. Summary of literature review

Heavy metals such as Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} may pose a health hazard when in solution. Electroplating processes are one of the major industrial contributors of heavy metal-laden effluents into the environment. Various remediation methods such as membrane technologies, precipitation, ion exchange or electrowinning have been developed to remove metal ions from the contaminated wastewaters. Among these technologies, chemical precipitation with lime or caustic soda is one of the most common conventional treatments. However, decreasing metal concentrations to acceptable levels requires a large excess of chemicals, which generates volumetric sludge and increases the costs. Finally, a discussion about the merits and the demerits of the above methods has led to the conclusion that there is a need for an efficient, cost effective and environmentally–friendly technology to treat electroplating wastewaters. From this review of literature, biosorption seems to be an economically feasible and technically efficient technology to remove and recover metals from solutions.

Although many studies have reported that brown and green algae are effective biosorbents to remediate heavy metal liquid pollution, few have investigated the potential of red algae. The major binding sites of red algae biomasses are suspected to be sulphate ester groups and hydroxyl groups of polysaccharides that occur in the cell wall and as extra-cellular polysaccharides. In addition, in most cases, the various physico-chemical parameters that could have an influence on the sorption capacity including algae concentration, pH, initial metal concentration, contact time and temperature have been studied separately.

Statistical methods of optimization such as the Central Composite Design (CCD) are an appropriate experimental approach to estimate which combination of the factors influencing metal uptake, should maximize uptake, while taking into account their interactions In the following two sections, sorption characteristics by *Palmaria palmata* under different experimental conditions will be presented. Section 3 focuses on the experimental procedure, the analytical method and modelling. Section 4 presents the results and discussion on the application of CCD in mono and binary metal systems.

3. MATERIALS AND METHODS

3.1. Algae biomass preparation

Red algae *Palmaria palmata* were supplied by Atlantic Mariculture Ltd. (Grand Manan, NB, Canada). The algae are harvested and sun-dried on the beach rocks prior to packaging. They are certified organic and have no additives. Several packages were combined to make a composite stock. The biomass was ground with a blender and sieved (with Canadian standard sieves of 32 and 48 mesh) to give particles in the size rang 0.3 to 0.5 mm. This was done to obtain a homogeneous particle-size distribution and thus obtain even intra-particular diffusion of the metal ions. The main physical and biochemical properties of the biosorbent are summarized in Table 7.

Characteristics	**Value**
Moisture (wt %)	12
Particle size (mm)	0.3-0.5
Bulk density (kg/m^3)	**343.6**
Tapped density (kg/m^3)	**420.6**
Particle density (kg/m^3)	**1674.8**
Porosity	**0.795**
Compressibility (%)	**18.3**
Specific surface area (m^2/g solid)	**169**
Proteins (%)	*21.5**
Carbohydrates (%)	*44.6**
Lipids (%)	*1.7**
Ash (%)	*32.2**

*Table 7. Physical and biochemical properties of the dried Palmaria palmata powder (**Mishra et al., 1995**; Atlantic Mariculture., 2000*).*

3.2. Biosorption batch experiments

Metal ion solutions containing the desired concentrations of Cd $^{2+}$, Pb $^{2+}$, Cu $^{2+}$, Zn $^{2+}$ and Ni $^{2+}$ were prepared by dissolving cadmium (II), lead (II), copper (II), zinc (II) and nickel (II) nitrate of analytical grade supplied by Sigma-Aldrich (Oakville, Ontario, Canada). Nitrate was chosen as the anion because it has a low tendency to form complexes with the metals used (Dean, 1999). The laboratory batch method as described in the EPA procedure (1991) was used for this project because of the low equipment, cost and time requirements. Biosorption experiments were carried out in 30-mL Teflon tubes provided by VWR Canlab to avoid any losses of metals caused by sorption on the borosilicate glass. Polyfluoroethylene (PTFE) is recommended as the best material to avoid losses of trace metals, especially at low concentration and relatively high pH (Massee and Maessen, 1981; Pellenbarg, 1978; King, et al., 1974).

Deionized water was used instead of buffer in the preliminary experiments with *Palmaria palmata* to avoid competition with the buffer constituents. Buffers were used only to study the pH effect. A 0.2 M hydrochloric acid- potassium chloride buffer was used to maintain the metal solution at pH = 2. The other pH levels (pH=3 to pH=6) were obtained by adding 0.2 M sodium acetate/acetic acid buffer. Hoyle and Atkinson (1979) and Ferguson and Bubela (1974) used these buffers in earlier adsorption studies. Solubility product data indicated that there should be no precipitation of the metals or anions contained in the buffers. In addition, complexes that might be formed between buffer anions and the metal ions are relatively unstable.

Palmaria palmata powder (0.02 g) was weighed and added to Teflon tubes containing the metal solution. All the tubes were sealed to minimise evaporation and shaken at 275 rpm. An Innova 3000 water bath shaker (New Brunswick) refrigerated by a cooling system MGW Lauda RM 20 (Brinkman) was used for the experiments conducted at 6 °C, and a rotary G 24 environmental incubator shaker (New-Brunswick scientific Inc.) was used for runs at temperatures of 23, 14.5, 31.5 and 40 °C. Control experiments without biomass or metal were run in the same manner.

The supernatant was then filtered through 0.45 μm PVDF membrane filters (Millipore filters HV 0.45 μm). The amount of metal removed by *Palmaria palmata* was calculated with the following equation:

$$q = [V (C_i - C_f)] / M \qquad \textbf{(6)}$$

where V = volume of solution (L), C_i = initial concentration of metal ions (mg/L) , C_f = solute equilibrium concentration or final concentration of metal ion in the aqueous phase (mg/L), M = mass of algae biomass (g).

3.3. Analytical analysis of metals in solution

Analysis was first to be done with a Varian SpectrAA-30 Zeeman atomic absorption spectrophotometer that was available at the Biotechnology Research Institute. When it was tested to determine the final metal concentration C_f it was found that the graphite furnace atomization method was not suitable for the levels of concentration encountered in the present research project.

The optimum metal concentration given by Varian depends on the metal but never exceeds 100 μg/L. When working at ppm levels, the graphite furnace analytical method would require high dilution factors with an inherent inaccuracy.

Finally, this method was judged to be too sensitive (Hendershot, 2001).

The metal concentrations in the filtrate were therefore determined by a Perkin-Elmer atomic adsorption spectrophotometer (AAS) using an air/acetylene flame. The instrument was first calibrated with standard solutions in the range covering the expected concentrations in diluted samples. The samples were analysed in triplicate. Each sample was injected into the AAS three times. The standard deviation of the measured values was less than 5%, indicating good reproducibility. In addition, procedural blanks were also analysed to check for contamination.

3.4. Sorption isotherms

During biosorption, a rapid equilibrium is reached between the adsorbed metal ion on the algae surfaces and the metal ions found in the solution. The sorption isotherm is representative of the distribution of the solute in the solid phase and in the liquid phase at equilibrium. The sorption curve or isotherm is obtained at a constant temperature for different initial metal concentrations. The adsorbed solute, q (in mg of metal per g of biomass, expressed in dry weight), is plotted against the residual or final concentration at equilibrium, C_f (in mg/L). Several models have been developed to describe adsorption isotherms.

The present study focused on three widely used sorption models: the Freundlich, the Langmuir and the BET models (Weber, 1985).

3.4.1. The Freundlich model

The main assumption is that the stronger binding sites are occupied first and that the binding strength decreases with an increasing degree of site occupation. The Freundlich model (Weber, 1985) is expressed as:

$$q = kC_f^{1/n} \qquad (7)$$

where q = amount of solute per unit dry weight of sorbent, (mg/ g) , C_f = solute equilibrium concentration (mg/L), k and 1/n = empirically determined constants.

The Freundlich equation is often linearized by logarithmic transfer of equation. (7):

$$\ln q = \ln k + \frac{1}{n} \ln C_f \quad \mathbf{(8)}$$

Thus, a plot of ln q versus ln C_f should give a straight line, from the intercept of which k may be estimated and from the slope of which 1/n may be calculated. k is related to the adsorption capacity and 1/n indicates the adsorption intensity.

3.4.2. The Langmuir model

The Langmuir isotherm (Langmuir, 1918) was derived from kinetic considerations taking into account the following assumptions:

1) There is only one sorbate.
2) The surface consists of adsorption sites.
3) One adsorbed species interacts with only one site and not with the other species.
4) The adsorption energy of the sites is equal.

A general expression of the Langmuir model is:

$$q = \frac{q_{max}\, b\, C_f}{1 + b\, Cf} \quad \textbf{(9)}$$

where q = metal uptake of species or adsorption capacity (mg/g), q_{max} = maximum metal uptake or maximum adsorption capacity (mg/g), C_f = solute equilibrium concentration (mg/L) and b is a constant related to energy of adsorption (L/mg).

Equation (9) is often expressed as:

$$\frac{1}{q} = \frac{1}{b\, C_f\, q_{max}} + \frac{1}{q_{max}} \quad \textbf{(10)}$$

The values of Langmuir's coefficients q_{max} and b can be derived from a linearization by plotting $1/q$ versus $1/C_f$.

3.4.3. The BET model

The BET model was first developed by Brunauer, Emmet and Teller for gases at temperatures not far removed from their condensation point (1938). This adsorption isotherm is an extension of the Langmuir model. The main assumption of the BET model is that the first layer of molecules adheres to the surface with an adsorption energy that is comparable to the heat of adsorption for monolayer sorption.

In addition, the subsequent layers are assumed to be formed by condensation reactions and have equal energies of adsorption.

The BET model takes the form (Weber, 1985):

$$q = \frac{Bq_{max}C_f}{(C_f - C_s)[1+(B-1)(C_f\ /C_s)]} \quad \textbf{(11)}$$

where C_S is the saturation concentration (solubility limit) (mg/L) of the solute, B is a constant related to the energy of adsorption and qmax (mg/g) is the amount of solute needed to form a complete unimolecular adsorbed layer. Equation (5) gives an S-shaped isotherm with two regions. Indeed, the low final concentration region will be concave to the final concentration axis.

At higher final concentration, the curve becomes convex to the final concentration axis. Moreover equation (11) can be linearized as:

$$\frac{C_f}{(Cs - C_f)q} = \frac{1}{Bq_{max}} + \left(\frac{B-1}{B\,q_{max}}\right)\left(\frac{C_f}{C_s}\right) \quad \textbf{(12)}$$

which shows that a plot of $\frac{C_f}{(Cs - C_f)q}$ against $\left(\frac{C_f}{C_s}\right)$ should give a straight line whose intercept is $\frac{1}{Bq_{max}}$ and whose slope is $\left(\frac{B-1}{B\,q_{max}}\right)$. The two constants B and q_{max} can be evaluated from the slope and the intercept.

3.5. Statistical design

A suitable uniform-precision rotatable central composite (CCD) for four test factors, each at five levels was selected. The four factors were pH (PH), temperature (TEMP), initial metal concentration (INCONC) and contact time (TIME). As described by Box and Hunter (1957) (section 2.7.1.5), this design includes 16 cube points, 8 axial points, and 7 replicates at the centre point, indicating that 31 treatments were required for the procedure. The use of unitless coded values in place of the input variables facilitates the construction of the experimental design.

A convenient coding formula for defining the coded variable is:

$$Z_i = \frac{\left(x_i - x_i^*\right)}{\Delta x_i}$$

where Z_i is the coded value of the i-th test variable, X_i is the uncoded variable of the i-th test variable, X_i * is the uncoded value of the of the i-th test variable at the center point and ΔX_i is the step change value. The coded values for the five levels used in this design are –2, -1, +1, 0, +1 and +2. The experimental design with coded factors is shown in Table 8.

The experiments were performed in duplicate and the mean values were taken for the statistical analysis. A commercial statistical program, SAS (SAS Institute, Inc.) was used to estimate the parameters of the model, by least-squares regression. More specifically, the RSREG procedure of SAS can fit the parameters of a complete quadratic response surface and can analyse the fitted surface to determine the factor levels of optimum response. Two analyses were performed:

1. The analysis of variance (ANOVA) to estimate the parameters of the model by least-squares regression, to evaluate the contribution of each factor to the statistical fit, and to check the model fitting by examining various sources of error.
2. The canonical analysis, to find the estimated stationary point and to study the over-all shape of the response surface (to see whether it is a maximum, a minimum or a saddle point).

Only the significant parameters obtained from the step (1) was retained for the final regression equation. In other words, a factor was eliminated when the probability of the F-test of the coefficient was greater than the significance level: $\alpha = 0.15$. The equation of regression with significant coefficients was then used in the three-dimensional graphical representation. The obtained response surface could describe the individual and cumulative effects of the variable, as well as their mutual interactions.

Exp N°	Coded factors			
	PH	TEMP	INCONC	TIME
1	-1	-1	-1	-1
2	-1	-1	-1	1
3	-1	-1	1	-1
4	-1	-1	1	1
5	-1	1	-1	-1
6	-1	1	-1	1
7	-1	1	1	-1
8	-1	1	1	1
9	1	-1	-1	-1
10	1	-1	-1	1
11	1	-1	1	-1
12	1	-1	1	1
13	1	1	-1	-1
14	1	1	-1	1
15	1	1	1	-1
16	1	1	1	1
17	-2	0	0	0
18	2	0	0	0
19	0	-2	0	0
20	0	2	0	0
21	0	0	-2	0
22	0	0	2	0
23	0	0	0	-2
24	0	0	0	2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0

Table 8. Central Composite Design matrix of four environmental factors in coded units

4. RESULTS AND DISCUSSION

4.1. Introduction

The following chapter is divided into 5 sections. This section describes the organization of the chapter. The preliminary experiments are described in section 4.2. The first was a comparison of copper sorption by treated (washed or homogenized algae) and untreated algae. The second experiment was an evaluation of the effect of biomass concentration on metal uptake, whereas the third involved a comparison of the affinities and the sorption capacities of Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+} and Cu^{2+} to the algae used. The two metals showing the greatest adsorption capacity for *Palmaria palmata* were selected for the Central Composite Design. A 24-h contact period was chosen to ensure that equilibrium was reached in the preliminary experiments of section 4.2. The results of further experiments aimed at determining the ranges of initial metal concentration, contact time, pH and temperature to be used in the different CCDs are presented in section 4.3. An application of the CCD on the copper sorption at an initial concentration range of 0-20 mg/L is presented in section 4.4. Based on these results, it was decided to extend the range for copper to 0-500 mg/L. Biosorption in systems with either copper or lead, as well as in the binary system (Cu^{2+} + Pb^{2+}), is discussed in section 4.5.

4.2. Preliminary experiments

The preliminary experiments were conducted to evaluate the necessity to pre-treat the algae, to estimate the algae concentration to be fixed in the sorption experiments, and to determine the metals exhibiting the greatest adsorption capacity. More specifically, the preliminary experiments presented in sections 4.2.1 and 4.2.2 were limited to copper and the results were extended to Cd^{2+} Pb^{2+}, Ni^{2+}, and Zn^{2+}. The objective of the first preliminary experiment was to study the effect of washing and homogenization on the copper sorption by *Palmaria palmata*. A second preliminary experiment aimed at estimating which biomass concentration was the most efficient on a unit mass basis, at sorbing copper.

Section 4.2.3 describes the sorption isotherms of Cd^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Cu^{2+} obtained by linearization of the Freundlich, Langmuir and BET model equations as explained in section 3.4. The model constants were useful to compare the sorption capacity of Cd^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Cu^{2+} by *Palmaria palmata*. The metals showing the highest sorption capacity were selected to be used in the CCD.

4.2.1. Effect of untreated and treated algae on copper sorption

The effect of washing and homogenization of *Palmaria palmata* on the copper sorption was tested here because there was some evidence in the literature that sorption might be affected. For instance, recent studies have shown that cations such as Na^+, and K^+ compete with heavy metals for ionic sorption sites and may diminish heavy metal sorption. (Leusch et al., 1996; Fourest et al., 1996). The removal of salts from the algae surface might result in the freeing of binding sites for metal sorption. On the other hand, homogenization might fragment the cells and increase the available surface area for sorption.

A batch of algae was washed five times with distilled water (pH = 6.5-7), filtered and then dried overnight in a dessicator. Another batch was homogenized in a Brinkman grinder (Rendale, Ontario, Canada). A known amount of untreated, washed or homogenized algae (0.01 g) was contacted for 24 h with 10 mL of an aqueous copper solution having an initial concentration of 10 mg/L. As shown in Appendix I, the copper uptakes were 9.3, 10.2, and 10.0 mg/g for the untreated, washed algae and homogenized algae, respectively. However, these differences were not significant according to the ANOVA. The results of ANOVA are shown in Table 9.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio
Treatments	2	1.24222	0.62111	0.15751
Error	6	7.88667	3.94333	
Total	8	9.12889		

Table 9. Analysis of Variance (ANOVA) for the treatment effects on the sorption of copper by Palmaria palmata without pH adjustment (pH = 6.5-7). Contact time = 24h. Temperature = 21±3° C. Agitation rate = 275 rpm.

The observed F value $F_{0.01}(2,6) = 0.15751$ is lower than the tabular F value $F_{0.01}(2, 6) = 10.92$ at the 1% level, indicating that the treatment effects are not significant on the sorption of copper by *Palmaria palmata*. Although it is possible that the washing and/or homogenization could have greater and significant effects under other conditions of pH, temperature, contact time and initial concentration of the metal, it was decided to use untreated dried algae in further experiments with copper and the other metals studied (Cd, Ni, Pb and Zn).

4.2.2. Determination of the algae concentration

The effect of the concentration of dried algae on the metal uptake was studied in this preliminary phase. The results are shown in Appendix II. The purpose was to determine what algae concentration should be used since the literature indicated that metal uptake per unit mass of biosorbent drops as biomass concentration increases (Fourest and Roux, 1992; Blanco et al., 1999; Dönmez et al., 1999). Copper was the only metal used for this experiment and the range of initial concentrations was 0 to 60 mg/L. The biomass concentration was varied from 0.5 to 2 g/L and the contact time held at 24h. The results shown in Figure 1 indicated that copper uptake per gram biomass decreased when the biomass concentration was increased from 0.5 g/L to 2 g/L, thus corroborating the discussion in section 2.6.4. According to some authors, this reduction in the uptake could be linked to the electrostatic interaction between cells which effectively reduces the availability of binding sites per unit mass (De Rome and Gadd, 1987). However, this was based on a study of filamentous living cells and may not be valid for dried algae.

In addition, as observed in Figure 1 the sorption capacity corresponding to the saturation plateau is about 4.5 mg/g for both 0.5 g/L and 1g/L biomass concentration, whereas it is about 3.5.mg/g at 2 mg/L. The saturation plateau is reached for an initial copper concentration of 13 mg/L, at both 0.5 g/L and 1g/L. However, an increase in the initial copper concentration from 2.4 mg/L to 13 mg/L results in three times greater copper uptake at 0.5 g/L and in seven times greater copper uptake at a biomass concentration of 1 g/L. Copper is removed more efficiently at a biomass concentration of 1 g/L in the range of initial copper concentrations of 0-60 mg/L.

A 1 g/L algae concentration was therefore used in other experiments involving other methods and a larger range of initial concentrations.

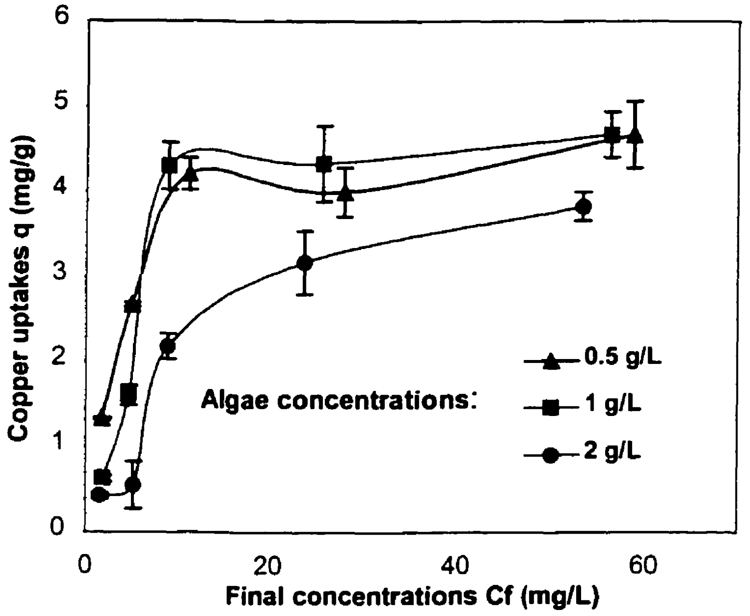


Figure 1. Effect of dried algae concentrations (0.5; 1 and 2 g/L) on the uptake of Cu^{2+} by Palmaria palmata.. Temperature 21 ± 3°C. No pH adjustment (pH = 6.5-7. Equilibrium time = 24h. Agitation rate = 275 rpm. Vertical bars denote standard error within triplicates.

4.2.3. Screening of metals

The literature review provided the background necessary to identify the metals that could pose a health hazard and that were present in the effluents of the PWB manufacturing activities, namely, cadmium, lead, copper, zinc and nickel. The objective of the following section is to understand the sorption properties of *Palmaria palmata* and to select the metals that are adsorbed to the greatest degree by *Palmaria palmata.* The metal uptake q (mg/g) was calculated by using equation (6) for Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}. Sorption isotherms (values of the metal uptake q plotted against various final metal concentrations C_f) were plotted to characterize the sorbent system behaviour. The experimental data were then fitted to the Langmuir, Freundlich and BET models. The constants derived from the models (such as the Langmuir parameters q_{max} and b) characterized the surface properties and affinity of the biosorbent. These parameters are also useful for comparing the affinity of *Palmaria palmata* to the different metal ions, and for screening the metals that exhibited the greatest adsorption capacities.

4.2.3.1. Sorption isotherms of various metals

The adsorption isotherms for each metal in mono-metal solutions were obtained at a biomass concentration of 1 g/L and a contact time of 24 hours (Figures 2 and 3). Raw data are presented in Appendix III. The saturation plateau reached at various residual concentrations C_f depended on the sorbed species. An initial concentration range of 0 to 60 mg/L was therefore used for all the metals in the first study. However, it seemed that saturation levels were reached at higher residual concentration for copper and lead C_f. In addition, the fits of the Freundlich, Langmuir and BET models (according to equations (8), (10) and (12), respectively) was not satisfying for the 0 to 60 mg/L initial metal concentration range. These are the reasons why the range of initial concentrations for copper and lead were extended to 0 to 600 mg/L.

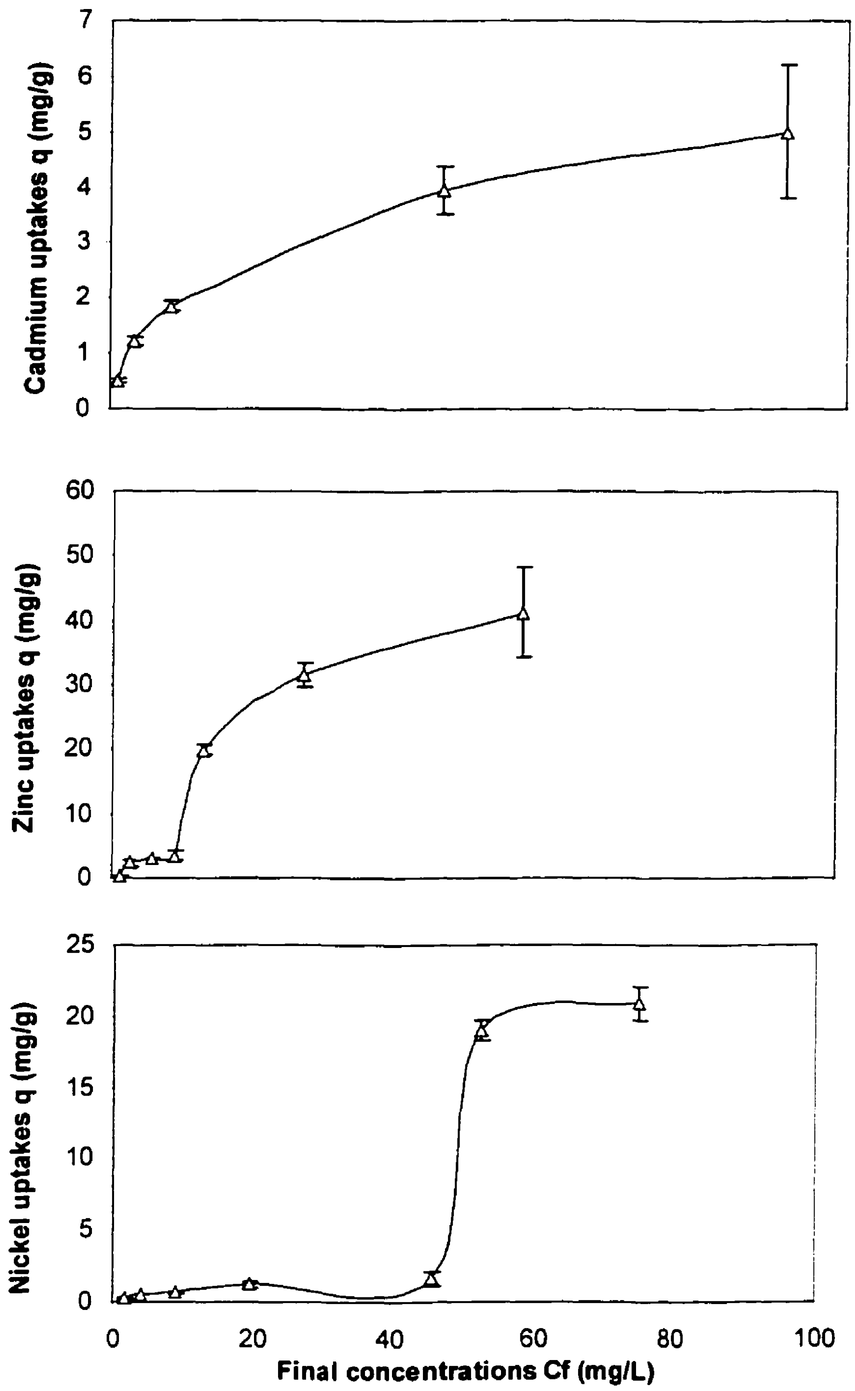


Figure 2. Sorption isotherms for Cd^{2+}, Ni^{2+} and Zn^{2+} uptakes by Palmaria palmata. Algae concentration : 1 g/L. Temperature 21 ± 3°C. No pH adjustment (pH = 6.5-7). Equilibrium time = 24h.. Agitation rate = 275 rpm. Vertical bars denote standard error.within triplicates. Horizontal scales are the same and vertical scales are different.

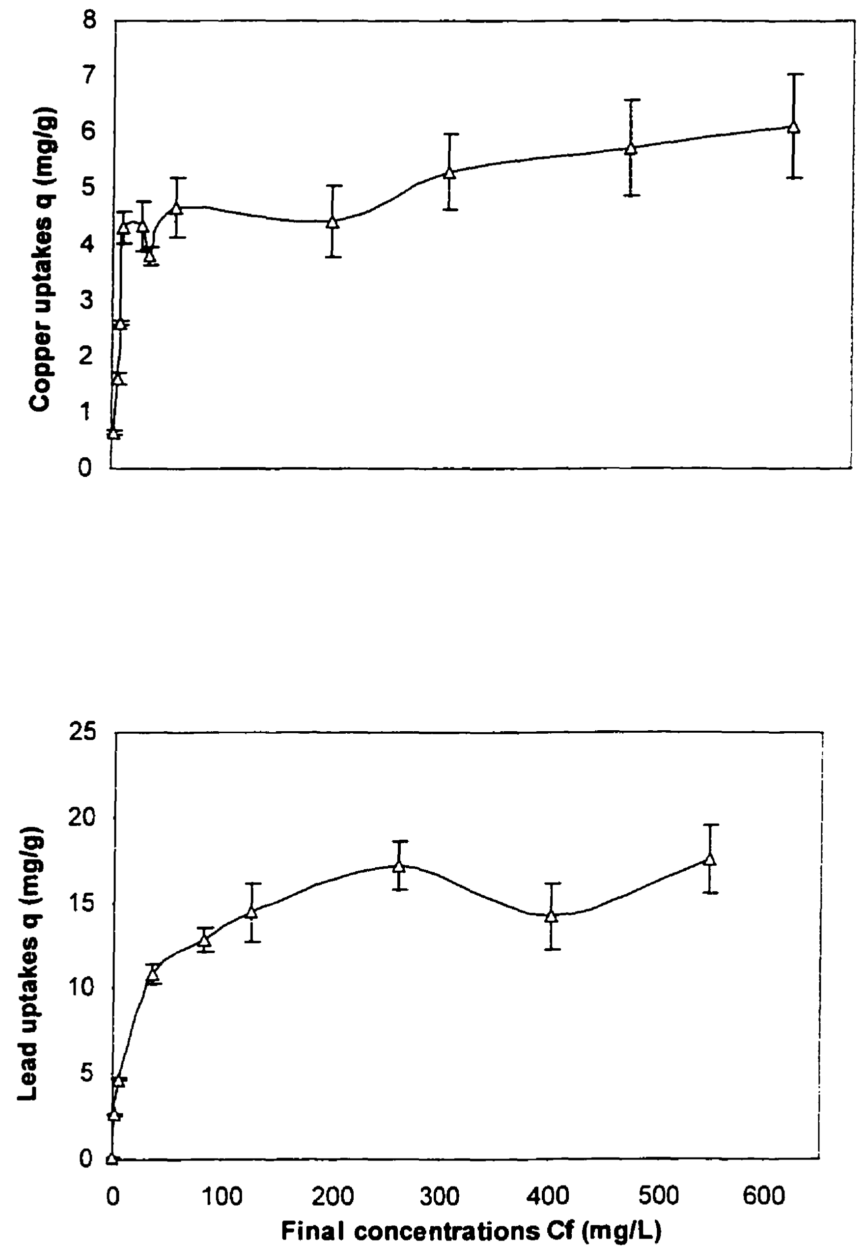


Figure 3. Sorption isotherms for Cu^{2+} and Pb^{2+} uptakes by Palmaria palmata. Algae concentration : 1 g/L.. Temperature 21 ± 3°C. No pH adjustment (pH = 6.5-7). Equilibrium time = 24h. Agitation rate = 275 rpm. Vertical bars denote standard error.within triplicates. Horizontal scales are the same and vertical scales are different.

The metal uptake results indicated that *Palmaria palmata* was an efficient biosorbent to remove Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Pb^{2+} from aqueous solutions. As shown in Figures 2 and 3, at low residual concentrations (C_f = 10 mg/L), *Palmaria palmata* sorbed approximately 3.5 mg/g Zn^{2+}, 1.8 mg/g Cd^{2+}, 0.7 mg/g Ni^{2+}, 4.3 mg/g Cu^{2+} and 5 mg/g Pb^{2+}. At higher residual concentrations (C_f = 60 mg/L) about 41 mg/g Zn^{2+}, 4 mg/g Cd^{2+}, 20 mg/g Ni^{2+}, 4.5 mg/g Cu^{2+} and 20 mg/g Pb^{2+} were sorbed by *Palmaria palmata.* Metal uptakes at equilibrium residual concentrations C_f of 200 mg/L were 4.5 mg/g and 17 mg/g, respectively, for copper and lead.

It should be mentioned that the lead uptake by *Palmaria palmata* was found to be generally higher than that of other red algae reported in the literature. For instance, Figure 3 shows that the lead uptake was about 17 mg/g at C_f = 240 mg/L. The lead uptake by *Palmaria palmata* at C_f = 243 mg/L was reported to be 13.5 mg/g (Volesky and Holan, 1995).

In addition, the lead uptake at C_f = 10 mg/L and C_f = 200 mg/L was reported to be 3 mg/g and 65 mg/g, respectively, for formaldehyde crosslinked *Chondrus crispus*, and 1 mg/g and 11 mg/g, respectively, for formaldehyde crosslinked *G.marginata (*Holan and Volesky, 1993). The nickel uptake found in the present study was of the same order or higher than the one investigated by these authors. The nickel uptakes at a C_f of 10 mg/L were 4.6 mg/g for native *C. cripus* and 0.8 mg/g for native *G.marginata* (Holan and Volesky, 1993).

4.2.3.2. Linearizations of the sorption isotherms

The experimental data were linearized by the Langmuir, Freundlich, and BET models according to equations (8), (10) and (12) to charactarize the sorption of metals by *Palmaria palmata* and to evaluate parameters of the models which are related to metal affinity. The results of the linearizations for Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Pb^{2+} are shown in Table 10. The R-squares are high in general, indicating that the models described the experimental data well. However, in the case of the zinc, the R-square of the BET model was too low to do any predictions. In this particular case, the model parameters were not determined..

Metal ion	Langmuir constants			Freundlich constants			BET constants		
	q_{max} (mg/g)	b (L/mg)	R^2	k $mg^{(1-1/n)}L^{1/n}/g$	n	R^2	qmax (mg/g)	B (L/mg)	R^2
Zn^{2+}	28.49	0.031	0.7740	0.669	0.926	0.8521	ND	ND	0.0277
Cd^{2+}	4.75	0.098	0.9969	0.557	1.963	0.9720	5.66	$8.84*10^4$	0.9923
Pb^{2+}	15.17	0.102	0.9948	2.682	3.113	0.9217	16.95	$2.95*10^4$	0.9812
Cu^{2+}	6.65	0.060	0.9649	1.108	3.383	0.7050	6.02	$8.31*10^4$	0.9892
Ni^{2+}	3.03	0.055	0.9505	0.215	1.814	0.9764	2.06	$1.21*10^5$	0.9803

Table 10. Constant parameters of the Langmuir, Freundlich and BET models for the biosorption of Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, and Ni^{2+}, by Palmaria palmata. Algae concentration : 1 g/L. Temperature 21 ± 3°C. No pH adjustment (pH = 6.5-7). Equilibrium time = 24h. Agitation rate = 275 rpm. ND = Not determined.

According to the system of isotherm classification of solid / solute adsorption, the shape of the isotherms shown in Figures 2 and 3 indicates that the metal adsorption isotherms belong to the S type curves (Giles et al., 1974).

An increase in initial metal concentration will induce a sorption rise, as long as binding sites are not saturated. The species that sorb first facilitate the sorption of the additional species (favourable or cooperative sorption). The slope of the curve drops because the progressive covering of the surface makes the vacant sites more and more difficult to find.

In addition, as shown in Table 10, based on higher coefficients of determination R^2, the linearized experimental data conformed best to the BET model (and to the Langmuir model to a lesser extent) than to the Freundlich model. However, Zn was an exception and no model could work well. Overall, due to the good fit to the BET model, the metal sorption on *Palmaria palmata* could be characterized as a multilayer sorption, in which a Langmuir equation applies to each layer (Langmuir, 1918; Webber, 1985). The Langmuir and Freundlich models are used in the majority of sorption studies. However, the adsorption of copper on *Rhizopus arrhizus* was reported to obey the BET model (De Rome and Gadd, 1987).

4.2.3.3. Interpretation of the parameters derived from the models

➢ Metal affinity for *Palmaria palmata*

The Langmuir parameter b corresponds to the ratio of the adsorption rate constant to the desorption rate constant. The value of b is thus commonly used to compare the affinity of an ion metal for a biosorbent (Chong and Volesky, 1995; Puranik and Packnikar, 1999). From Table 10 and based on the value of b, the affinity sequence of metal for *Palmaria palmata* was:

$$Pb^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+} > (Zn^{2+})$$

The affinity of Zn^{2+} is uncertain due to its low coefficient of determination R^2, when fitted to the Langmuir model. The same sorption affinity sequence has been reported by Puranik and Packnikar (1999) for sorption on *S. cinnamoneum* ($Pb^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} = Co^{2+} > Ni^{2+}$). Tobin et al. (1984) found $UO^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+}$ as an affinity order for *Rhizopus arrhizus*.

However, due to the variability and the complexity of biosorption by different types of biomass, different affinity sequences have been reported. The affinity of a metal for binding sites depends mainly on its physical and chemical properties. The more important ones are:

- *The atomic weight.* According to Haug and Smidrod (1967), the metal affinity is correlated to stereo-chemical effects. According to them, larger ions could better fit a binding site with two distant functional groups.
- *The Pauling electronegativity.* More electropositive metal ions are more strongly attracted to the negative surface of the biosorbent. This correlation has been observed by Chong and Volesky (1995) for sorption by *Ascophyllum nodosum* (where the affinity order was $Cu^{2+} > Cd^{2+} > Zn^{2+}$).
- *The ionic radius.* According to Tobin et al. (1984), a smaller ionic radius implies more molecules can sorb onto a fixed surface area of biosorbent.
- *The covalent index.* The covalent index was introduced by Nieboer and McBryde (1973) and is computed using the expression $X_m^2\,(r + 0.85)$, where X_m is the electronegativity, r is the cationic crystal radius and 0.85 stands for the contribution of N or O donors to the bond distance. In general, the higher the covalent index of a metal ion, the higher its potential to form covalent bonds with biological material.

The previous described parameters are listed for Pb, Cd, Cu Ni and Zn in Table 11.

Parameter	Pb^{2+}	Cd^{2+}	Cu^{2+}	Ni^{2+}	Zn^{2+}
Atomic weight	207.2	112.4	63.4	58.7	65.38
Ionic radius (A)	1.21	0.97	0.72	0.69	0.74
Covalent index	6.61	5.20	6.32	4.99	4.54
Electronegativity of the atom	2.33	1.69	1.9	1.91	1.6

Table 11. Physical and chemical properties of Pb^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} metal ions (Moeller, 1972).

The Table 11 indicates that, in general, relative orders of the physico-chemical properties such as atomic weight, ionic radius, covalent index and electronegativity are suitable criteria to predict the relative affinity order of Pb^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} for *Palmaria palmata*. It should be mentioned that more than one parameter could influence the sorption characteristic of metals, especially in heterogeneous systems such as algae. Last, but not least, the nature of the algal surface and the preference of the surface functional groups for certain metals, are also determining factors.

- Screening of metals using the q_{max} value

Since the parameter q_{max} corresponds to the maximum uptake of a metal by the biosorbent, it can be used to screen the metals on the basis of their sorption performances. However, Zn^{2+} is arbitrarily eliminated for two reasons: its sorption capacity is weak at low final concentrations, and the behaviour is not well described by the Langmuir linearization. In the range 0-10 mg/L C_f, the observed uptakes never exceeded 3.5 mg/g. As shown in Table 10, the sorption performance order on the basis of the q_{max} obtained from the Langmuir model (and BET model) is: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$.

In addition, as mentioned in the literature review (section 2.2.2), Pb and Cu are the main metals used commonly in the plating process steps of PWB manufacturing. For these reasons, Pb^{2+} and Cu^{2+} were selected for the main experiments in this study. The q_{max} values for Cd^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and Pb^{2+} obtained with various biosorbents are listed in Appendix IV.

The q_{max} values obtained in this study are generally lower than those commonly found in the literature. However, the performance of a raw *Palmaria palmata* material cannot be compared to that of crosslinked or pre-treated biomass.

4.3. Determination of the ranges of physico-chemical parameters for the Central Composite Design (CCD)

A comprehensive literature review (section 2.6) was helpful in determining the factors that have a significant effect on the sorption and that should be varied in the statistical design. The parameters of interest were contact time, pH, temperature and initial metal concentration.

However, before applying the Central Composite Design (CCD), it is necessary to know by how much and to which point the factors should be varied. In this optimization process, including the study of the multi-factor interactions, the ranges of the factors should be delimited. For instance, it is not necessary to involve contact times longer than that needed to reach equilibrium, since no more sorption is expected to occur. In addition, it is essential to know the behaviour of the biological material at extreme values of the factors. For example, it is crucial to know whether structural damage occurs in the *Palmaria palmata* cells at low pH or at high temperature. A one-factor at a time approach was therefore used first to determine the particular factor spaces, or in other words, the ranges to be used for contact time, pH, temperature and initial metal concentration, in the CCD.

4.3.1. Initial metal concentration

The effect of the initial concentrations of copper and lead on the metal uptake by *Palmaria palmata* was studied in section 4.2.3. No significant increase in metal uptake was observed above an initial lead concentration of 200 mg/l. or above an initial copper concentration of 500 mg/L. The ranges used in the CCD for these metals were therefore 0-200 mg/L and 0-500 mg/L, respectively.

4.3.2. Contact time

A set of experiments with contact time of 48 h was carried with mono-Cu solutions and mono-Pb solutions in order to determine the necessary contact time for the CCD. Results are shown in Figure 4. Raw data are presented in Appendix V. The copper and lead uptake graphics presented two phases. The first phase was non-linear and was characterized by rapid uptake in the first 80 minutes. Nearly 70% and 100% of the total copper and lead uptakes, respectively, were reached in the first 10 minutes. The copper uptake further increased to 83% and 95% at 15 min and 30 min, respectively, while the lead uptake decreased by 18% from 90 min to 650 min contact time .The saturation plateaux were reached within 5 h for copper and 11 h for lead. This two-phases behaviour is in agreement with other studies on the uptake of divalent cations (Kuyucak and Volesky, 1989; Zhou et al., 1998).

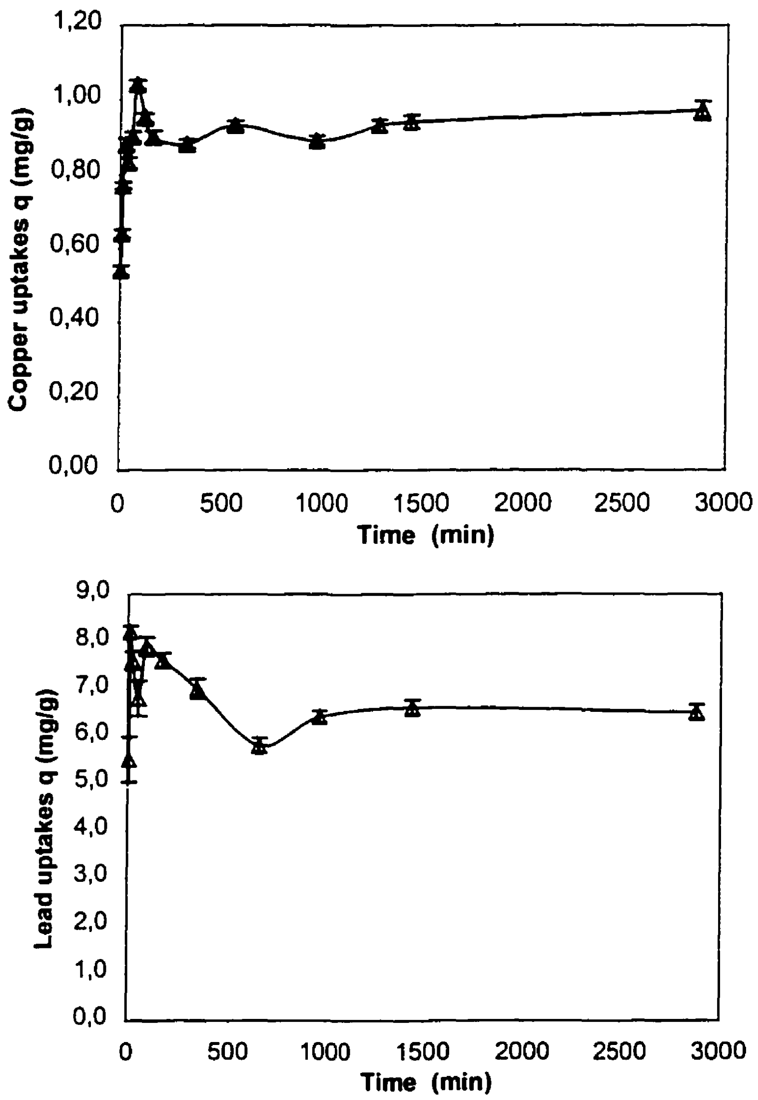


Figure 4. Effect of contact time on the uptakes of Cu^{2+} and Pb^{2+} by Palmaria palmata .Algae concentration: 1 g/L. Initial Cu^{2+} concentration: 10 mg/L, initial Pb^{2+} concentration: 100 mg/L. Temperature: 23°C. pH =4. Agitation rate= 275 rpm. Vertical bars denote standard error withintriplicates.

Some studies have reported that the first phase was fast, and was independent of metabolic energy (Kuyucak and Volesky, 1989; Zhou and Banks, 1991). This first phase was attributed to adsorption on the cell surface, mainly by anion exchange (Volesky and Holan, 1995). The second phase was slower and energy dependent (White and Gadd, 1987). Actually, this second phase is representative of ion diffusion into the cells (Volesky and Holan, 1995).

The highest variations in the removal of copper and lead were observed from 5 min to 2 h. The range of contact times to apply in the CCD was therefore decided to be from 10 to 170 min.

4.3.3. pH

The range for pH was set at 2-6. This range was used because most of the industrial effluents containing heavy metals are acidic (Macchi et al., 1993) and to prevent precipitation, as it is expected to occur under more basic conditions Indeed, hydroxides (or oxides) of Cu and Pb appear at pH of 5.1 and 6.5, respectively. Moreover precipitation of copper and lead in the bulk solution may occur for pH values above 6.5 and 7, respectively (Baes and Mesmer, 1976). Although precipitation may contribute to the overall removal of metals from solution, it interferes with the pure biosorption phenomenon. For scientific purposes, it is therefore preferable to work in a range of pH where precipitation would not occur.

Lastly, extreme values may pose a problem. Very acidic or moderately alkaline conditions pH could damage the physical structure of the algae cell wall. Some distorted cells have been observed under microscope and significant weight losses have been reported (Kuyucak and Volesky, 1989). No serious effects of this kind are expected to occur in the range of pH 2-6. The pH effect was studied on a mono-Cu^{2+} solution and on a mono-Pb^{2+} solution, at initial copper concentration of 10 mg/L and initial lead concentration of 100 mg/L for 90 minutes (the mean value of the contact time range determined in section 4.3.2 and is represented in Figure 5. Raw data are shown in Appendix VI.

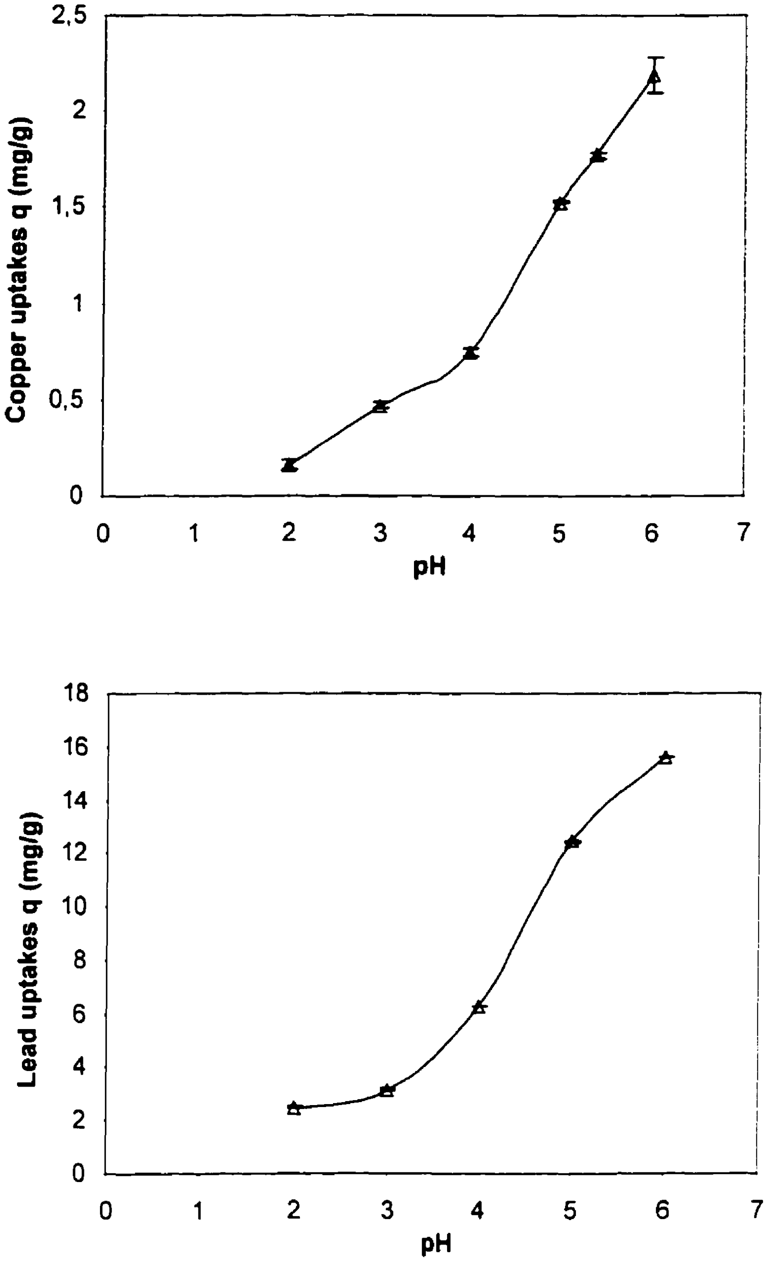


Figure 5. Effect of pH on the uptake of Cu^{2+} and Pb^{2+} by Palmaria palmata. Algae concentration: 1g/L. Initial Cu^{2+} concentration: 10 mg/L, initial Pb^{2+} concentration: 100 mg/L. Temperature: 23°C. Equilibrium time = 90 min. Agitation rate = 275 rpm. Vertical bars denote standard error within triplicates.

Figure 5 clearly showed that pH had a great influence on the sorption of copper and lead by *Palmaria palmata*, indicating that the process might be governed by an ion-exchange mechanism (Puranik and Paknikar, 1997). Under highly acidic concentrations (pH = 2), there was little sorption of copper. These low levels of sorption could be due to the higher concentrations of hydrogen (H^+) ions that may compete for the binding sites on the cell surface (Gadd, 1988; Tobin et al., 1984). At low enough pH, virtually all sites become protonated. Moreover, loss of sorbing activity has been reported at pH lower than 2 (Tanigushi, 2000). This explains why it is easy to desorb the metal by washing the biosorbent with dilute acids such as hydrochloric acid, nitric acid or 0.1 M EDTA (De Rome and Gadd, 1987; Puranik and Paknikar, 1997).

At a pH of 4, less than 8% and 7% of the initial copper and lead, respectively, are removed from solution by *Palmaria palmata*. Figure 6 shows that the metal ion binding increased with an increasing pH. When pH was increased from 4 to 5, the copper and lead removal were about twice as high (the removal varied from 7.6% to 16.2%, and from 6.45% to 13%, respectively). It is generally agreed that the sorption of metal cations increases with a rise in pH (Tsezos and Volesky, 1981; Darnall et al., 1986; Fourest and Roux, 1992; Holan et al., 1993; Ricou et al.; 1999; Tanigushi, 2000). This could result from the change of the total surface charge. The algal biopolymer provides various functional groups such as carboxyl, sulfate or phosphate groups as binding sites for the metal cations. Increasing the pH results in exposing more ligands carrying negative. The sorption of metal action is thus increased. Generally, the overall negative charge of the biopolymer occurs when the isoelectric point is reached (which is usually found at a pH of 3 for algae (Gadd, 1988)).

Furthermore, the S-shaped curve shown in Figure 5 could be compared to a titration curve, where the steep part might correspond to the dissociation of ionic groups of the algae (at pK_a = pH). Therefore, the rapid rise in the binding capacity (between pH = 4 and pH = 6), could be explained by the electrostatic attractions between positively charged copper (II) cations and negatively charged binding sites. More specifically, this may be mainly due to the dissociation of carboxylic groups whose pK_a in the marine algae has been reported to range between 3.5 and 5.5 (Kratchovil and Volesky, 1998).

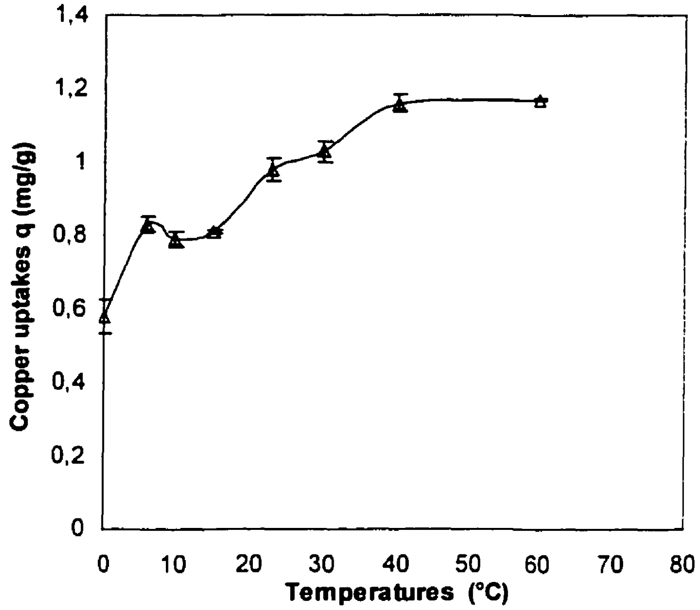
Finally, the solubility of the metal complex decreases at high pH. Besides, formation of hydrolyzed species having a lower degree of hydratation was reported to enhance sorption on biomass, resulting in removal of larger amount of metal (Puranik and Packnikar, 1997), at high pH.

According to Figure 5, copper and lead uptakes increased continuously as pH increased from 2 to 6. In addition, no serious damage or precipitation were observed in this experiment. Thus, the 2-6 pH range is used in the statistical design.

4.3.4. Temperature

The temperature effect was studied on a mono-Cu^{2+} solution and on a mono-Pb^{2+} solution. Initial metal concentrations were 10 mg Cu/L and 100 mg Pb/L, and the contact time was 24 hours in both cases. Sorption data are presented in Appendix VII.

Figure 6 shows that the copper uptake increased, whereas the lead uptake decreased with temperatures. For example, an increase from 0 °C to 6 °C increased the copper uptake by 43%. A further increase of temperatures to 23°C and to 40°C increased the copper removal by 70% and 100%, respectively. No increase in the copper uptake was observed at 60°C. On the other hand, an increase from 5°C to 23°C, to 40°C and to 60°C resulted in a decrease of 38%, 45% and 58%, respectively, in the lead uptake. Since the greatest changes in metal uptake occurred between 5°C and 40°C, this range was chosen for the CCDs.



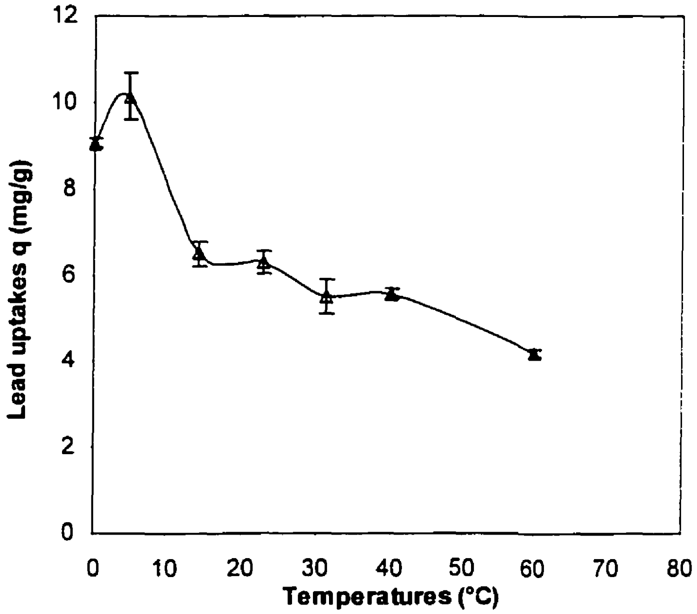


Figure 6. Effect of temperature on the uptake of Cu^{2+} and Pb^{2+} by Palmaria palmata. Algae concentration: 1g/L. Initial Cu^{2+} concentration: 10 mg/L initial Pb^{2+} concentration: 100 mg/L. pH = 4. Equilibrium time = 90 min. Agitation rate = 275 rpm. Vertical bars denote standard error within triplicates.

4.4. CCD results for copper at a 0-20 ppm concentration range

In the following experiments, the response surface-models for a uniform-precision rotatable central composite design were fit to the experimental data and used to evaluate the effects of pH, temperature, initial concentration, and contact time on the metal uptake by *Palmaria palmata*.

4.4.1. Experimental design

This first CCD focused on the copper uptake by *Palmaria palmata* in a restricted range of initial copper concentrations (0-20 mg/L). Table 12 shows the experimental ranges and the relationship between factor levels in natural units and coded values. The design matrix with the observed and predicted values is given in Appendix VIII.

Coded factors	Symbols	Factor levels				
		-2	-1	0	+1	+2
pH	PH	2	3	4	5	6
Temperature (°C)	TEMP	6	14.5	23	31.5	40
Initial concentration C_i (mg/L)	INCONC	0	5	10	15	20
Contact time (min)	TIME	10	50	90	130	170

Table 12. Experimental range and levels of independent factors of the CCD of Cu^{2+} (at a 0-20 mg/L $INCONC_{Cu}$ range).

In the first place, the four factors (pH, temperature, initial metal concentration, and contact time) are considered to check for the model fitting and to seek for a possible optimum response. In the second place, each combination of the most significant factors is computed to construct and interpret two-dimensional response surfaces.

4.4.2. Study of the four-factor interactions

4.4.2.1. Adequacy of the fitted model

Table 13 shows the ANOVA output by SAS for the lack of fit test and for the test of significance of the model on the response surface model used on the CCD involving copper only, with the range of initial concentrations of 0-20 mg/L.

Sources of variation	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
Lack of fit	10	0.191375	0.019138	1.684	0.2705
Pure error	6	0.068200	0.011367		
Total error	16	0.25975	0.16223		
Total model	14	12.30037	-	54.156	0.0000

Table 13. Analysis of Variance (ANOVA) for the quadratic model of the CCD of Cu (at a 0-20 mg/L $INCONC_{Cu}$ range). R-square = 0.9793.

These results show that there was no lack of fit (LOF) (since Prob>F is greater than the significance level of 0.15). The model is highly significant and the R^2 was found to be 0.9793, and thus explains almost all of the variability in the data aside from the 0.5% due to pure error. Thus, the quadratic response surface model must be considered very adequate for the system studied.

4.4.2.2. Optimization of the parameters

The solution for optimum response generated from PROC RSREG of the SAS program (SAS, 1998) for the pH, temperature, initial concentration, and contact time effects is listed in Appendix IX.

The predicted value at the stationary point was $\hat{y}_o = 0.27313$, and the eigenvalues were λ_1 = 0.60394, $\lambda_2 = 0.11936$, $\lambda_3 = -\ 0.07262$ and $\lambda_4 = -\ 0.38068$.

The canonical equation for the copper predicted uptake $\hat{Y}$ can thus be written as:

$$\hat{Y} = 0.27313 + 0.60394 W_1^2 + 0.11936 W_2^2 - 0.07262 W_3^2 - 0.38068 W_4^2$$

Where W_1, W_2, W_3, and W_4 are the transformed independent variables of the canonical form. Since the eigenvalues are of mixed signs, the stationary point of the fitted surface is a saddle point (or minimax point). There is no maximum response optimizing the four parameters, in the neighbourhood of the stationary point (otherwise all the eigenvalues would have been negative). However, the results of the canonical analysis indicate maximum responses at the stationary point corresponding to a temperature of 31 °C and a pH value of 2. On the other hand, minimum responses at the stationary point were obtained at an initial copper concentration $INCONC_{Cu}$ of 2.8 mg/L and a contact time of 174 minutes.

4.4.3. Study of the two-factor interactions

To simplify the geometrical representation of the studied model, three-dimensional diagrams are used in which two dimensions accommodate two significant variables (PH and TEMP, PH and INCONC$_{Cu}$, or PH and INCONC$_{Cu}$ for example), while the third dimension accommodates the response q_{Cu} (copper uptake). The resulting response surface corresponds to the average surface of the total variation. For instance when the combination (PH*TEMP) is considered, the part of the response attributed to the other factors INCONC$_{Cu}$ or TIME, is included in the error sum of squares (Dutilleul, 2001).

4.4.3.1. *Determination of the most significant factors*

The Fisher F-test and its corresponding p-value are used to check the significance of each factor in the model and are given in Table 14. The larger the F-value and the smaller the p-value, the more significant is the corresponding factor.

Factors	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
PH	5	8.238299	1.64766	101.6	0.0000* *
TEMP	5	0.383088	0.076618	4.723	0.0077* *
INCONC	5	4.315731	0.863146	53.204	0.0000* *
TIME	5	0.127370	0.025474	1.570	0.2246 (ns)

*Table 14. Analysis of Variance (ANOVA) for each factor of the CCD of Cu in the mono-Cu system (at a 0-20 mg/L INCONCCu range). Confidence level: ns (not significant), * : $p< 0.15$, ** : $p < 0.015$.*

From Table 14, pH, TEMP, and INCONC$_{Cu}$ had a significant effect on the copper uptake by *Palmaria palmata*. On the contrary, the factor TIME exhibited a probability of the F-test greater than 15% and was thus eliminated. Then three RSEG procedures including only the combinations of two significant factors were run to determine the coefficients of the regression equation.

4.4.3.2. *Study of the response surfaces*

The response surfaces for the effect of the combined factors (INCONC$_{Cu}$ and PH, TEMP and PH, INCONC$_{Cu}$ and TEMP) on copper uptake and their second-order regression equation are shown in Figure 7, 8 and 9, respectively.

$INCONC_{Cu}$ and PH interaction

The predicted copper uptake by *Palmaria palmata* ranged from 0 to 4 mg/g depending on the pH (PH) and the initial copper concentration ($INCONC_{Cu}$) (Figure 7). More precisely, at low pH range (from 2 to 3), the copper uptake was negligible (from 0 to 0.5 mg/g), for an $INCONC_{Cu}$ ranging from 0 to 20 mg/L. At low pH values (from 2 to 4), the effect of changes in $INCONC_{Cu}$ was negligible, while at higher pH values (from 4 to 6), the copper uptake increased rapidly with an increasing $INCONC_{Cu}$. For instance, a ten-time increase in $INCONC_{Cu}$ (from 2 mg/L to 20 mg/L) at a pH of 4 increased the predicted copper uptake more than 6 times, about 5 times at a pH of 5, and about 4 times at a pH of 6. All things considered, a concomitant increase of PH and $INCONC_{Cu}$ increased the predicted uptake, due to the quite high value of the bilinear term PH* $INCONC_{Cu}$ in the regression equation. The highest predicted value (3.88 mg/g) was obtained for pH = 6 and $INCONC_{Cu}$=20 mg/L.

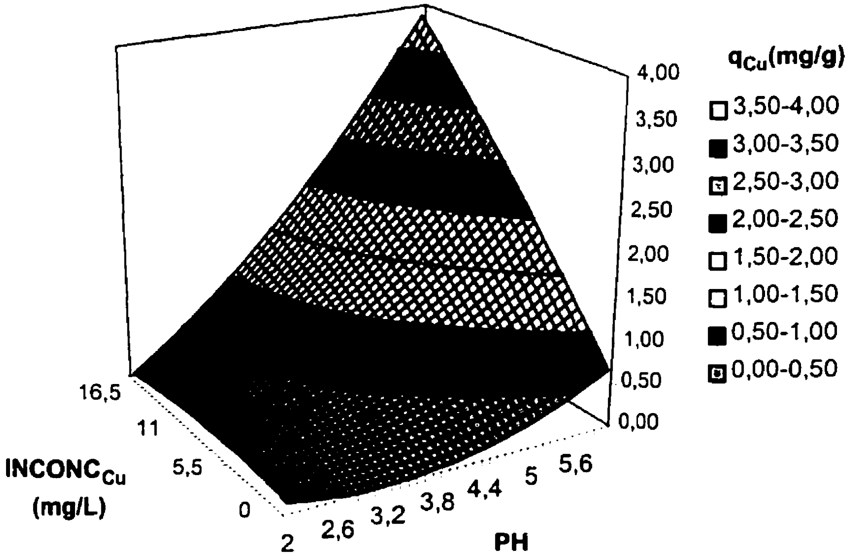


Figure 7. Response surface representing the regression equation: q_{Cu} = 0.93519+1.10583*PH + 0.76917* $INCONC_{Cu}$ + 0.33909 $(PH)^2$ + 0.82750 PH* $INCONC_{Cu}$ – 0.99591 $(INCONC_{Cu})^2$ *where pH (PH) and intial copper concentration ($INCONC_{Cu}$) have a significant effect on copper uptake (q_{Cu}). The order of the legend items directly follow the order in which they appear in the figure.*

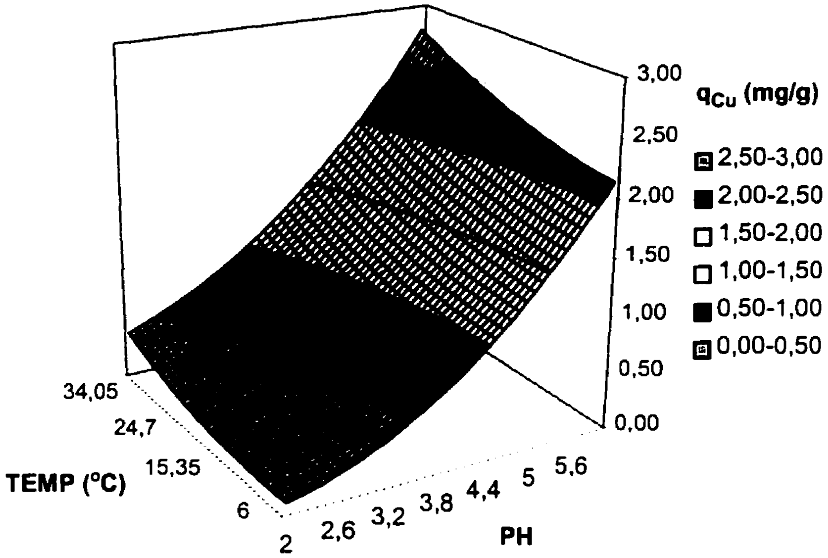


Figure 8. Response surface representing the regression equation $q_{Cu} = 0.8925 + 1.10583\ PH + 0.2325\ TEMP + 0.3569\ (PH)^2 + 0.0825\ PH * TEMP + 0.10688\ (TEMP)^2$ *where pH (PH) and temperature (TEMP) have a significant effect on copper uptake* q_{Cu}*. The order of the legend items directly follow the order in which they appear in the figure.*

TEMP and PH interaction

According to the regression equation of Figure 8, the effect of the linear term PH on the response q_{Cu} is the most important. The predicted copper uptake increased proportionally to the PH. For example, at a temperature of 23°C, when PH increased from 2 to 4, q_{Cu} increased 6 times. A further increase from PH = 2 to PH = 6 increased the predicted copper uptake 17 times. The highest predicted copper uptakes (ranging from 2.5 mg/g to 2.8 mg/g) were obtained for a PH of 6 in the temperature range of 33 to 40 °C. As shown in Appendix VIII, the actual copper uptake observed for PH = 6, TEMP = 23 °C and $INCONC_{Cu}$ = 10 mg/L, TIME = 90 min, was 2.3 mg/g, which agreed with the predicted values.

$INCONC_{Cu}$ and TEMP interaction

The predicted copper uptake was affected essentially by the $INCONC_{Cu}$ factor, which exhibited the highest coefficient in the regression equation of Figure 9. As observed in Figure 9, at $INCONC_{Cu}$ = 20 mg/L, the response surface reached a maximum (about 2 mg/g) at a temperature range of 37 to 40 °C and dropped slowly with decreasing temperature. At $INCONC_{Cu}$ = 20 mg/L, the response surface reached a maximum (about 2 mg/g) at a temperature range of 37 to 40 °C and dropped slowly with decreasing temperature. For example, at $INCONC_{Cu}$ = 20 mg/L, only a 25% decrease in q_{Cu} was predicted for a 10 °C drop (from 36 to 26°C). At low $INCONC_{Cu}$ (from 0 to 3 mg/L), there was no effect of the temperature on the predicted copper uptake.

The actual values of Appendix VIII are of the same order to the predicted value for $INCONC_{Cu}$ = 20mg/L. Besides, the observed copper uptake at $INCONC_{Cu}$ = 20 mg/L, pH = 4, TEMP = 23 °C and TIME = 90 min, was 1.62 mg/g.

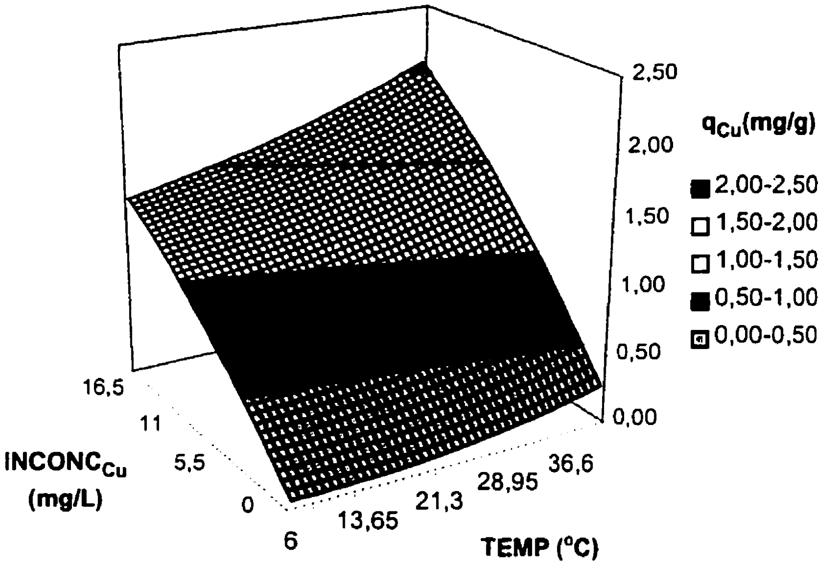


Figure 9. Response surface representing the regression equation: $q_{Cu} = 0.99288 + 0.76917 * INCONC_{Cu} + 0.2325 * TEMP - 0.11995 (INCONC_{Cu})^2 + 0.1175\ INCONC_{Cu} * TEMP + 0.06505\ (TEMP)^2$ *where initial copper concentration* ($INCONC_{Cu}$) *and temperature (TEMP) have a significant effect on copper uptake* (q_{Cu}). *The order of the legend items directly follow the order in which they appear in the figure.*

Conclusion

Simultaneously increasing the pH from 2 to 6 and the initial copper concentration from 0 to 20 mg/L resulted in an increase of the predicted copper uptake up to a value of 4 mg/g. In addition, as shown by the two-factor analyses, (PH, TEMP) and (INCONC, TEMP), the predicted uptake was higher at higher temperature. The highest predicted uptake always occurred near the upper limit of the chosen range. Nevertheless, the pH and temperature should not be increased further so as to avoid precipitation and structural damage of the algal cells. However, as discussed in section 4.2.3, the one-by-one factor approach indicated that higher initial copper concentrations could be used to increase the uptake.

The next section therefore describes the results of a CCD in which the range of initial copper concentrations was extended to 0 to 500 mg/L. Furthermore, a binary system involving copper and lead was used to evaluate the possible competition between the two species.

4.5. CCD results for copper and lead in mono-metal and binary metal systems

The experiments reported in the following sections were designed to estimate the effects of pH, temperature, initial concentration, and contact time on the uptake of copper and lead by *Palmaria palmata*. Three rotatable central composite designs were used. Two of them were applied to mono-metal systems (one for copper and one for lead), while the third was applied to a binary system involving both copper and lead.

4.5.1. Experimental design

The respective ranges of initial concentrations of copper and lead were 0-500 mg/L and 0-200 mg/L. Table 15 shows the experimental ranges and the relationship between factor levels in natural units and coded values. The design matrices of the variables in coded and natural units along with the observed and predicted values of uptake for the copper and lead mono-systems, are shown in Appendix X, Appendix XII, respectively. The results for copper and lead uptakes in the binary system are given in Appendix XIV and Appendix XVI.

Coded factors		**Symbols**	**Factor levels**				
			-2	-1	0	+1	+2
pH		PH	2	3	4	5	6
Temperature (°C)		TEMP	6	14.5	23	31.5	40
Initial metal concentration (mg/L)	**Mono-Cu^{2+} system**	$INCONC_{Cu}$	0	125	250	375	500
	Mono-Pb^{2+} system	$INCONC_{Pb}$	0	50	100	150	200
	(Cu^{2+} + Pb^{2+}) system	$INCONC_{Cu}$	0 (0)	125 (50)	250 (100)	375 (150)	500 (200)
Contact time (min)		TIME	10	50	90	130	170

Table 15. Experimental range and levels of independent factors in the Cu^{2+} or Pb^{2+} mono-metal systems and in the binary (Cu^{2+}+ Pb^{2+}) system.

The methodology described in section 4.4 was also used in these experiments. After considering the four factors (pH, temperature, initial concentration, and contact time) in the mono-metal and binary systems, the most significant factors were selected for the two-dimensional response surface analyses.

4.5.2. Comparison of copper uptake in a mono and binary systems

4.5.2.1. Adequacy of the fitted model

Table 16 shows the analyses of variance (ANOVA) generated by Statistical Analysis System (SAS, 1998) for the two experiments involving copper. The lack of fit tests for the models of copper uptake corresponding to the CCD for the mono-Cu^{2+} and binary (Cu^{2+}+Pb^{2+}) systems indicate that LOF exists in the model for the mono-system but not in that for the binary system.

Sources of variation	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
Mono-Cu system					
Lack of fit	10	281.2626	28.126260	15.14	0.0017
Pure error	6	11.148143	1.858024		
Total error	16	292.410743	18.275671		
Total model	14	1350.66659	-	5.28	0.0011
(Cu + Pb) system					
Lack of fit	10	70.370525	7.037053	2.32	0.1579
Pure error	6	18.221886	3.036981		
Total error	16	88.592411	5.537026		
Total model	14	387.61398	-	5.00	0.0015

Table 16. Analysis of Variance (ANOVA) for the quadratic model in the mono-Cu^{2+} system (at a 0.-500 mg/L $INCONC_{Cu}$ range). (R-square = 0.8220) and in the (Cu^{2+} + Pb^{2+}) system (R-square = 0.8140).

The lack of fit may be due to outliers, to incorrect distributional assumptions, or to an incorrect model (Bayne and Rubin, 1986). However, according to some analysts, this approach to modelling is robust with respect to distributional assumptions (Peter Alvo, 2001). Recalling that the analysis for the experiment on copper in which the initial range of concentrations was only 0-20 mg/L (section 4.4), as well as data from the preliminary experiments that showed that adsorption of metals tends to a limit as initial concentration increases (at fixed biomass concentration) (section 4.2.3), the problem of lack of fit in the present case is most likely due to the fact that the range of initial concentrations of copper is too wide. Essentially, the quadratic model does not adequately represent a curvature that is evidently S-shaped over the wider range of initial concentrations. There is no LOF in the binary system at a significance level of 15%, although the probability of a greater F for the test is on the limit (0.1579).

Table 16 shows that the models for both systems explain a significantly greater portion of the variability than can be attributed to error. However, only the model for the binary system should be considered to be adequate since it does not suffer from LOF.

Although the R-square ($R^2 = 0.8220$ for the mono-Cu^{2+} system and $R^2 = 0.8140$ for the binary system) values are reasonably high, leading us to believe that predictions based on the response surfaces may be reasonable, the model for the mono-Cu^{2+} system should be used with caution and that for the binary system should only be used within the factor space, not for extrapolation (Peter Alvo, 2001).

In addition, in Appendix X, each of the observed values (q_{Cu}) is compared with predicted value ($\hat{q}_{Cu}$) calculated from the regression equation including the four factors for the mono-Cu system. The calculated residuals ($q_{Cu} - \hat{q}_{Cu}$) indicate that the difference between observed and predicted value was the largest for experiments (N = 1, 2, 11, 12,15,16,18 to 20, and 22 to 24).

Therefore comparison should be avoided near the corresponding regions of the factor space.

As far the binary system is concerned, the calculated residuals (q_{Cu} - $\hat{q}_{Cu}$) is shown in Appendix XIV.

The largest difference between predicted and observed values were obtained for experiments N = 1, 2 and 19 to 22.

4.5.2.2. Optimization of the parameters

The interaction of the four factors (pH, temperature, initial concentration, and contact time) on copper sorption was studied in the mono-metal solution and in the binary system. The results of the canonical analysis generated by PROC RSREG of the SAS program (SAS, 1998) are reported in Appendix XI, for the mono-Cu^{2+} system, and in Appendix XV for the (Cu^{2+} + Pb^{2+}) system.

Since the eigenvalues are of mixed signs in both systems, the stationary points of the fitted surfaces are saddle points (or minimax points). There are therefore no maximum responses optimizing the four factors at the same time in the factor space.

The plotting of three-dimensional diagrams will help to represent the effect of two combined factors on the copper sorption in the mono-Cu system and in the binary (Cu^{2+}+ Pb^{2+}) system. In addition, it will allow us to assess quantitatively the possible effect of a secondary metal (Pb^{2+}) presence on the uptake of the primary metal (Cu^{2+}).

4.5.2.3. Study of the two-factor interactions

- Determination of the most significant factors

Three-dimensional diagrams are used to visualize the effects of pairs of significant factors on the response, based on the F-tests on the factors from the ANOVA (Table 17).

The pH, temperature and initial concentration of copper all had significant effects on the uptake by *Palmaria palmata*. Contact time was not found to be significant at the 15 % level, and was eliminated from the equations for the response surfaces.

Factors	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
Mono-Cu^{2+} system					
PH	5	889.312089	1777.862418	9.73	0.0002* *
TEMP	5	177.475331	35.495066	1.94	0.1430*
INCONC$_{Cu}$	5	493.307561	98.661512	5.40	0.0043* *
TIME	5	26.316841	5.263368	0.29	0.9127 (ns)
(Cu^{2+} +Pb^{2+}) system					
PH	5	94.874263	18.974853	3.43	0.0270 *
TEMP	5	57.470769	11.494154	2.08	0.1219*
INCONC$_{Cu}$	5	324.332286	46.866457	8.46	0.0004* *
TIME	5	7.618986	1.523797	0.028	0.9200 (ns)

*Table 17. Analysis of Variance (ANOVA) for each factor of the CCD for Cu in the mono-Cu system (at a 0.-500 mg/L INCONC$_{Cu}$ range). Confidence level: ns (not significant), * : $p< 0.15$,** : $p < 0.015$ in mono-Cu system and (Cu + Pb) system.*

Then three RSEG procedures including only the combination of two significant factors were run to determine the coefficients of the regression equation.

➢ Study of the response surfaces

The response surfaces for the effect of the combined factors (INCONC$_{Cu}$ and PH, TEMP and PH, INCONC$_{Cu}$ and TEMP) on copper uptake in mono-Cu^{2+} are shown in Figure 10, 11 and 12, respectively. Similarly, Figures 13, 14 and 15 represent the influence of the combined factors (INCONC$_{Cu}$ and PH, TEMP and PH, INCONC$_{Cu}$ and TEMP) on copper uptake in the (Cu^{2+} + Pb^{2+}) system.

INCONC$_{Cu}$ and PH interaction:

The response surface for the mono-Cu^{2+} system, shown in Figure 10, exhibits the same shape than the one for the mono-Cu^{2+} system with an copper initial concentration range of 0 – 20mg/L. The predicted copper uptake by *Palmaria palmata* ranged from about 0 to 43.2 mg/g depending on the pH (PH) and the copper initial concentration (INCONC$_{Cu}$). A concomitant increase of PH and INCONC$_{Cu}$ will increase the predicted uptake because of the quite high value of the bilinear term PH* INCONC$_{Cu}$ in the regression equation. The highest predicted value (q_{Cu} = 43.2 mg/g) was obtained for PH=6 and INCONC$_{Cu}$ =500 mg/L.

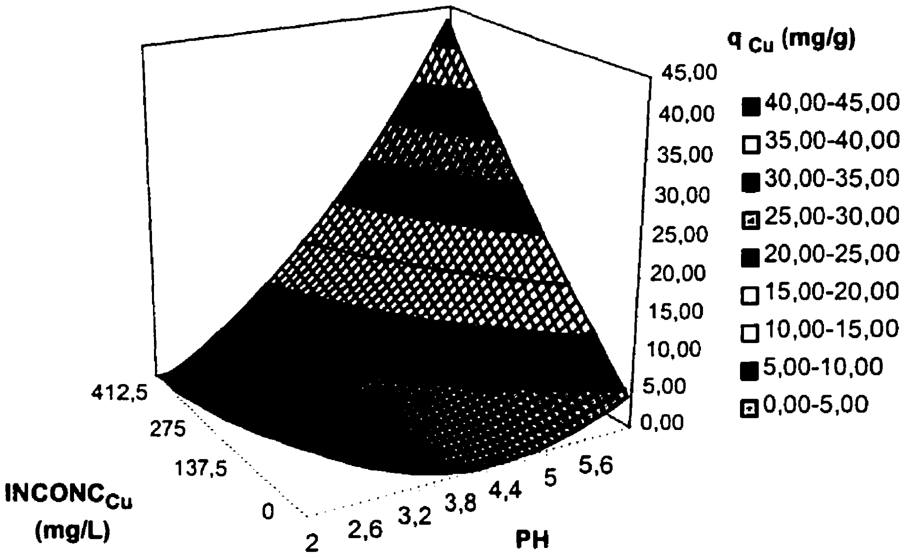


Figure 10. Response surface representing the regression equation: q_{Cu} = 6.66077+10.05833 PH + 7.5 INCONC$_{Cu}$+ 6.14885 $(PH)^2$ + 11.935 PH INCONC$_{Cu}$ + 0.89385 $(INCONC_{Cu})^2$ where pH (PH) and intial copper concentration (INCONC$_{Cu}$) have a significant effect on copper uptake q_{Cu} in the mono-Cu^{2+} system. The order of the legend items directly follow the order in which they appear in the figure.*

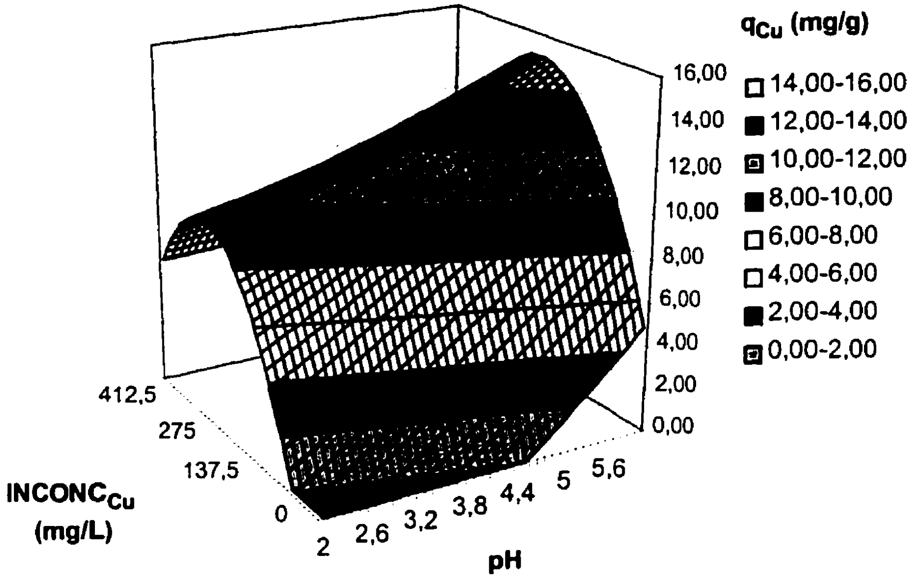


Figure 11. Response surface representing the regression equation : $q_{Cu} = 11.24827 + 3.53833\ PH + 4.03917\ INCONC_{Cu} + 0.23947\ (PH)^2 - 2.7825 PH^{*}\ INCONC_{Cu} - 8.85553\ (^{*}\ INCONC_{Cu})^2$ *where pH (PH), and intial copper concentration (INCONC$_{Cu}$) have a significant effect on copper uptake in the (Cu^{2+} + Pb^{2+}) system. The order of the legend items directly follow the order in which they appear in the figure.*

The response surface for the (Cu^{2+} + Pb^{2+}) system is represented in Figure11. The term PH* INCONC$_{Cu}$ has a lower coefficient in the binary system than in the mono-Cu system. In addition, the quadratic effect $(INCONC_{Cu})^2$ of the binary system was higher in terms of absolute values. This characteristic explains the parabolic contour form of the response surface shown in Figure 11. The response surface of Figure 11 also showed that the effect of changes in pH was greatest within the copper initial concentration range 200-400 mg/L. The highest predicted copper uptake ($q_{Cu} = 15.06$ mg/g) was obtained for PH = 6 and INCONC$_{Cu}$ =275 mg/L. The corresponding value in the mono-Cu system was $q_{Cu} = 24.82$ mg/g.

It should be mentioned here that, in the binary system, at pH = 6 and INCONC$_{Cu}$ =250 mg/L, the observed value was q_{Cu} = 14.65 mg/g, which agreed with the predicted value. On the other hand, in the mono-Cu system, the actual value for pH = 6 and INCONC$_{Cu}$ =250 mg/L, TEMP = 23 °C and TIME = 90 min (experiment N = 18) was $q_{Cu} = 14.67$ mg/g, which is lower than the predicted value. The large difference between the observed and predicted value is due to the lack of fit of the mono-Cu model for the $INCONC_{Cu}$ range of 0-500 mg/L, as previously discussed in section 4.5.2.1.

The mono-copper system and the binary system can be compared quantitatively in Figures 10 and 11. At low pH (from 2 to 3.5), the copper uptake in the mono-Cu^{2+} system was comparable to the one in the binary system, at any $INCONC_{Cu}$, and never exceeded 11 mg/g. At higher pH (from 3.5 to 6), the difference in uptake depends on the $INCONC_{Cu}$.

For $0<INCONC_{Cu}<150$ mg/L, the copper uptake was similar in both systems; the highest value being 15 mg/g. The uptake was generally lower in the upper part of the range in the binary system $150<INCONC_{Cu}<500$ mg/L. The difference between systems was more pronounced at higher pH. For example, in the pH range 5.5-6 and for $INCONC_{Cu}$, ranging from 425 mg/L to 500 mg/L, the copper uptake was predicted to vary from 7.13 mg/g to 11.56 mg/g in the binary system and from 29.62 to 43.2 mg/g for the mono-copper system. Overall, a 75% decrease in the copper uptake could be observed when copper was in the presence of lead. The inhibition of copper sorption is possibly due to competition between copper and lead for the binding sites on *Palmaria palmata*. This decrease is all the more sensitive at higher initial copper concentrations since the binding sites might become gradually "saturated".

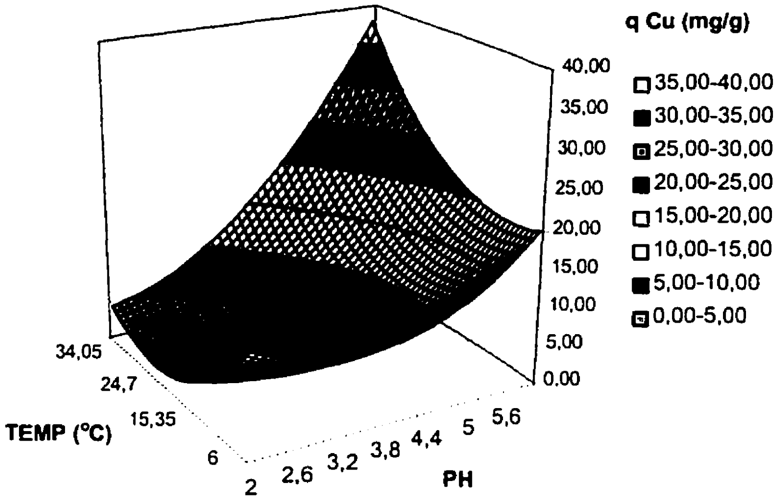


Figure 12. Response surface representing the regression equation: $q_{Cu} = 5.40538+10.05833\ PH + 2.14167\ TEMP + 6.67192\ (PH)^2 + 6.74\ PH*TEMP + 6.85692\ (TEMP)^2$ where pH (PH) and temperature (TEMP) have a significant effect on copper uptake q_{Cu} in the mono-Cu^{2+} system. The order of the legend items directly follow the order in which they appear in the figure.

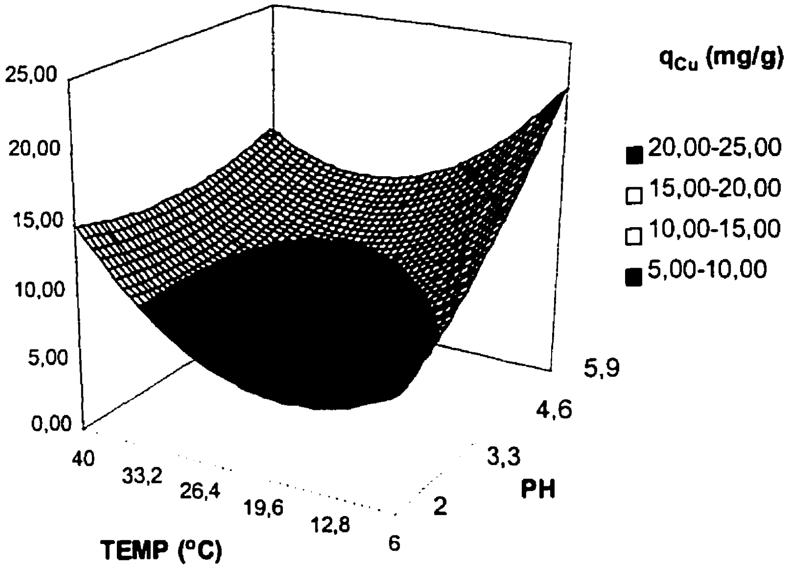


Figure 13. Response surface representing the regression equation: $q_{Cu} = 8.27596+3.535833\ PH + 0.0825\ TEMP + 1.47793\ (PH)^2 - 3.2825 PH*\ TEMP + 5.26293\ (TEMP)^2$ where pH (PH), and temperature (TEMP) have a significant effect on copper uptake q_{Cu} in the $(Cu^{2+} + Pb^{2+})$ system. The order of the legend items directly follow the order in which they appear in the figure.

TEMP and PH interaction

According to the regression equations in Figures 12 and 13, the effect of the quadratic term $(PH)^2$ on the response q_{Cu} is important in both systems. This explains the hollow shapes of the two response surfaces. In addition, at low pH (pH=2 to pH=3), the effect of a temperature change was negligible in both systems. Figure 12 shows that from a pH of about 5.5, the predicted copper uptake increased proportionally to the temperature. For instance, at pH=5.5, an increase from 20 to 40°C, caused an increase in copper uptake from 20.mg/g to 38 mg/g. The highest predicted copper uptakes in the Cu-mono system were obtained in the pH range 5.5-6 and for a TEMP ranging from 36.5 to 40°C. These highest copper uptakes were predicted to range from 26.85 mg/g to 37.87 mg/g. Under the same conditions of PH and TEMP in the binary system, the predicted copper uptake ranged from 13.22 to 15.35 mg/g, or half of that in the single-metal system. However, the binary system showed higher predicted copper uptakes at low temperatures (about 6 °C) and high pH (about 6). For instance, in the pH range 5.5-6 and for a TEMP range of 6 to 9.5 °C, the copper uptake was predicted to range from 17 mg/g to 21.8 mg/g.

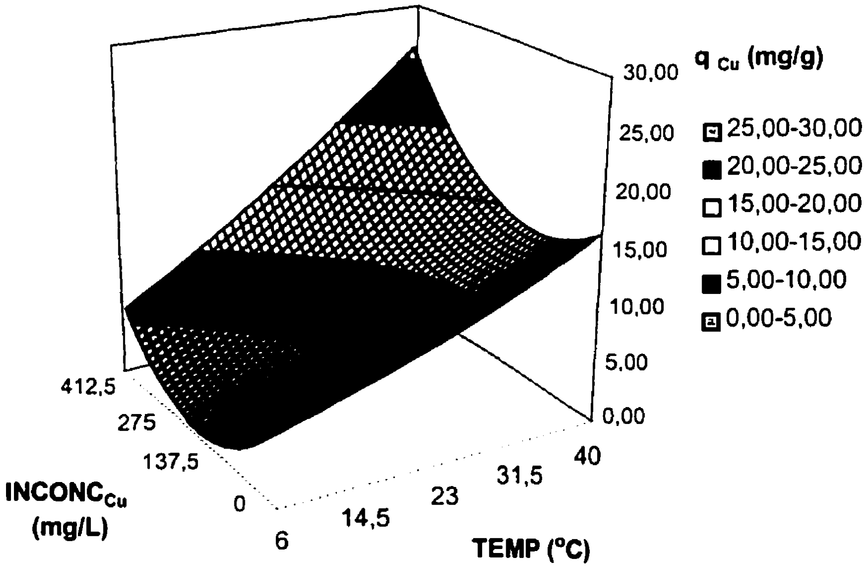


Figure 14. Response surface representing the regression equation: $q_{Cu} = 6.61808 + 7.5\ INCONC_{Cu} + 2.14167\ TEMP - 0.91163\ (INCONC_{Cu})^2 + 2.54\ INCONC_{Cu} * TEMP + 6.35163\ (TEMP)^2$ *where temperature (TEMP) and intial copper concentration ($INCONC_{Cu}$) have a significant effect on copper uptake q_{Cu} in the mono-Cu system. The order of the legend items directly follow the order in which they appear in the figure.*

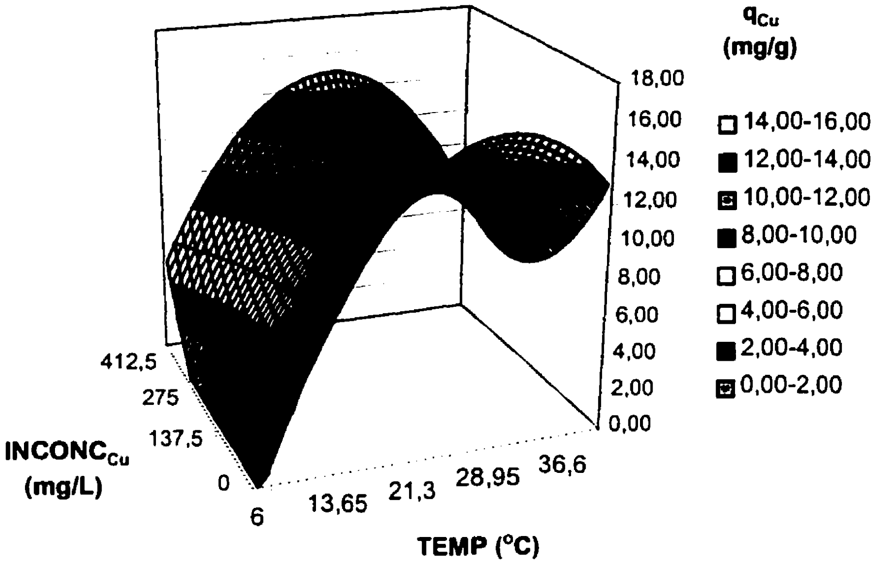


Figure 15. Response surface representing the regression equation: $q_{Cu} = 10.37481 + 4.03917\ INCONC_{Cu} + 0.0825\ TEMP - 8.49159\ (INCONC_{Cu})^2 - 2.7775\ INCONC_{Cu}*TEMP + 4.38841\ (TEMP)^2$ *where temperature (TEMP) and intial copper concentration (*$INCONC_{Cu}$*) have a significant effect on copper uptake* q_{Cu} *in the (*$Cu^{2+} + Pb^{2+}$*) system. The order of the legend items directly follow the order in which they appear in the figure.*

INCONC$_{Cu}$ and TEMP interaction

The effects of copper initial concentration and temperature in the mono-Cu^{2+} system are shown in Figure 14. The predicted copper uptake was affected essentially by the linear INCONC$_{Cu}$ and TEMP factors, and the quadratic term $(INCONC_{Cu})^2$, which exhibited the highest coefficient in the regression.equation. For a given INCONC$_{Cu}$, q_{Cu} increased proportionally to the temperature. As observed in Figure14, the response surface reached a maximum (about 26.06 mg/g) at INCONC$_{Cu}$ = 500 mg/L at a temperature of 40 °C.

In the binary system, INCONC$_{Cu}$ and TEMP affected the predicted copper uptake in a more complex manner. Similarly to the single metal system, q_{Cu} increased gradually with the temperature up to about 20°C where it approached a saddle point (Figure 15). Within the ranges 20 to 30°C 125 to 350 mg/l, q_{Cu} was stabilized in the range 10-12 mg/g. The highest predicted uptakes (from 14 to 16 mg/g) were obtained at moderate temperatures (20 to30°C) for either low $\text{INCONC}_{\text{Cu}}$ levels (0 to 25 mg/L) or high $\text{INCONC}_{\text{Cu}}$ levels (475 to 500 mg/L). When compared to the mono-Cu^{2+} system, the decrease in the maximum predicted copper uptake was less than twice.

These results indicate an apparent competition between copper and lead in all of the studied two-factor interactions. The impact of competition on lead uptake is discussed below.

4.5.3. Comparison of lead uptake in a mono and binary systems

4.5.3.1. *Adequacy of the fitted model*

Table 18 shows that the models of lead uptake in a mono and a binary system involving copper both suffered from LOF.

Sources of variation	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
Mono-Pb^{2+} system					
Lack of fit	10	55.287917	5.528792	10.97	0.0042
Pure error	6	3.023571	0.503929		
Total error	16	58.311488	3.644468		
Total model	14	528.47319	-	10.36	0.0000
(Pb^{2+} + Cu^{2+}) system					
Lack of fit	10	18.452917	1.845292	3.57	0.0662
Pure error	6	3.097143	0.5161190		
Total error	16	21.550060	1.346879		
Total model	14	201.30365	-	10.68	0.0000

Table 18. Analysis of Variance (ANOVA) for the quadratic model for Pb^{2+} (at a0-200 mg/L $\text{INCONC}_{\text{Pb}}$ range) in the mono-Pb^{2+} system (R-square = 0.9006) and in (Cu^{2+} + Pb^{2+}) system (R-square = 0.9033).

Table 18 also shows that, not considering LOF, the models explained a significantly greater portion of the variability than could be due to error (R^2 = 0.9006 for the mono-Pb^{2+} system, and R^2 = 0.9033 for the binary system). Nevertheless, the models should be viewed with extreme caution as explained in the previous section.). Although the R^2 are high, the lack of fit is an indication that the presumed model does not describe the true shape of the surface.

Furthermore, as shown in Appendix XII and XVI, the largest differences between predicted and observed values were obtained for experiments N = 7, 8, 11, 12, 16, 18 and 21 and N = 9 to 12, 15, 16, and 18 to 20 for lead in the mono-system and in the binary system, respectively.

4.5.3.2. Optimization of the parameters

The influence of the four factors (pH, temperature, initial concentration, and contact time) on the lead sorption was studied in the mono-metal solution and in the binary system. The results of the canonical analysis generated from PROC RSREG of the SAS program (SAS, 1998) are reported for the mono-Pb^{2+} system in Appendix XIII and for the (Pb^{2+}+ Cu^{2+}) system in Appendix XVII.

The eigenvalues were found to be of mixed signs in both systems. Again, the stationary point of the fitted surface is a saddle point and there is no single maximum or minimum in either system with respect to lead uptake.

4.5.3.3. Study of the two-factor interactions

- Determination of the most significant factors

Table 19 indicates that PH and $INCONC_{Pb}$ were significant in both systems. In the mono-Pb system, neither TEMP nor TIME were significant, whereas TEMP was significant in the binary system.

Factors	Degrees of freedom	Sums of squares	Mean squares	F-ratios	Prob > F
Mono-Pb^{2+} system					
PH	5	357.454984	71.490997	19.62	0.0000* *
TEMP	5	20.635391	4.127078	1.13	0.3832 (ns)
$INCONC_{Pb}$	5	204.341535	40.868307	11.21	0.0001* *
TIME	5	4.021154	0.804231	0.22	0.9484 (ns)
(Cu^{2+} +Pb^{2+})					
PH	5	69.841439	13.968288	10.37	0.0001 *
TEMP	5	91.822325	18.364465	13.63	0.0000* *
$INCONC_{Pb}$	5	46.671492	9.334298	6.93	0.0013* *
TIME	5	1.660916	0.332183	0.25	0.9355 (ns)

*Table 19. Analysis of Variance (ANOVA). for each factor of the CCD for Pb^{2+} at a0-200 mg/L $INCONC_{Pb}$ range) in the mono- Pb system and in the (Cu^{2+} + Pb^{2+}) system. Confidence level: ns (not significant), * : $p< 0.15$,** : $p < 0.015$.*

Table 19 shows that PH and INCONC$_{Pb}$ had a significant effect on the lead uptake by *Palmaria palmata* in both systems. TEMP and TIME were not significant in the mono-Pb^{2+} system and were eliminated from the model equation. TIME was the only non-significant factor in the binary system.

- ➢ Study of the response surfaces

INCONC$_{Pb}$ and PH interaction

The response surfaces for the combined effects INCONC$_{Pb}$ and PH on lead uptake in the mono-Pb^{2+} and in the binary (Pb^{2+} + Cu^{2+}) system are shown in Figures 16 and 17, respectively.

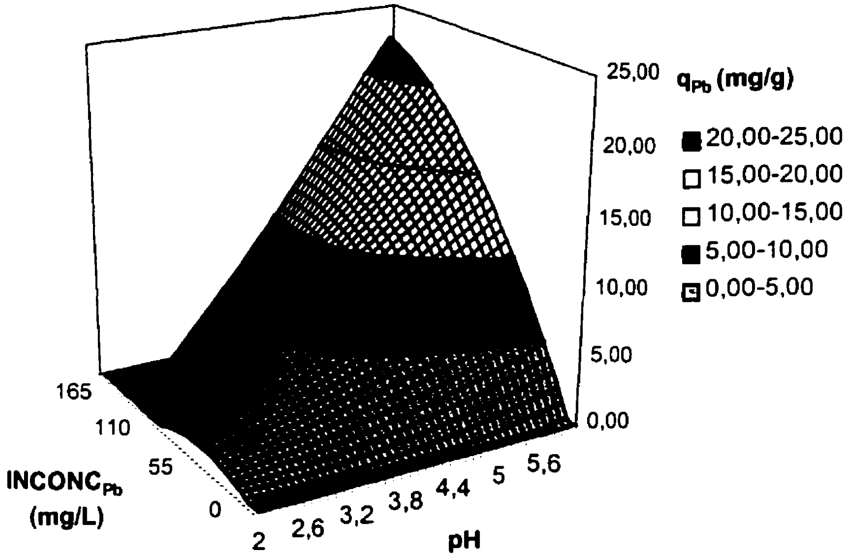


Figure 16. Response surface representing the regression equation: $q_{Pb} = 6.86923 + 7.13333\ PH + 4.45\ INCONC_{Pb} + 0.88365\ (PH)^2 + 7.1\ PH^* \ IINCONC_{Pb} - 3.84135\ (INCONC_{Pb})^2$ *where pH (PH) and intial lead concentration (INCONC$_{Pbw}$) have a significant effect on lead uptake q_{Pb} in the mono-Pb^{2+} system. The order of the legend items directly follow the order in which they appear in the figure.*

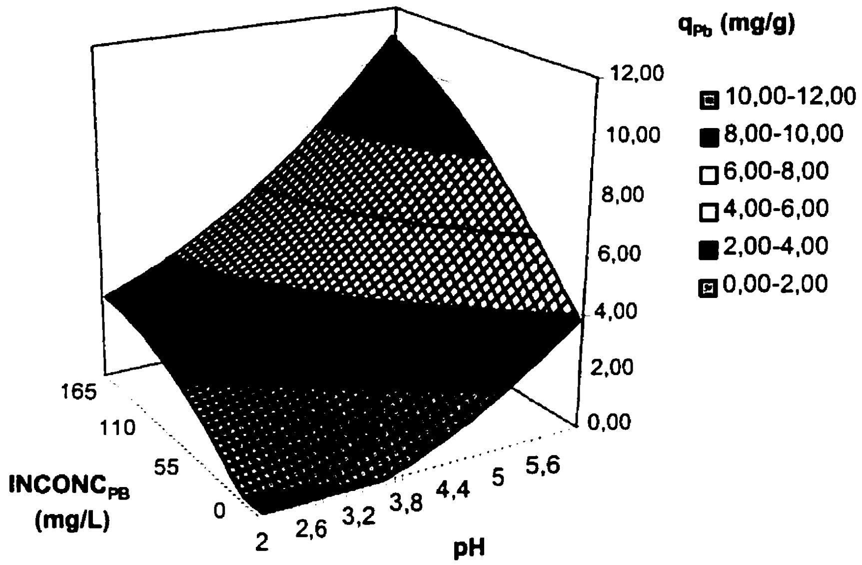


Figure 17. Response surface representing the regression equation: $q_{Pb} = 4.13654+3.15833\ PH + 2.70833\ INCONC_{Pb} + 1.02644\ (PH)^2 + 0.825\ PH^* INCONC_{Pb} - 0.92356\ (INCONC_{Pb})^2$ *where pH (PH) and lead intial concentration* ($INCONC_{Pb}$) *have a significant effect on lead uptake* q_{Pb} *in the* ($Pb^{2+} + Cu^{2+}$) *system. The order of the legend items directly follow the order in which they appear in the figure.*

The response surface for the mono-Pb^{2+} system exhibits approximately the same shapes as those of the mono-Cu^{2+} system of section 4.5.2 and (Pb^{2+}+Cu^{2+}) system. A concomitant increase of PH and $INCONC_{Pb}$ increased the predicted uptake. However, the highest predicted lead uptake, obtained at pH = 6 and $INCONC_{Pb}$ = 200 mg/L, was 22.59 mg/g for the mono-Pb system and 10.93 mg/g for the binary system. The actual values shown in Appendix XII indicate that the highest lead uptake in the mono system was 16.75 mg/g, and was observed for pH = 6, a temperature of 23 °C, an initial lead concentration of 100 mg/L and a contact time of 90 min. On the other hand, Appendix XVI shows that the corresponding observed lead uptake in the binary system was 6.85 mg/g, confirming the predicted reduction in the lead uptake in presence of a co-cation. As discussed for the copper in section 4.5.2, this decrease is probably due to the competition between copper and lead for the sorption sites on *Palmaria palmata.*

TEMP and PH interaction

Figure 18 represents the response surface for the effect of TEMP and PH on the predicted lead uptake in the binary (Pb^{2+} + Cu^{2+}) system. The high value for the coefficient of the quadratic term $(TEMP)^2$ in the regression equation explains the hollow curvature in front of the TEMP axis. At 40°C, the response surface reached a maximum at a pH = 6, and dropped rapidly with decreasing pH and decreasing TEMP. Due to the high coefficient of the bilinear term PH*TEMP of the regression equation, the response of the predicted lead uptake was largely affected by both PH and TEMP effects.

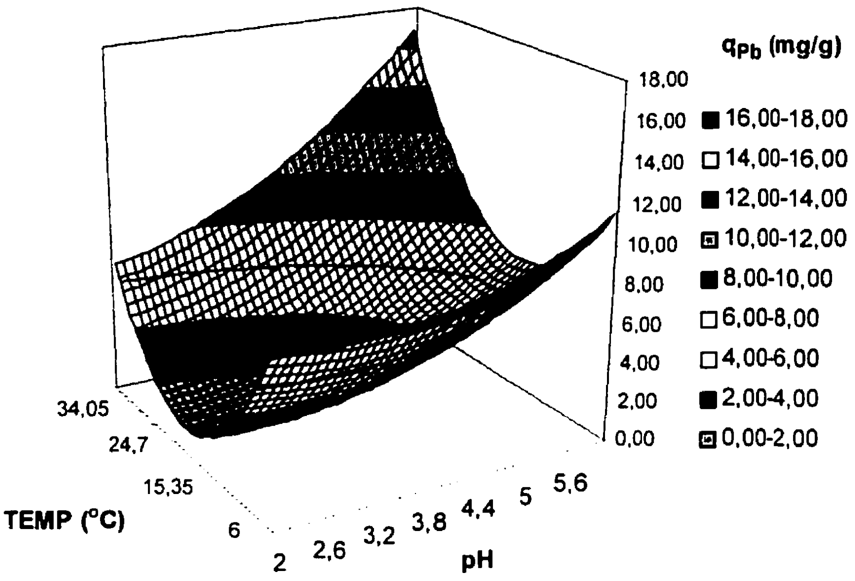


Figure 18. Response surface representing the regression equation: q_{Pb} = 2.50961+3.15833 PH + 0.75 TEMP + 1.70433 $(PH)^2$ + 1.85 PH TEMP + 6.80433 $(TEMP)^2$ where pH (PH) and temperature (TEMP) have a significant effect on lead uptake q_{Pb} in the (Pb^{2+} +Cu^{2+}) systemThe order of the legend items directly follow the order in which they appear in the figure.*

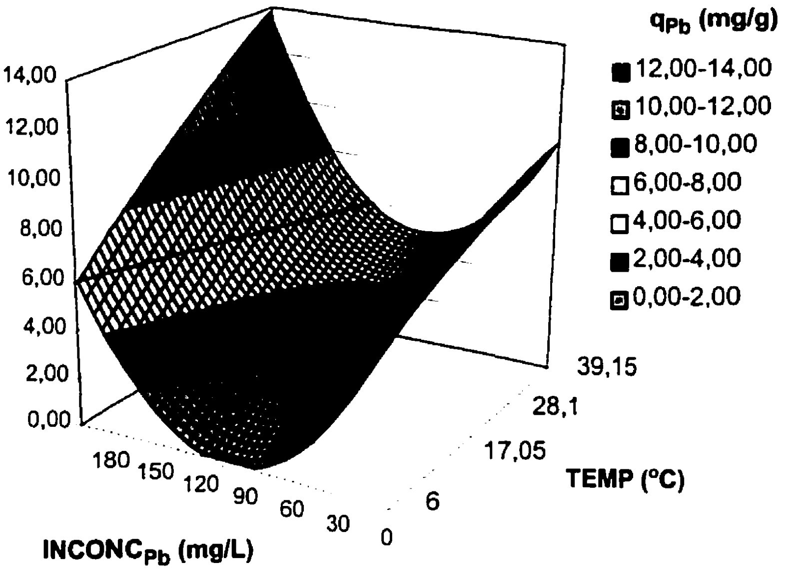


Figure 19. Response surface representing the regression equation: $q_{Pb} = 2.95961 + 0.75\ INCONC_{Pb} + 2.70833\ TEMP + 6.61683\ (INCONC_{Pb})^2 + 1.2\ INCONC_{Pb} * TEMP - 0.43317\ (TEMP)^2$ *where temperature (TEMP), and lead intial concentration* ($INCONC_{Pb}$) *have a significant effect on lead uptake* q_{Pb} *in the* ($Cu^{2+} + Pb^{2+}$) *system. The order of the legend items directly follow the order in which they appear in the figure.*

$INCONC_{Pb}$ and TEMP interaction

The effects of initial lead concentration and temperature in the binary (Cu^{2+}+ Pb^{2+}) system are shown in Figure 19. The predicted lead uptake was affected essentially by the linear TEMP factor, and the quadratic term $(INCONC_{Pb})^2$ which exhibited the highest coefficient in the regression. In the $INCONC_{Pb}$ range 80-120 mg/L, the variation in the predicted lead uptake was small, ranging from 0 to about 6 mg/g. At higher $INCONC_{Pb}$ (125 to 200 mg/L), q_{Pb} increased with increasing temperatures. As observed in Figure 19, the response surface reached a maximum (13.80 mg/g) at $INCONC_{Pb}$ = 200 mg/L at a temperature of 40 °C.

The competition between copper and lead is again apparent in all of the studied two-factor interactions. The next step will aim at evaluating the impact of this competition on the lead uptake.

4.5.4. Quantitative comparison of the copper and lead sorption with 2-D representations

Equations derived from the Central rotatable Composite Design (CCD) model could be adequately represented by three-dimensional response surfaces, as described in sections 4.4 and 4.5. Only Three-dimensional diagrams could represent the evaluation of the influence of two factors on the metal sorption. However, selected cuts through the 3-D diagrams presented from Figure 10 to Figure 19 better reveal the quantitative trends observed in the two-metal uptake systems. Furthermore, the effect of the presence of a second metal can be more easily quantified from the two-dimensional diagrams.

The figures in sections 4.5.2 and 4.5.3 showed the effects of two combined factors on the metal uptake. The following sets of diagram actually represents sections through the corresponding 3-D uptake surfaces by planes at a constant pH, metal initial concentration or temperature. In this case, one factor becomes a parameter of the 2-D sorption plot where the metal uptake remains the studied variable (or response) on the vertical axis, as usual. In this section, the individual effect of the pH, metal initial concentration, and temperature on the metal sorption in mono-metal and binary systems are studied.

4.5.4.1. *Influence of the pH*

Figure 20 results from the cut of the 3-D diagrams representing the influence of the INCONC-PH interactions on metal sorption at a fixed $INCONC_{Cu}$ = 500 mg/L (corresponding to a $INCONC_{Pb}$ = 200 mg/L). First, it should be mentioned that when copper uptake and lead uptake in their respective mono-metal system were added, the resulting cumulative metal uptake was always higher than the total metal uptake in the binary system (except for pH =3). For instance the total metal uptake was reduced by 2, 3 and 3.6 times at pH = 4, 5, and 6, respectively.

In the mono-metal systems, it was generally observed that an increasing pH results in an increase in the metal uptake. For instance, an increase from pH = 3 to pH = 4 increased the copper uptake 1.7 times (respectively it increased the lead uptake 12 times). A further increase from pH = 3 to pH = 6 increased the copper uptake 7 times (respectively it increased the lead uptake 38 times).

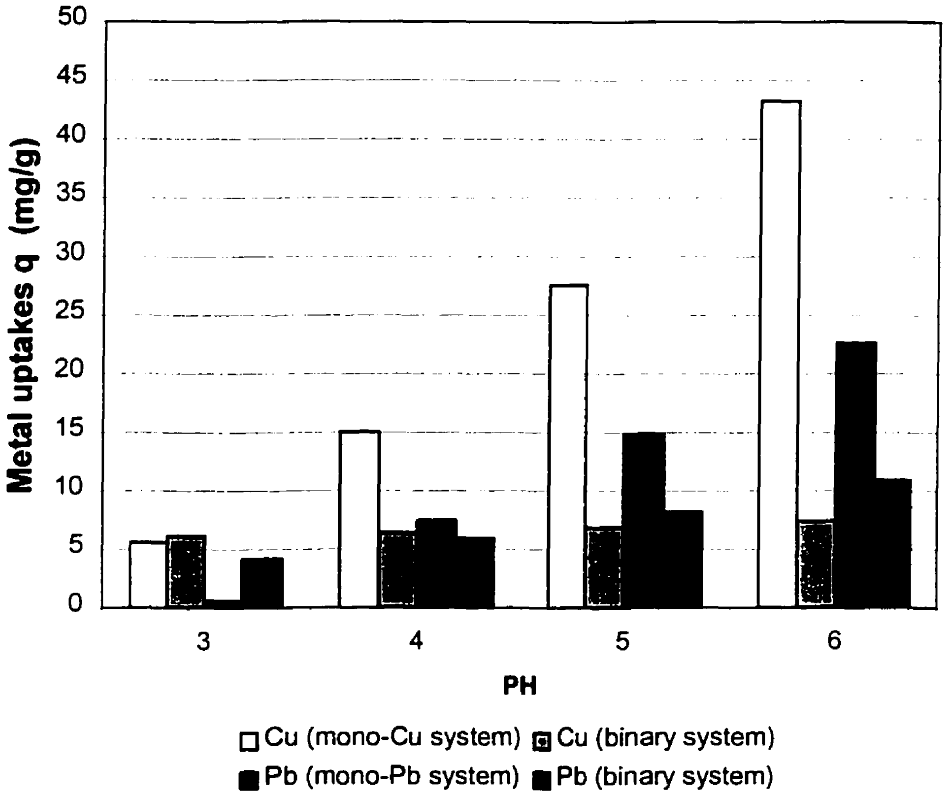


Figure 20. Influence of the pH on the copper uptake (respectively lead uptake) in mono-copper system (respectively mono-lead system) and in binary (Cu^{2+} + Pb^{2+}) system at $INCONC_{Cu}$ = 500 mg/L (respectively $INCONC_{Pb}$ = 200 mg/L).

The actual values for the mono-metal systems shown in Appendix X and XII confirmed this trend. For instance, at an initial copper concentration of 250 mg/L, for a contact time of 90 min and for a temperature of 23 °C, the observed copper uptake doubled (from 6.57 mg/g to 14.65 mg/g when the pH increased from 2 to 6). Similarly, at an initial lead concentration of 100 mg/L, for a contact time of 90 min and a temperature of 23 °C, the observed lead uptake at pH = 6 (16.75 mg/g) was seven times greater than the observed copper uptake at pH = 2 (2.25 mg/g).

When the biosorbent was exposed to the binary system, the predicted copper uptake was reduced by 57 % and 83% at a pH of 4 and 6, respectively, in comparison with the mono-copper system. When compared to the mono-lead system, the predicted lead uptake was reduced by 21% and 52% at a pH of 4 and 6 respectively.

All in all, in the binary system, copper uptake by *Palmaria palmata* was more affected by the presence of a co-cation than lead uptake, especially at high pH.

4.5.4.2. Influence of the metal initial concentration

Section 4.5.4.1 indicated that the highest predicted metal uptake was observed at pH = 6. In addition, the competition between Cu and Pb was the most significant at this pH. Figure 21 results from the "slicing" of the 3-D representation of the INCONC-PH interactions on metal uptake in mono-metal and binary system at pH = 6.

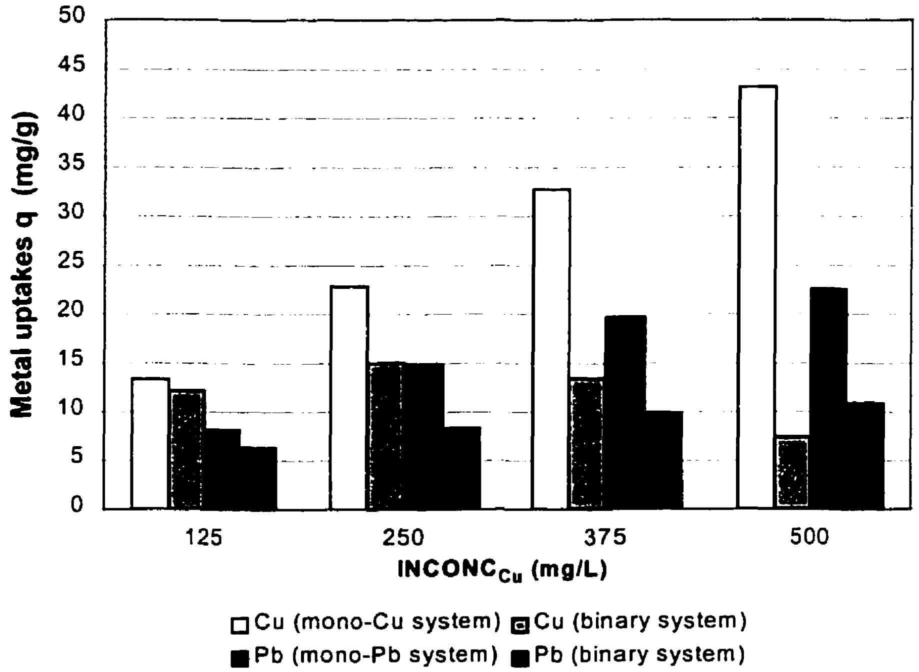


Figure 21. Influence of the initial metal concentration on the copper uptake (respectively lead uptake) in mono-copper system (respectively mono-lead system) and in binary (Cu^{2+} + Pb^{2+}) system at pH = 6.

As shown in Figure21, and as observed in section 4.5.4.1, the total amount of metal sequestered by *Palmaria palmata* was always lower when the biosorbent was exposed to the (Cu^{2+} + Pb^{2+}) binary system than when the metal was present alone. This decrease in the total uptake was proportional to the increases in $INCONC_{Cu}$ or $INCONC_{Pb}$ for the respective cases. For instance, the total metal uptake decreased by 100% at $INCONC_{Cu}$ = 125 mg/L ($INCONC_{Pb}$ = 50 mg/L), by 1.7 times at $INCONC_{Cu}$ = 250 mg/L ($INCONC_{Pb}$ = 100 mg/L) and by 3.6 times at $INCONC_{Cu}$ = 500 mg/L ($INCONC_{Pb}$ = 200 mg/L).

The predicted uptakes of copper and lead in mono-metal solution increased proportionally to the increases in $INCONC_{Cu}$ and $INCONC_{Pb}$ respectively. For example, the predicted copper uptake increased of 71% when $INCONC_{Cu}$ increased from 125 mg/L to 250 mg/L, while the predicted lead uptake increased of 83%, when $INCONC_{Pb}$ increased from 50 mg/L 100 mg/L. A further increase of $INCONC_{Cu}$ to 375 mg/L and 500 mg/L (respectively of $INCONC_{Pb}$ to 150 mg/L, and 200 mg/L) increased q_{Cu} (respectively q_{Pb}) by 1.5 and 2 times respectively. The actual values for the mono-metal systems are shown in Appendix IX and XI and indicated the same trend. For instance, at pH = 5, for a contact time of 130 min and for a temperature of 31.5 °C, the observed copper uptake increased from 9.89 mg/g to 28.28 mg/g when the initial copper concentration increased from 125 to 375 mg/L. Similarly, the observed lead uptake increased from 5.55 mg/g to 12.45 mg/g when the lead initial concentration increased from 50 to 150 mg/L.

In the binary (Cu^{2+} + Pb^{2+}) system, the inhibitory effect of the presence of a secondary metal depends also on metal initial concentration. As an illustration, q_{Cu} decreased by 9% when in presence of lead, for $INCONC_{Cu}$ = 125 mg/L. Then the decrease in the q_{Cu} (34%, 59% and 83% for a $INCONC_{Cu}$ of 250, 375 and 500 mg/L respectively) was proportional to $INCONC_{Cu}$. The predicted copper uptake became gradually smaller, at higher initial copper concentrations, possibly due to the "saturation" of sites within *Palmaria palmata*.

The predicted lead uptakes were also decreased due to the presence of a co-cation in the binary (Cu^{2+} + Pb^{2+}) system but to a lesser extent. The decrease in q_{Pb} depended also on the metal initial concentration. For example, the decrease in q_{Pb} was 22%, 50%, and 52% for $INCONC_{Pb}$ of 50, 150 and 200 mg/L, respectively. At high $INCONC_{Pb}$, the decrease in predicted lead uptake tended to stabilize at a value of 50%, which would indicate that perhaps some specific sites for lead were not yet saturated.

Overall, when exposed to the binary system, the biosorbent demonstrated a preference for sorbing Pb^{2+} over $Cu.^{2+}$ At $INCONC_{Cu}$ of 125 mg/L and 250 mg/L, only 35% of the total uptake were due to copper uptake. At respective $INCONC_{Cu}$ of 375 mg/L and 500 mg/L, 42% and 60% of the total uptake were due to lead uptake. However, it should be recalled that the initial copper concentration is higher than that of lead in the binary system, the ratio $INCONC_{Cu}$ / $INCONC_{Pb}$ being 2.5 to reflect the ratio of atomic weights. It would therefore be more accurate to compare the ratio q_{Cu} / q_{Pb} for different $INCONC_{Cu}$. With increasing $INCONC_{Cu}$ (from 125 to 500 mg/L), the ratio q_{Cu} / q_{Pb} decreased from 1.93 to 0.68, confirming the preferred sorption of lead on copper by the biosorbent. The actual values shown in Appendix XIV and XVI exhibited the same trend in the binary system. For instance, at pH = 5, for a contact time of 130 min, and for a temperature of 31.5 °C, the ratio q_{Cu} / q_{Pb} decreased from 2.02 to 0.93 as initial copper concentration increased from 125 to 375 mg/L.

4.5.4.3. *Influence of the temperature*

As seen in section 4.5.4.2, the maximum copper and lead uptakes in mono and binary system were obtained at pH = 6, for a $INCONC_{Cu}$ of 500 mg/L (and a $INCONC_{Pb}$ of 200 mg/L, respectively). Under these conditions, the competition between copper and lead was the most significant. The effect of temperature on copper and lead sorption in mono and binary systems are represented in Figure 22.

The 2-D diagram comes from the cut through the 3-D diagram corresponding to the TEMP-PH interactions at pH = 6, and $INCONC_{Cu}$ = 500 mg/L. Figure 22 shows that at a TEMP of 6 °C, the cumulative metal uptake amount in mono- systems was approximately equal to the total uptake amount in the (Cu^{2+} + Pb^{2+}) binary system. However, at TEMP of 23 °C, 31.5 °C, and 40°C, the total uptake in the binary system was half that in the mono-systems.

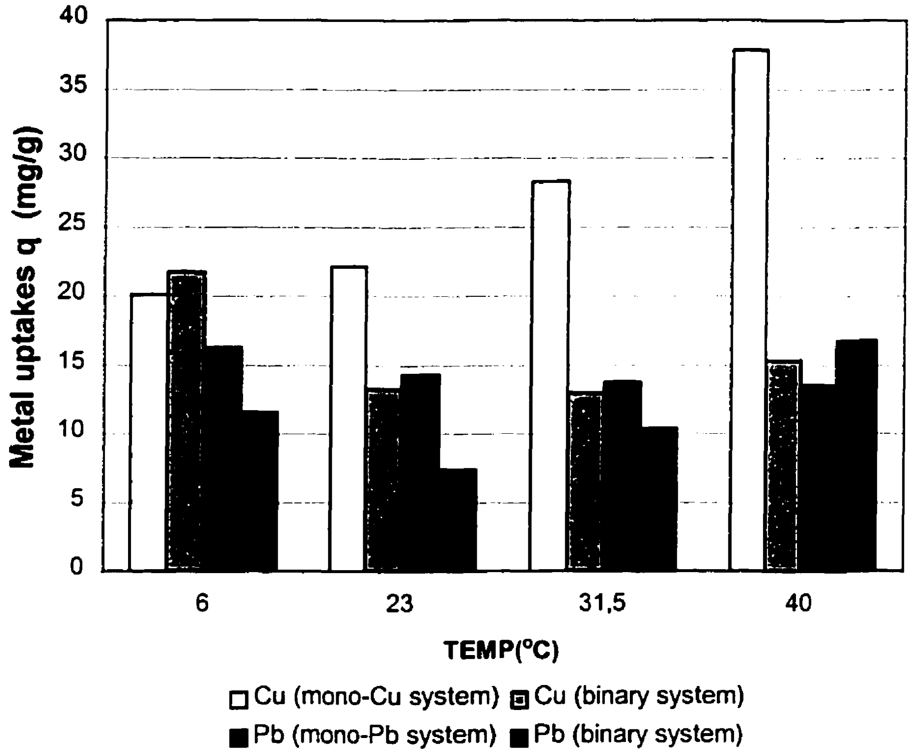


Figure 22. Influence of the temperature on the copper uptake (respectively lead uptake) in mono-copper system (respectively mono-lead system) and in binary (Cu^{2+} + Pb^{2+}) system at $INCONC_{Cu}$ = 500 mg/L (respectively $INCONC_{Pb}$ = 200 mg/L) and at pH = 6.

In the mono-metal solutions, it was observed that the copper uptake increased with the temperature, whereas the lead uptake decreased with the temperature. For example, when temperature increased from 6 °C to 23 °C, the predicted copper uptake q_{Cu} was increased by 10% (respectively the predicted lead uptake q_{Pb} was decreased by 10%). A further increase to 31.5 °C and 40 °C increased q_{Cu} by 41% and 88%, respectively, and reduced q_{Pb} by 15% and 17%, respectively. This trend was already observed in the one-factor at a time approach of section 4.3.4. Besides, when compared to the actual values of Appendix X, XII, XIV and XVI, the effect of the temperature on the observed copper and lead uptake is similar to the one observed for the predicted values. In the mono-metal systems, at pH = 4, and for a contact time of 90 min indeed, an increase from 6 °C to 40°C results in an increase from 6.43 mg/g to

9.40 mg/g for observed copper uptake, and in a decrease from 10.9 mg/g to 8.55 mg/g for observed lead uptake.

As mentioned in the literature review, copper binding was usually considered as an endothermic reaction (with a rising equilibrium constant correlated to an increasing temperature), while lead uptake rather belonged to exothermic reactions. In the (Cu^{2+} + Pb^{2+}) system, the mutual interaction between copper and lead on the metal sorption depended on the temperature. When compared to the copper uptake in the mono-copper system, the relative copper uptake of the binary system decreased relatively to increasing temperatures. However an exception could be noticed at low temperatures. At TEMP = 6 °C indeed, the predicted copper uptake in the binary system was similar to the one in the mono-copper system, probably due to the inhibition of uptake at low temperatures in both system. On the other hand, q_{Cu} decreased by 40%, 54% and 59% for a TEMP of 23 °C, 31.5 °C and 40 °C respectively, when in presence of Pb^{2+}.

The predicted lead uptake also decreased in presence of a co-cation in the binary (Cu^{2+} + Pb^{2+}) system but to a lesser extent. The decrease in q_{Pb} in the binary system compared to that of q_{Pb} in the mono-lead system depended also on the temperature. For instance, q_{Pb} decreased by 29%, 49%, and 25% for respective temperatures of 6 °C, 23°C and 31.5 °C. However, at TEMP = 40 °C, an increase of 24% could be observed, showing that the presence of copper enhanced the lead sorption on *Palmaria palmata* at high temperature.

As a general rule, mutual inhibition of the two metals on the uptake was observed. However, when exposed to the binary system, Pb^{2+} was preferably sorbed over Cu^{2+}, as it was demonstrated by the q_{Cu} / q_{Pb} ratio for different temperatures. In the binary system, the q_{Cu} / q_{Pb} ratio decreased gradually from 1.88 to 0.91 with increasing TEMP (from 6 °C to 40°C). This trend can be also observed in the actual data for the binary system: at pH = 4, and contact time = 90 min, the q_{Cu} / q_{Pb} ratio decreased from 1.13 to 0.2 while increasing the temperature from 6 °C to 40°C.

As a general rule, there was mutual inhibition of the uptake of both metals. However, when exposed to the binary system, Pb^{2+} was sorbed preferably over Cu^{2+}, as was demonstrated by the q_{Cu} / q_{Pb} ratios for different temperatures. In the binary system, the q_{Cu} / q_{Pb} decreased gradually from 1.88 to 0.91 with increasing TEMP. (from 6 °C to 40°C). This trend can be also observed in the actual data for the binary system: at pH = 4, and contact time = 90 min, q_{Cu} / q_{Pb} decreased from 1.13 to 0.2 as the temperature rose from 6 °C to 40°C.

Maximum inhibition of copper uptake should be expected at low temperatures, because of the endothermic characteristic of the reaction. This is the case in mono-copper system. However, there is an apparent contradiction in the binary system. This observation confirms the fact that the results in a binary system cannot be predicted on the basis of mono metal studies. Metal uptake studies in both mono and binary systems are required to permit conclusions.

4.5.4.4. Conclusion on the Copper / Lead competition

All things considered, 2-D diagrams were useful for studying the sorption behaviour of copper and lead in mono and binary systems. A mutual and unequal inhibitory effect of the presence of co-cations on the metal uptake could be observed. Uptakes of copper and lead were lower when present together in solution than when alone in solution. The extent of this inhibition depended on the physico-chemical parameters pH, metal initial concentration and temperature. Generally, the inhibition in the metal uptake was found to be the highest at pH = 6 and $INCONC_{Cu}$ = 500 mg/L (and $INCONC_{Pb}$ = 500 mg/L) for the two metals, and at a temperature of 40°C and 23°C for copper and lead respectively. Overall, in all cases, copper sorption capacity was reduced to a greater extent in the presence of lead, than was that of lead in the presence of copper.

Unequal inhibition was observed in numerous sorption studies. For instance, in a study of the effect of various co-ions (cadmium, copper, cobalt and nickel) on lead sorption by *S. cinnamoneum* and *P. chrysogenum* at pH = 4, Puranik and Packnikar. (1999) found that the presence of copper in a binary system resulted in the maximum inhibition of lead uptake. Furthermore, Kapoor et al. (1999) investigated the removals of heavy metal by the fungus *Aspergillus niger* in three binary systems including copper, cadmium and lead. They observed that the removals of copper, cadmium and lead were higher when they were present alone in aqueous solutions. In addition, lead uptake was found to be more sensitive to the presence of copper than to that of cadmium.

The extent of the metal uptake depends on the metal chemistry, affinity for binding sites and the type of metal binding. (Tzesos, 1990). The values of the Langmuir model constants were commonly used to predict the preferential sorption order of metals in multicomponent solutions. For instance, Puranik et al. classified the metal cations on the basis of Langmuir parameters evaluated in binary systems as following: high affinity (Pb^{2+}), intermediate affinity (Cd^{2+}, Cu^{2+} and Zn^{2+}) and low affinity (Ni^{2+} and Co^{2+}). Moreover, in the Langmuir equation, $q = (qmax *b\ C_f) / (1+b\ C_f)$, b is a constant related to energy of adsorption (mg/L). The higher are the values of b, the higher is the affinity for metal cations (Chong and Volesky, 1995). The values of the Langmuir model parameters were determined in mono-metal solutions in section 4.2.3. The value of b calculated for lead (0.102 L/mg) was higher than that for copper (0.060 L/mg), demonstrating the higher affinity of *Palmaria palmata* for the lead.

There are various reasons for the preferential affinity of the biosorbent for lead. They are listed in section 4.2.3 and include atomic weight, Pauling electronegativity, and ionic radius of the metal ion. However, the variation in the sorption preference could be mainly attributed to the difference in the covalent index. Nieboer and McBride (1973) suggested that the metal ions could be classified on the basis of their covalent indices (section 4.2.3) into two groups: class b ion and borderline ions. The higher the covalent index of a metal ion, the higher is its degree of class b character, and the higher is the binding strength with biological materials. More specifically, lead belonged to class b, while copper was classified as a borderline ion.

The precise nature of the mutual inhibition cannot be strictly defined from this study. However, various mechanisms of metal uptake have been proposed in the literature. For instance, Tobin et al. (1988) argued that direct competition for the uptake sites of *Rhizopus arrhizus* was the main mechanism involved in the binary system (Cd^{2+} + Zn^{2+}). But sorption studies on other binary systems such as (Ag^{+} + Cd^{2+}), revealed that a fraction of binding sites selectively bound particular cations, below saturation concentration ranges. Similarly, the results of the present study might not be only explained by direct competition between copper and lead for the same binding sites of *Palmaria palmata*. Indeed preferential uptake of lead was observed in the binary system at various copper initial concentrations. And as mentioned for the binary system in section 4.5.4.2, the decrease in the lead uptake was found to be independent of the co-cation concentration. These observations supported the hypothesis of the presence of specific binding sites.

5. SUMMARY AND CONCLUSIONS

5.1. Overall project goals

The primary goals of this project were to estimate the effectiveness of a dried alga, *Palmaria palmata*, to act as a biosorbent for heavy metals common to the printed wiring board manufacturing industry. This involved an investigation of the effect of various physico-chemical parameters such as initial metal concentration, contact time, pH and temperature on the metal uptake by *Palmaria palmata*. Secondary objectives included the application of a statistical design that would reduce the number of trials needed to measure the effects of changes in environmental factors and their mutual interactions on the biosorption, as well as to provide an idea of the optimal conditions for maximum uptake. These objectives were met by conducting batchwise experiments and modelling the experimental data with suitable isotherm models. The mutual interactions between the physico-chemical parameters, on the metal sorption by *Palmaria palmata*, were successfully described by the statistical Central Composite Design (CCD).

5.2. General sorption characteristics of *Palmaria palmata*

1) A cheap and abundant form of biomass, *Palmaria palmata* possesses a relatively high ability to remove copper and lead from aqueous solutions.
2) A biomass concentration of 1 g/L was found to give the highest sorption of copper from aqueous solutions.
3) The Langmuir and BET models were used to describe the sorption of Pb^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} on *Palmaria palmata*. The good fits of these models to the experimental data reflects that the sorption on *Palmaria palmata* was a multi - layer sorption, but was reduced to the Langmuir model when the limit of adsorption was a mono - layer.
4) The maximum sorption capacities q_{max} could be derived from the Langmuir model. The highest maximum sorption capacity q_{max} was 15.17 mg/g for lead and 6.65

mg/g for copper at a pH of 5.5-6. On the other hand, the highest observed uptakes were 17 mg/g for lead and 6.1 mg/g for copper at a pH of 5.5-6.

5) The affinity of metals for *Palmaria palmata* was found to decrease in the order: $Pb^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$.
6) The factors influencing copper and lead uptake were found to be contact time, pH, initial concentration and temperature.
7) Biosorption of copper and lead is a rapid process, 70% and 100% of the respective uptakes occurring within the first 10 minutes.

5.3. Statistical modelling

1) The Central rotatable Composite Design (CCD) was a powerful tool for describing factor interactions in the multifactor system. When studying the contact time, pH, initial concentration and temperature interactions on the copper and lead uptake in mono-metal and binary systems, the (CCD) model was generally significant. However, several of the models suffered from lack of fit, which could be due to the fact that some of the base relationships between the response (metal uptake) and predictor variables were neither linear nor 2nd degree, but rather logarithmic in nature.
2) There was no optimum combination of the four physico-chemical factors to obtain the highest metal uptake, in the studied factor spaces. Some saddle (or minimax) points were found.
3) The factors having a significant effect on the copper and lead uptake in mono-metal and binary systems were generally found to be initial metal concentration, pH and temperature.
4) Three-dimensional response surfaces (metal uptake as a function of the values of two physico-chemical factors) were successfully used in the evaluation of the effects of two combined factors on the one and two-metal sorption performance.
5) Two-dimensional sections of the 3-D uptake surfaces allowed quantitative estimation on the influence of one physico-chemical parameter on the metal uptake in mono-metal and binary systems, and on the metal uptake inhibition due to the influence of the other metal in the binary system.

6) The presence of a co-metal ion in the sorption system always lowered the uptake of the other metal, indicating an apparent case of sorption competition and mutual inhibition.
7) In the binary (Cu^{2+} + Pb^{2+}) system, the inhibitory effect of the presence of a secondary metal depended on the metal initial concentration, the temperature and the pH.
8) A mutual and unequal inhibitory effect of the presence of co-cations on the metal uptake could be observed when *Palmaria palmata* was exposed to the (Cu^{2+} + Pb^{2+}) binary system. Due to a lower affinity of copper for *Palmaria palmata*, the copper uptake was always reduced to a larger extent than the lead uptake by the presence of the secondary metal.
9) The hypothesis of specific binding sites for copper and lead was preferred to the assumption of direct competition for the same binding sites on *Palmaria palmata*.

6. RECOMMENDATIONS

1) This work was based on response surface methodology to examine the performance of the dried red algae, *Palmaria palmata*, at removing heavy metals from aqueous solutions, and to determine under what conditions of pH, temperature, initial metal concentration and biomass concentration the maximum uptake may be expected. This approach was used to examine the system with either one or two metals present. It was found that no maximum existed within the ranges of the used for the predictor variables. From the two-factor interaction 3-D diagrams (section 4.5), it could be generally observed that if the variables were increased together, then an increase in the uptake was still possible. Most of the obtained response surfaces could be related to a rising ridge system, where saturation did not seem to be reached. Therefore further studies should be performed with different ranges for the predictors. However, it should be noticed that a first attempt has been done in the present study by changing the initial copper concentration range from 0-20 mg/L to 0-500 mg/L. However, no difference was observed in the trend of copper uptake. It should be also stressed that increasing the pH may result in the formation of hydroxides and precipitation of the metals. In addition, the structure of *Palmaria palmata* cells may be damaged at high alkaline conditions or high temperatures.

2) A deeper investigation of the reasons for lack of fit in three of the statistical designs is necessary. The non-center points are likely at fault. Another possible reason is the inadequacy of the quadratic model to fit the experimental data, especially when the ranges of parameters were wide. For instance, the analysis for the experiment on copper in which the initial range of concentrations was only 0-20 mg/L (section 4.4) showed that there was no lack of fit. On the other hand, models of copper uptake in the mono-Cu^{2+} system (section 4.5.2) and lead uptake in the mono-Pb^{2+} system and binary system involving Pb^{2+}(section 4.5.3) suffered from lack of fit. The initial ranges of metal concentrations were 0-500 mg/L and 0-200 mg/L, respectively for copper and lead. In further experiments, another model (such as an exponential or

logarithmic model) should be tested, using relatively narrow ranges of initial metal concentrations.

3) This project mainly focused on copper and lead uptake. It would be interesting to study the biosorption of other metals on *Palmaria palmata* in mono, binary and multi - metal systems. For instance, it would be interesting to look at competition with Ca^{2+} or Na^{+}, which might be a hindrance in a real industrial waste stream. It would also be relevant to use different sorbent materials using a similar experimental approach.

4) Desorption studies are required to know the exact mechanisms involved in the metal uptake by *Palmaria palmata*. For instance, the present study focused on the general sorption phenomena. In further investigations, adsorption should be distinguished from absorption.

5) From this study, it could be concluded that *Palmaria palmata* favoured the uptake of lead to that of copper in mono-metal and binary systems. Further studies should be performed to elucidate the basis for metal biosorption preferences. The exact causes for one inhibition of the sorption of one metal by another metal(s) should also be investigated.

6) The present work indicated the possibility of the existence of different and specific binding sites for copper and lead uptake. Therefore it would be important to know the precise chemical composition of the binding sites (which could include sulfate and carboxylate).

7) In this work, the raw dried *Palmaria palmata* biomass was used in its natural state. Short studies on the effect of a pre-treatment on the algae metal uptake were conducted in section 4.2.1. They showed that copper sorption by *Palmaria palmata* could be slightly increased by washing or homogenizing the algae. However the preliminary experiment of the present study focused only on copper at a given pH,

temperature, contact time and initial copper concentration. Further investigations on the physical or chemical *Palmaria palmata* pre-treatment (such as washing, homogenization, heating and formaldehyde crosslinking for example) are needed.

8) The use of *Palmaria palmata* to remove metal ions from aqueous solutions was studied at the bench scale. Feasibility of the application of the biosorbent in a fixed packed bed process to decontaminate real effluents from current PWB manufacturing facilities should be investigated in column studies.

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8. APPENDICES

APPENDIX I: Sorption data for copper, for untreated algae, washed algae and homogenized algae, without pH adjustment (pH = 6.5-7), contact time = 24h, temperature = 21±3° C, agitation rate = 275 rpm. Initial and final concentrations are the mean of three replicates

	Untreated algae		Washed algae		Homogenized algae	
In. Conc. Ci (mg/L)	Fin. Conc. Cf (mg/L)	Copper uptake q (mg/g)	Fin. conc Cf (mg/L)	Copper uptake q (mg/g)	Fin. Conc. Cf (mg/L)	Copper uptake q (mg/g)
14.3	5	9.3	4.1	10.2	4.3	10.0

APPENDIX II: Sorption data for copper at various algae concentrations without pH adjustment (pH = 6.5-7), contact time = 24h, temperature = 21±3° C, agitation rate = 275 rpm. Initial and final concentrations are the mean of three replicates.

	Algae 0.5 g/L		Algae 1 g/L		Algae 2 g/L	
In. Conc. Ci (mg/L)	Fin. Conc. Cf (mg/L)	Copper uptake q (mg/g)	Fin. conc Cf (mg/L)	Copper uptake q (mg/g)	Fin. Conc. Cf (mg/L)	Copper uptake q (mg/g)
2.4	1.7	1.33	1.8	0.63	1.5	0.43
6.1	4.8	2.67	4.5	1.6	5	0.55
13	10.89	4.21	8.7	4.3	8.6	2.18
30	28	4	25.7	4.33	23.7	3.17
61	58.7	4.67	56.3	4.67	53.3	3.83

APPENDIX III: Sorption data for various metals, at algae concentration = 1 g/L without pH adjustment (pH = 6.5-7), contact time = 24h, temperature = 21±3° C, agitation rate = 275 rpm. Initial and final concentrations are the mean of three replicates.

Cadmium			**Zinc**			**Nickel**		
In. Conc.	**Fin. Conc.**	**Cd^{2+} uptake**	**In. Conc.**	**Fin. Conc.**	**Zn^{2+} uptake**	**In. Conc.**	**Fin. Conc.**	**Ni^{2+} uptake**
C_i (mg/L)	C_f (mg/L)	q (mg/g)	C_i (mg/L)	C_f (mg/L)	q (mg/g)	C_i (mg/L)	C_f (mg/L)	q (mg/g)
1.7	1.2	0.5	5.03	2.6	2.42	1.83	1.58	0.25
4.55	3.34	1.21	8.68	5.59	3.09	4.59	4.05	0.53
9.91	8.07	1,84	12.17	8.66	3.51	9.42	8.74	0.68
49.42	45.48	3.95	32.43	12.55	19.88	20.55	19.32	1.22
96.74	91.74	5	57.81	26.25	31.56	47.31	45.7	1.61
			97.82	56.56	41.26	71.31	52.33	18.98
						96.2	75.27	20.94

Copper			**Lead**		
In. Conc.	**Fin. Conc.**	**Cu^{2+} uptake**	**In. Conc.**	**Fin. Conc.**	**Pb^{2+} uptake**
C_i (mg/L)	C_f (mg/L)	q (mg/g)	C_i (mg/L)	C_f (mg/L)	q (mg/g)
2.4	1.8	0.63	0.27	0.16	0,. 1
6.1	4.5	1.6	4.66	2.03	2.63
9.01	7.21	1.8	9,56	4.84	4.72
13	8.7	4.3	47.39	36.55	10.84
30	25.7	4.33	95.99	83.13	12.85
36.9	33.1	3.8	140	125.5	14.5
196.7	192.3	4.3	276.10	258.9	17.2
61	56.3	4.67	416.40	402.2	14.2
300.6	295.3	5.4	563.7	546.2	17.5
458.2	452.5	5.7			
605	589.9	6.1			

APPENDIX IV: Comparison of metal uptakes by various types of sorbents

Sorbent type	Sorbent class	Metal uptake q_{max}(mg/g)					References
		Cu^{2+}	Pb^{2+}	Ni^{2+}	Zn^{2+}	Cd^{2+}	
Rhizopus arrhizus	Fungus	16	91	18	20	30	Tobin et al., 1984
Ascophyllum nodosum	Brown marine algae	-	-	30	-	215	Holan et al., 1993
Chondrus crispus	Red marine algae	-	195	-	-	-	Volesky and Holan, 1995
Galaxaura marginata	Red marine algae	-	25	-	-	-	Volesky and Holan, 1995
Saccharomyces cerevisiae	Yeast	17-40	-	-	70	20-40	Volesky et al., 1993
Australian natural zeolite	Mineral	-	16.6	-	-	-	Matheickal et al., 1996
Powdered activated carbon	GAC	-	20.7	-	-	-	Matheickal et al., 1996
Duolite GT-73	Ion Exchange resin	-	136	-	-	67	Volesky and Holan, 1995
S.cinnamoneum	Fungus	8.9	74.6	5.87	11.18	21.35	Puranik and Paknikar, 1999
P.chrysogenum	Fungus	12.71	70.45	1.17	9.16	12.36	Puranik and Paknikar, 1999

APPENDIX V: Sorption kinetic data for C_i = 10 mg Cu^{2+}/L concentration, and C_i = 100 mg Pb^{2+}/L, algae concentration = 1 g/L without pH adjustment (pH = 6.5-7), temperature = 23° C, agitation rate = 275 rpm. Initial and final concentrations are the mean of three replicates.

Copper	
Contact time (mn)	**Cu^{2+} uptake** q (mg/g)
5	0.54
10	0.64
15	0.77
30	0.88
40	0.83
60	0.90
80	1.04
120	0.95
160	0.90
320	0.88
554	0.93
960	0.89
1280	0.93
1440	0.94
2880	0.97

Lead	
Contact time (mn)	**Pb^{2+} uptake** q (mg/g)
5	5.5
10	8.2
20	7.6
50	6.8
90	7.9
170	7.6
340	7.0
650	5.8
960	6.4
1440	6.6
2880	6.5

APPENDIX VI: Sorption data for pH effect, for C_i = 10 mg Cu^{2+}/L concentration, and C_i = 100 mg Pb^{2+}/L, algae concentration = 1 g/L, contact time = 90 min, temperature = 23° C, agitation rate = 275 rpm. Initial and final concentrations are the mean of three replicates.

pH	**Cu^{2+} uptake** q (mg/g)	**Pb^{2+} uptake** q (mg/g)
2	0.16	2.5
3	0.47	3.15
4	0.75	6.27
5	1.53	12.45
5.5	1.77	
6	2.19	15.6

APPENDIX VII: Sorption data for temperature effect, for for C_i = 10 mg Cu^{2+}/L concentration, and C_i = 100 mg Pb^{2+}/L,, algae concentration = 1 g/L, contact time = 90 min, agitation rate = 275 rpm Initial and final concentrations are the mean of three replicates.

Temperature °C	**Cu^{2+} uptake q (mg/g)**
0	0.58
6	0.83
10	0.79
15	0.81
23	0.98
30	1.03
40	1.16
60	1.17

Temperature °C	**Pb^{2+} uptake q (mg/g)**
0	9.05
5	10.13
14.5	6.5
23	6.27
31,5	5.5
40	5.55
60	4.2

APPENDIX VIII: Central Composite Design matrix of 4 factors in coded and uncoded units along with the observed and predicted response for Cu^{2+} 0-20 mg/L Observed responses are means of two replicates.

Regression equation:

$$\hat{q}_{cu} = 0.93 + 1.10583\ PH + 0.2325\ TEMP + 0.76917\ INCONC_{Cu} + 0.08417\ TIME + 0.34125\ (PH)^2 + 0.0825\ TEMP{*}PH + 0.09125\ (TEMP)^2 + 0.8275\ INCONC_{Cu}{*}\ PH + 0.1175\ INCONC_{Cu}{*}\ TEMP - 0.09375\ (INCONC_{Cu})^2 + 0.0375\ TIME{*}PH - 0.1525\ TIME{*}TEMP - 0.2275\ TIME{*}NCONC_{Cu} - 0.06875\ (TIME)^2$$

	Coded factors				Uncoded factors				Uptake values (mg/g)		Residua
Exp N	PH	TEMP	INCONC	TIME	PH	TEMP	INCONC	TIME	q_{Cu} observed	$\hat{q}_{Cu}$ predicted	
1	-1	-1	-1	-1	3	14.5	5	50	0.14	-0.31	0.45
2	-1	-1	-1	1	3	14.5	5	130	0.23	0.55	-0.32
3	-1	-1	1	-1	3	14.5	15	50	0.42	-0.20	0.62
4	-1	-1	1	1	3	14.5	15	130	0.54	-0.26	0.80
5	-1	1	-1	-1	3	31.5	5	50	0.35	0.06	0.29
6	-1	1	-1	1	3	31.5	5	130	0.39	0.31	0.08
7	-1	1	1	-1	3	31.5	15	50	0.93	0.64	0.29
8	-1	1	1	1	3	31.5	15	130	0.56	-0.03	0.59
9	1	-1	-1	-1	5	14.5	5	50	0.83	0.01	0.82
10	1	-1	-1	1	5	14.5	5	130	0.93	1.01	-0.08
11	1	-1	1	-1	5	14.5	15	50	1.99	3.42	-1.43
12	1	-1	1	1	5	14.5	15	130	1.94	3.52	-1.58
13	1	1	-1	-1	5	31.5	5	50	1.02	0.71	0.31
14	1	1	-1	1	5	31.5	5	130	1.2	1.10	0.10
15	1	1	1	-1	5	31.5	15	50	2.45	4.59	-2.14
16	1	1	1	1	5	31.5	15	130	2.25	4.08	-1.83
17	-2	0	0	0	2	23	10	90	0.19	0.08	0.11
18	2	0	0	0	6	23	10	90	2.3	4.51	-2.21
19	0	-2	0	0	4	6	10	90	0.83	0.83	0.00
20	0	2	0	0	4	40	10	90	1.16	1.76	-0.60
21	0	0	-2	0	4	23	0	90	0	-0.98	0.98
22	0	0	2	0	4	23	20	90	1.62	2.09	-0.47
23	0	0	0	-2	4	23	10	10	0.56	0.49	0.07
24	0	0	0	2	4	23	10	170	1.11	0.82	0.29
25	0	0	0	0	4	23	10	90	0.97	0.93	0.04
26	0	0	0	0	4	23	10	90	0.69	0.93	-0.24
27	0	0	0	0	4	23	10	90	0.95	0.93	0.02
28	0	0	0	0	4	23	10	90	0.96	0.93	0.03
29	0	0	0	0	4	23	10	90	0.99	0.93	0.06
30	0	0	0	0	4	23	10	90	0.98	0.93	0.05
31	0	0	0	0	4	23	10	90	0.97	0.93	0.04

APPENDIX IX: SAS printout of the location of the stationary point x_0, the predicted value at x_0 and the coefficients, λ_1, λ_2, λ_3, and λ_4 of the canonical analysis of response surface.

CCD of Cu^{2+} 0-20 mg/L

```
                     Canonical Analysis of Response Surface
                   (based on coded data)


                      Critical Value
           Factor         Coded          Uncoded


           PH           -0.867137        2.265726
           TEMP          0.456397       30.758750
           INCON        -0.714306        2.856935
           TIME          1.051296      174.103680

           Predicted value at stationary point     0.273133


                          Eigenvectors
   Eigenvalues       PH          TEMP         INCONC        TIME

    0.603943      0.837817     0.139186     0.521715    -0.080644
    0.119362     -0.214690     0.886225     0.045270    -0.407999
   -0.072624      0.158497     0.441785    -0.241085     0.849460
   -0.380681     -0.476287    -0.007621     0.817094     0.324731


                Stationary point is a saddle point.
```

APPENDIX X: Central Composite Design matrix of 4 factors in coded and uncoded units along with the observed and predicted response for Cu^{2+} 0-500mg/L in the mono-Cu^{2+} system. Observed responses are means of two replicates.

Regression equation:

$$\hat{q}_{cu} = 4.10286 + 10.05833\ PH + 2.14167\ TEMP + 7.5\ INCONC_{Cu} - 0.25667\ TIME + 7.21464\ (PH)^2 + 6.74\ TEMP*PH + 7.39964\ (TEMP)^2 + 11.935\ INCONC_{Cu}*\ PH + 2.54\ INCONC_{Cu}*\ TEMP + 1.95964\ (INCONC_{Cu})^2 - 1.18\ TIME*PH + 0.465\ TIME*TEMP + 0.22\ TIME*NCONC_{Cu} + 3.68464\ (TIME)^2$$

Exp	Coded factors				Uncoded factors				Uptake values (mg/g)		Residual
N	PH	TEMP	INCONC	TIME	PH	TEMP	INCONC	TIME	q_{Cu} observed	$\hat{q}_{Cu}$ predicted	
1	-1	-1	-1	-1	3	14.5	125	50	3.29	25.64	-22.35
2	-1	-1	-1	1	3	14.5	125	130	3.29	26.11	-22.82
3	-1	-1	1	-1	3	14.5	375	50	8.41	11.25	-2.84
4	-1	-1	1	1	3	14.5	375	130	7.42	12.60	-5.18
5	-1	1	-1	-1	3	31.5	125	50	2.97	10.43	-7.46
6	-1	1	-1	1	3	31.5	125	130	2.97	12.77	-9.80
7	-1	1	1	-1	3	31.5	375	50	4.79	6.20	-1.41
8	-1	1	1	1	3	31.5	375	130	8.08	9.42	-1.34
9	1	-1	-1	-1	5	14.5	125	50	7.42	10.76	-3.34
10	1	-1	-1	1	5	14.5	125	130	7.42	6.52	0.90
11	1	-1	1	-1	5	14.5	375	50	20.28	44.11	-23.83
12	1	-1	1	1	5	14.5	375	130	20.28	40.75	-20.47
13	1	1	-1	-1	5	31.5	125	50	10.39	22.52	-12.13
14	1	1	-1	1	5	31.5	125	130	9.82	20.13	-10.31
15	1	1	1	-1	5	31.5	375	50	30.2	66.03	-35.83
16	1	1	1	1	5	31.5	375	130	28.28	64.52	-36.24
17	-2	0	0	0	2	23	250	90	0.79	12.84	-12.05
18	2	0	0	0	6	23	250	90	14.67	53.08	-38.41
19	0	-2	0	0	4	6	250	90	6.43	29.42	-22.99
20	0	2	0	0	4	40	250	90	9.4	37.98	-28.58
21	0	0	-2	0	4	23	0	90	0	-3.06	3.06
22	0	0	2	0	4	23	500	90	4.95	26.94	-21.99
23	0	0	0	-2	4	23	250	10	4.94	19.35	-14.41
24	0	0	0	2	4	23	250	170	3.46	18.33	-14.87
25	0	0	0	0	4	23	250	90	3.96	4.10	-0.14
26	0	0	0	0	4	23	250	90	6.44	4.10	2.34
27	0	0	0	0	4	23	250	90	3.96	4.10	-0.14
28	0	0	0	0	4	23	250	90	1.98	4.10	-2.12
29	0	0	0	0	4	23	250	90	4.95	4.10	0.85
30	0	0	0	0	4	23	250	90	3.96	4.10	-0.14
31	0	0	0	0	4	23	250	90	3.47	4.10	-0.63

APPENDIX XI: SAS printout of the location of the stationary point x_0, the predicted value at x_0 and the coefficients, λ_1, λ_2, λ_3, and λ_4 of the canonical analysis of response surface.

CCD of Cu^{2+} 0-500 mg/L in the mono-Cu^{2+} system

```
Canonical Analysis of Response Surface Based on Coded Data

                        Critical Value
            Factor        Coded        Uncoded
            pH          -0.585374      2.829251
            Temp         0.163965     25.787404
            InConc      -0.233800    191.550046
            Time        -0.062270     85.018428

            Predicted value at stationary point: 0.465733

                              Eigenvectors
   Eigenvalues       pH          Temp        InConc        Time
    13.246610     0.731145     0.516855     0.444450    -0.027433
     5.425194    -0.377211     0.834728    -0.336718     0.218086
     3.626092     0.060020    -0.165107     0.153291     0.972440
    -2.039325    -0.565276     0.093967     0.815835    -0.077761

              Stationary point is a saddle point.
```

APPENDIX XII: Central Composite Design matrix of 4 factors in coded and uncoded units along with the observed and predicted response for Pb^{2+} 0-200mg/L in the mono-Pb^{2+} system. Observed responses are means of two replicates.

Regression equation:

$$\hat{q}_{Pb} = 6.86429 + 7.1333\ PH - 1.45\ TEMP + 4.45\ INCONC_{Pb} + 0.58333\ TIME + 0.88571\ (PH)^2 + 0.075\ TEMP*PH + 0.18571\ (TEMP)^2 + 7.1\ INCONC_{Pb}*PH - 2.75\ INCONC_{Pb}*TEMP - 3.83929\ (INCONC_{Pb})^2 + 0.575\ TIME*PH - 0.625\ TIME*TEMP + 1.1\ TIME*INCONC_{Pb} - 0.16429\ (TIME)^2$$

Exp	Coded factors				Uncoded factors				Uptake values (mg/g)		Residua
N	PH	TEMP	INCONC	TIME	PH	TEMP	INCONC	TIME	q_{Pb} observed	$\hat{q}_{Pb}$ predicted	
1	-1	-1	-1	-1	3	14.5	50	50	1.20	-1.46	2.66
2	-1	-1	-1	1	3	14.5	50	130	1.85	-2.39	4.24
3	-1	-1	1	-1	3	14.5	150	50	1.85	-3.46	5.31
4	-1	-1	1	1	3	14.5	150	130	3.15	0.01	3.14
5	-1	1	-1	-1	3	31.5	50	50	1.10	2.39	-1.29
6	-1	1	-1	1	3	31.5	50	130	1.25	-1.04	2.29
7	-1	1	1	-1	3	31.5	150	50	1.35	-10.61	11.96
8	-1	1	1	1	3	31.5	150	130	2.00	-9.64	11.64
9	1	-1	-1	-1	5	14.5	50	50	3.10	-2.39	5.49
10	1	-1	-1	1	5	14.5	50	130	4.65	-1.03	5.68
11	1	-1	1	-1	5	14.5	150	50	13.4	24.01	-10.61
12	1	-1	1	1	5	14.5	150	130	15.05	29.77	-14.72
13	1	1	-1	-1	5	31.5	50	50	6.2	1.46	4.74
14	1	1	-1	1	5	31.5	50	130	5.55	0.32	5.23
15	1	1	1	-1	5	31.5	150	50	9.95	16.86	-6.91
16	1	1	1	1	5	31.5	150	130	12.45	20.12	-7.67
17	-2	0	0	0	2	23	100	90	2.25	-4.01	6.26
18	2	0	0	0	6	23	100	90	16.75	24.82	-8.07
19	0	-2	0	0	4	6	100	90	12.05	10.51	1.54
20	0	2	0	0	4	40	100	90	5.55	4.71	0.84
21	0	0	-2	0	4	23	0	90	0	-17.39	17.39
22	0	0	2	0	4	23	200	90	9.55	0.41	9.14
23	0	0	0	-2	4	23	100	10	8.65	5.04	3.61
24	0	0	0	2	4	23	100	170	8.25	7.37	0.88
25	0	0	0	0	4	23	100	90	5.7	6.86	-1.16
26	0	0	0	0	4	23	100	90	7.35	6.86	0.49
27	0	0	0	0	4	23	100	90	6.3	6.86	-0.56
28	0	0	0	0	4	23	100	90	7	6.86	0.14
29	0	0	0	0	4	23	100	90	7	6.86	0.14
30	0	0	0	0	4	23	100	90	6.8	6.86	-0.06
31	0	0	0	0	4	23	100	90	7.9	6.86	1.04

APPENDIX XIII: SAS printout of the location of the stationary point x_0, the predicted value at x_0 and the coefficients, λ_1, λ_2, λ_3, and λ_4 of the canonical analysis of response surface.

CCD of Pb^{2+} 0-200 mg/L in the mono-Pb^{2+} system

```
Canonical Analysis of Response Surface Based on Coded Data

                        Critical Value
               Factor     Coded       Uncoded
               pH       -1.532836     0.934328
               Temp     -1.077884     4.675976
               InConc   -0.553411    44.658888
               Time     -0.709502    33.239818

               Predicted value at stationary point: 0.740359


                              Eigenvectors
   Eigenvalues       pH        Temp       InConc       Time

    3.041990     0.819901   -0.242925    0.485953    0.180555
    0.375972     0.352528    0.868500   -0.031689   -0.347027
   -0.348058    -0.052174    0.386168   -0.060370    0.918971
   -6.002047    -0.448068    0.193827    0.871322   -0.049649

                 Stationary point is a saddle point.
```

APPENDIX XIV: Central Composite Design matrix of 4 factors in coded and uncoded units along with the observed and predicted response for Cu^{2+} 0-500mg/L in the binary system (Pb^{2+}+Cu^{2+}). Observed responses are means of two replicates.

Regression equation:

$$\hat{q}_{cu} = 9.74143 + 3.53583\ \text{PH} + 0.0825\ \text{TEMP} + 4.03917\ \text{INCONC}_{Cu} + 0.42083\text{TIME}+0.86732\ (\text{PH})^2 - 3.2825\ \text{TEMP*PH} + 4.65232\ (\text{TEMP})^2 - 2.7825\ \text{INCONC}_{Cu}\text{* PH}-2.7775\ \text{INCONC}_{Cu}\text{* TEMP} - 8.2277\ (\text{INCONC}_{Cu})^2 - 0.0025\ \text{TIME*PH} - 0.5075\ \text{TIME*TEMP} + 0.0025\text{TIME*NCONC}_{Cu}+ 1.87732\ (\text{TIME})^2$$

Exp N	Coded factors				Uncoded factors				Uptake values (mg/g)		Residu
	PH	TEMP	INCONC$_{Cu}$	TIME	PH	TEMP	INCONC$_{Cu}$	TIME	q_{Cu} observed	$\hat{q}_{Cu}$ predicted	
1	-1	-1	-1	-1	3	14.5	125	50	5.05	-8.52	13.57
2	-1	-1	-1	1	3	14.5	125	130	4.04	-6.66	10.70
3	-1	-1	1	-1	3	14.5	375	50	10.10	10.68	-0.58
4	-1	-1	1	1	3	14.5	375	130	9.60	12.54	-2.94
5	-1	1	-1	-1	3	31.5	125	50	5.05	4.78	0.27
6	-1	1	-1	1	3	31.5	125	130	4.04	4.61	-0.57
7	-1	1	1	-1	3	31.5	375	50	11.11	12.87	-1.76
8	-1	1	1	1	3	31.5	375	130	14.14	12.70	1.44
9	1	-1	-1	-1	5	14.5	125	50	7.58	10.69	-3.11
10	1	-1	-1	1	5	14.5	125	130	8.59	12.54	-3.95
11	1	-1	1	-1	5	14.5	375	50	15.15	18.75	-3.60
12	1	-1	1	1	5	14.5	375	130	17.17	20.61	-3.44
13	1	1	-1	-1	5	31.5	125	50	9.60	10.86	-1.26
14	1	1	-1	1	5	31.5	125	130	11.11	10.68	0.43
15	1	1	1	-1	5	31.5	375	50	12.12	7.81	4.31
16	1	1	1	1	5	31.5	375	130	8.08	7.64	0.44
17	-2	0	0	0	2	23	250	90	6.57	6.14	0.43
18	2	0	0	0	6	23	250	90	14.65	20.28	-5.63
19	0	-2	0	0	4	6	250	90	13.64	28.19	-14.55
20	0	2	0	0	4	40	250	90	15.15	28.52	-13.37
21	0	0	-2	0	4	23	0	90	0.00	-31.25	31.25
22	0	0	2	0	4	23	500	90	3.03	-15.09	18.12
23	0	0	0	-2	4	23	250	10	10.61	16.41	-5.80
24	0	0	0	2	4	23	250	170	12.63	18.09	-5.46
25	0	0	0	0	4	23	250	90	9.09	9.74	-0.65
26	0	0	0	0	4	23	250	90	12.12	9.74	2.38
27	0	0	0	0	4	23	250	90	9.60	9.74	-0.14
28	0	0	0	0	4	23	250	90	7.07	9.74	-2.67
29	0	0	0	0	4	23	250	90	11.62	9.74	1.88
30	0	0	0	0	4	23	250	90	10.10	9.74	0.36
31	0	0	0	0	4	23	250	90	8.59	9.74	-1.15

APPENDIX XV: SAS printout of the location of the stationary point x_0, the predicted value at x_0 and the coefficients, λ_1, λ_2, λ_3, and λ_4 of the canonical analysis of response surface.

CCD of Cu^{2+} 0-500 mg/L in the binary system (Cu^{2+}+Pb^{2+})

```
Canonical Analysis of Response Surface Based on Coded Data

                     Critical Value
          Factor        Coded        Uncoded


          pH         -1.940056       0.119887
          Temp       -0.506513      14.389278
          InConc      0.658980     414.745104
          Time       -0.182277      75.417813

          Predicted value at stationary point: 7.583188


                        Eigenvectors
   Eigenvalues       pH        Temp       InConc       Time


    5.332780    -0.326137   0.940702   -0.062885   -0.068985
    1.866005    -0.082303   0.044830    0.005299    0.995584
    0.606019     0.927259   0.313088   -0.195249    0.063596
   -8.635519     0.164473   0.122659    0.978721    0.002864

          Stationary point is a saddle point.
```

APPENDIX XVI Composite Design matrix of 4 factors in coded and uncoded units along with the observed and predicted response for Pb^{2+} 0-200 mg/L in the binary system (Pb^{2+}+Cu^{2+}). Observed responses are means of two replicates.

Regession equation:

$$\hat{q}_{Pb} = 2.44286 + 3.15833\ PH + 0.75\ TEMP + 2.70833\ INCONC_{Pb} + 0.1\ TIME + 1.73214\ (PH)^2 + 1.85\ TEMP*PH + 6.83214\ (TEMP)^2 + 0.825\ INCONC_{Pb}*\ PH + 1.2\ INCONC_{Pb}*\ TEMP - 0.21786\ (INCONC_{Pb})^2 - 0.725\ TIME*PH - 0.4\ TIME*TEMP - 0.675\ TIME*NCONC_{Pb} + 0.50714\ (TIME)^2$$

Exp N	Coded factors				Uncoded factors				Uptake values (mg/g)		Residu
	PH	TEMP	INCONC	TIME	PH	TEMP	INCONC	TIME	q_{Pb} observed	$\hat{q}_{Pb}$ predicted	
1	-1	-1	-1	-1	3	14.5	50	50	1.30	2.95	-1.65
2	-1	-1	-1	1	3	14.5	50	130	1.40	6.75	-5.35
3	-1	-1	1	-1	3	14.5	150	50	3.00	5.67	-2.6
4	-1	-1	1	1	3	14.5	150	130	3.20	6.77	-3.57
5	-1	1	-1	-1	3	31.5	50	50	0.65	2.85	-2.20
6	-1	1	-1	1	3	31.5	50	130	2.50	5.05	-2.55
7	-1	1	1	-1	3	31.5	150	50	4.65	10.37	-5.72
8	-1	1	1	1	3	31.5	150	130	4.25	9.87	-5.62
9	1	-1	-1	-1	5	14.5	50	50	3.65	12.77	-9.12
10	1	-1	-1	1	5	14.5	50	130	3.70	13.67	-9.97
11	1	-1	1	-1	5	14.5	150	50	5.8	18.79	-12.9
12	1	-1	1	1	5	14.5	150	130	6.55	16.99	-10.4
13	1	1	-1	-1	5	31.5	50	50	5.85	12.67	-6.82
14	1	1	-1	1	5	31.5	50	130	5.5	11.97	-6.47
15	1	1	1	-1	5	31.5	150	50	10.25	23.49	-13.2
16	1	1	1	1	5	31.5	150	130	8.65	20.09	-11.4
17	-2	0	0	0	2	23	100	90	2.4	-0.65	3.05
18	2	0	0	0	6	23	100	90	6.85	19.39	-12.5
19	0	-2	0	0	4	6	100	90	10.9	28.27	-17.3
20	0	2	0	0	4	40	100	90	8.55	31.27	-22.7
21	0	0	-2	0	4	23	0	90	0	-3.85	3.85
22	0	0	2	0	4	23	200	90	5.35	6.99	-1.64
23	0	0	0	-2	4	23	100	10	3.25	4.27	-1.02
24	0	0	0	2	4	23	100	170	3.55	4.67	-1.12
25	0	0	0	0	4	23	100	90	1.4	2.44	-1.04
26	0	0	0	0	4	23	100	90	2.75	2.44	0.31
27	0	0	0	0	4	23	100	90	2.25	2.44	-0.19
28	0	0	0	0	4	23	100	90	2.5	2.44	0.06
29	0	0	0	0	4	23	100	90	3.35	2.44	0.91
30	0	0	0	0	4	23	100	90	1.7	2.44	-0.74
31	0	0	0	0	4	23	100	90	3.15	2.44	0.71

APPENDIX XVII: SAS printout of the location of the stationary point x_0, the predicted value at x_0 and the coefficients, λ_1, λ_2, λ_3, and λ_4 of the canonical analysis of response surface.

CCD of Pb^{2+} 0-200 mg/l in the binary system (Cu^{2+}+Pb^{2+})

```
Canonical Analysis of Response Surface Based on Coded Data

                       Critical Value
              Factor       Coded        Uncoded


              pH        -1.349691      1.300617
              Temp      -0.077197     21.687647
              InConc     2.531864    353.186436
              Time       0.591158    137.292649

              Predicted value at stationary point: 3.740645


                          Eigenvectors
      Eigenvalues        pH        Temp       InConc       Time


       7.068156     0.179789    0.978305    0.092804    -0.044529
       1.728463     0.917753   -0.199283    0.183440    -0.290456
       0.462814     0.337136    0.002595   -0.244225     0.909223
      -0.405862    -0.108371   -0.056560    0.947677     0.294899

                Stationary point is a saddle point.
```