CHAPTER III

METHODOLOGY

3.1 Overview of Experiments

This study is categorized into six phases as described in Section 1.4 and shown in Figure 1.2. These included preparation of PSMC biosorbent from agricultural waste, advanced characterisation of biosorbent, optimisation of heavy metal biosorption in synthetic solutions, evaluation of existing models, application of biosorbent and development of ANN model. The study involved three scopes of work, namely, field work for sampling, laboratory work and mathematical analysis including modelling. Table 3.1 shows activities involved in laboratory work of this study which correspond to the objectives. Number of samples used in the various activities is also shown in the Table 3.1. All samples were prepared in duplicates. Each experimental design and procedure is described in the following sections.

Table 3.1 Major phases of laboratory work in this study

Activity	Objective	Number of samples prepared in duplicates
Preparation of biosorbent	To establish the physic-chemical characteristics of PSMC biosorbent in relation to its biosorption mechanism	45
Advanced characterisation of biosorbent		18
Biosorption study	To optimise and evaluate the biosorption efficiency of Pb(II), Cu(II) and Ni(II) from synthetic heavy metal solutions	262
Application study	To evaluate the potential application of PSMC biosorbent in the treatment of industrial wastewater and its subsequent recovery of heavy metal from biosorbent	8

3.2 Sampling Locations and Sampling Procedures

The biosorbent sample used was collected from C & C Mushroom Cultivation Farm Sdn. Bhd. at Lot 6, Kawasan Perindustrian Grisek 84700 Grisek, Muar, Johor, Malaysia. A 5 kg sample of raw *Pleurotus ostreatus* spent mushroom substrate compost (R-PSMC) in plastic bags was taken and transported to laboratory within 24 hours. Upon arrival, the sample was processed in the laboratory. The name of the fungus was given as *Pleurotus ostreatus* by the mushroom grower and no taxonomic identification work was carried out to verify the *Pleurotus ostreatus* fungus.

The wastewater sample was taken from Perodua Manufacturing Sdn. Bhd. at Lot 1896, Sungai Choh, Mukim Serendah, 48000 Rawang, Selangor, Malaysia. A two-litre sample was collected from phosphating tank using the grab sampling method and stored in an air tight plastic container. The two-litre wastewater sample was kept at 4 ^{o}C when it was transported to laboratory. Upon arrival, the sample was continuously kept at 4 ^{o}C until used (APHA, 1992). Figure 3.1(a) illustrates general sampling locations and Figure 3.1(b) shows the sampling point at Perodua Manufacturing Sdn. Bhd.

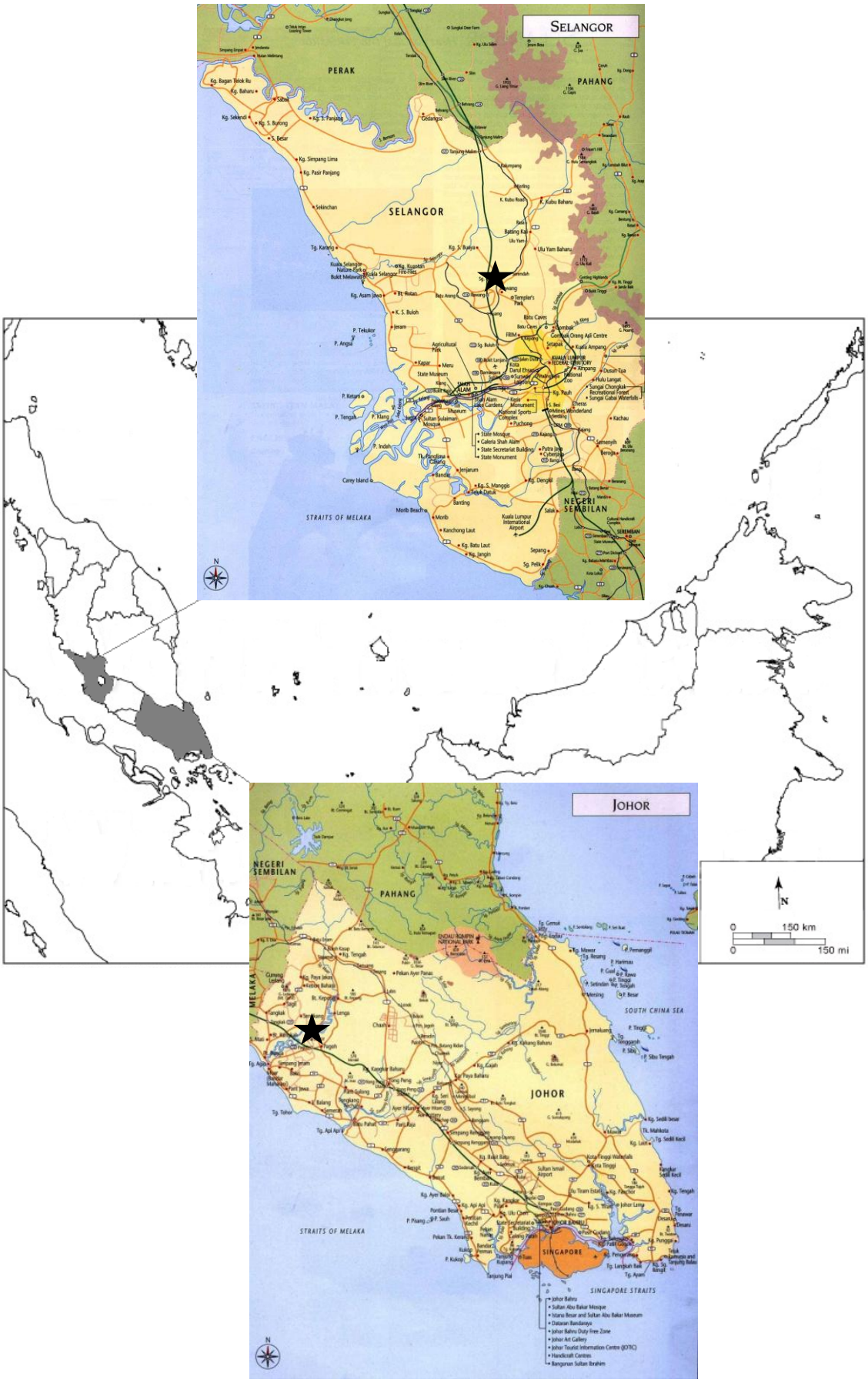

Figure 3.1 (a) General sampling locations (★)

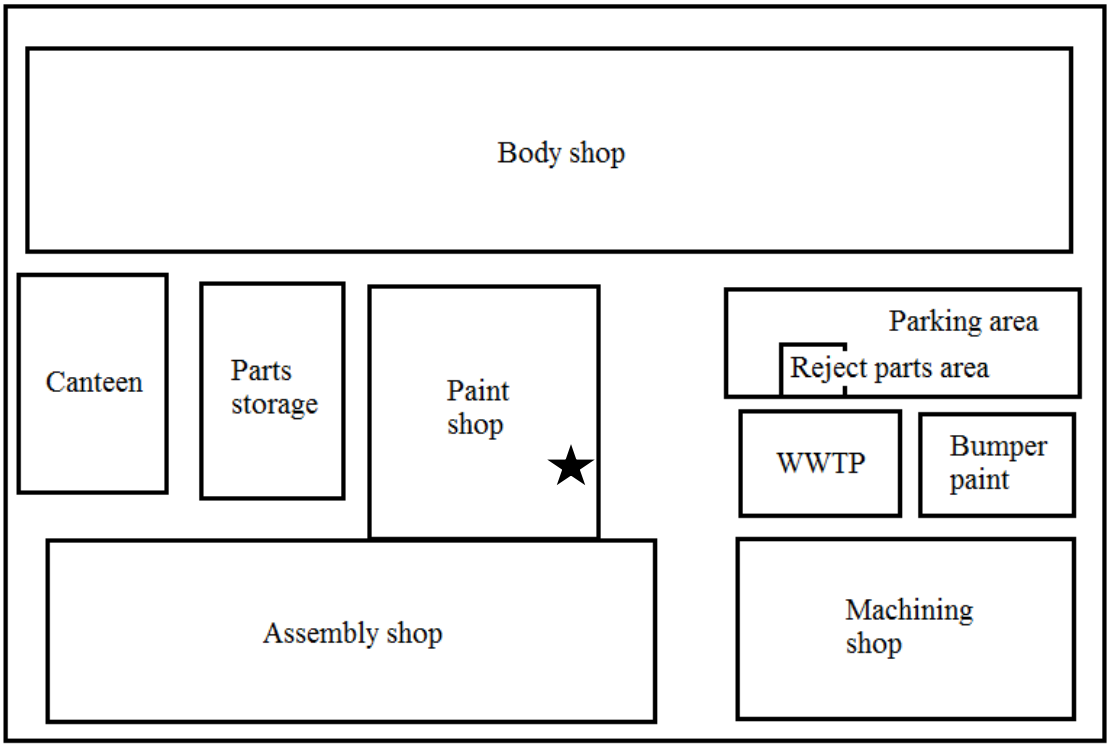

Figure 3.1(b) Specific wastewater sampling location at phosphating tank, Perodua Manufacturing Sdn. Bhd. (★)

3.3 Material and Apparatus

Materials and apparatus used in this study are described in the following sections.

3.3.1 Biosorbent Material

The PSMC biosorbent consists of *Pleurotus ostreatus* mycelium, rubber tree sawdust, rice bran husk, recycled substrate and commercial limestone. This material is an agricultural waste generated from *Pleurotus ostreatus* mushroom cultivation, which have undergone the biodestruction by enzyme complex of *Pleurotus ostreatus* (Dalimova and Akhmedova, 2001).

3.3.2 Apparatus Used in This Study

All apparatus used in this study are listed in Table 3.2

Table 3.2 Apparatus used in sample preparation and running of experiments

Apparatus	Brand
1000 mL volumetric flask	Isolab, Germany
250 mL volumetric flask	Isolab, Germany
10 mL volumetric flask	Isolab, Germany
500 mL beaker	Schott duran, Germany
250 mL beaker	Schott Duran, Germany
100 mL beaker	Schott duran, Germany
250 mL conical flask	Schott duran, Germany
25 mL pipette	Isolab, Germany
Mortar bland and pastle	Simax, Czech Republic
Micropipettes	Eppendorf, Germany
Easypet	Eppendorf, Germany
Dispensatte	Eppendorf, Germany
50 mL centrifuge tube	BD Falcon, United States of America
15 mL centrifuge tube	BD Falcon, United States of America

3.3.3 Chemicals

All reagents used were of analytical grade. Chemicals used in this study are listed in Table 3.3.

Table 3.3 Chemicals used in this study

Chemicals	Brand
Lead (II) nitrate salt ($Pb(NO_3)_2$)	Merck, Germany
Copper (II) sulphate anhydrous salt ($CuSO_4$)	Merck, Germany
Nickel (II) nitrate hexahydrate salt ($Ni(NO_3)_2 . 6H_2O$)	Merck, Germany
Nitric acid (HNO_3)	Merck, Germany
Hydrochloric acid (HCl)	Merck, Germany
Sodium hydroxide salt (NaOH)	Merck, Germany

3.4 Apparatus Cleaning Method for Heavy Metal Analysis

All apparatus used including glass and plastic wares for heavy metal analysis were washed, rinsed and soaked for a minimum of 36 hours in 10 % HNO_3. This is followed by rinsing with tap water and ultra pure water (UPW). Finally, all apparatus were put in an oven at a constant temperature of 60 ^oC for drying (APHA, 1992).

3.5 Equipments Used in This Study

All equipment used in this study is divided into two categories, namely, equipments for sample preparation and for sample analysis. These equipments are listed in Table 3.4 and Table 3.5.

Table 3.4 Equipments used for sample preparation

Equipments	Model	Function
Autoclave	Hirayama HVE-50, Japan	To sterilise PSMC sample
Benchtop Planetary Mill	Fritsch Pulverisette 7 Premium line, Germany	To grind sample into fine particle size
Laboratoty Test Sieve Shaker	Endecottes EFL 2000/2, United States of America	To select particle size
Incubator Shaker	Thermo Scientific MaxQ 5000, United States of America	To run biosorption experiments
Laboratory Water Purification System	Alga Purelab Ultra, United Kingdom	To produce ultra pure water of 18.2 MΩ-cm
Electronic drying cabinet	Weifo, Taiwan	To keep the prepared PSMC
Drying Oven	Binder ED 720, Germany	To dry sample
Analytical balance	Sartorius, United States of America	To weigh sample
Centrifuge	Eppendorf 5810 R, Germany	To separate the supernatant and solid

Table 3.5 Equipments used for sample analysis

Equipments	Model	Function
pH meter	Metrohm 827 pH lab, Switzerland	pH measurement
Inductively coupled plasma - optical emission Spectrometry (ICP-OES)	Perkin Elmer 7300DV, United States of America	Analysis of metals
Brunauer Emmett Teller (BET) surface area analyser	BELSORP MINI-II, Japan	Measurement of surface area
Elemental of CHNS analyser (CHNS analyser)	Leco TruSpec CHN, United States of America	Analysis of carbon, hydrogen and nitrogen
Elemental of oxygen analyser (O analyser)	Leco CHNS-932, United States of America	Analysis of oxygen
Scanning electron microscopy combined with energy dispersive X-ray (SEM/EDX)	SEM LEO 1455 VP/ EDX Oxford 300, United Kingdom	Analysis of surface morphology and heavy metal
X-ray photoelectron spectrometer (XPS)	Shimatzu Kratos Axis Ultra, Japan	Analysis species of heavy metal
Zeta potential	Malven Zetasizer Nano ZS, United Kingdom	Determine zeta potential
Fourier transformed infrared spectroscopy system (FTIR)	Perkin Elmer Series 100, United States of America	Analysis of functional groups
^{13}C solid state nuclear magnetic resonance (^{13}C ssNMR)	Bruker Oxford 400, Germany	Analysis of functional groups and mobility of carbon atoms

3.6 Preparation of Heavy Metal Solutions

Heavy metal solutions were prepared by using analytical grade anhydrous lead (II) nitrate salt $Pb(NO_3)_2$, copper (II) sulphate anhydrous salt $CuSO_4$ and nickel (II) nitrate hexahydrate salt $Ni(NO_3)_2$. $6H_2O$. All reagents were prepared in UPW with resistance of 18.2 MΩ.cm.

3.7 Analytical Instrument for Metals and Standard Calibration

A Perkin Elmer Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) was used for metals determination. Compressed air, purified argon gas and purified nitrogen gas were used for ICP-OES operation. All samples were filtered with 0.45 µm IC Millex hydrophilic PTFE syringe filter (Milipore, United State of America) prior to analysis. Measurements were taken in triplicates under specific wavelength as shown in Table 3.6. The unit was calibrated using blank and standards with concentrations of 0.01, 0.1, 1 and 10 mg/L for all metals. These calibration solutions were prepared daily. Unless specified otherwise, UPW was used in all the serial dilution for preparing standard calibration solutions. For every set of ten samples, a spiked sample containing all standards was run to check for interference and cross contamination.

Table 3.6 Specific wavelength for metals according to Perkin Elmer ICP-OES application note (Sarojam, 2010)

No.	Metals	Wavelength (nm)
1	Aluminium (Al)	396.158
2	Calcium (Ca)	317.933
3	Copper (Cu)	327.393
4	Ferum (Fe)	238.204
5	Potassium (K)	766.490
6	Magnesium (Mg)	285.213
7	Manganese (Mn)	257.610
8	Nickel (Ni)	231.604
9	Lead (Pb)	220.353
10	Sodium (Na)	589.592
11	Zinc (Zn)	206.200

3.8 Preparation of Biosorbent

The R-PSMC in bags was sterilised at 121 ^{o}C, under pressure of 18 psi for 15 minutes in order to sterilise the material and later air-dried in an oven at the temperature of 60 ^{o}C until a constant weight was attained. Subsequently, dried sample of R-PSMC was ground and sieved to the particle size of 710 µm. This sample was referred to as non-washed pre-treated biosorbent (NW-PSMC).

Washing pre-treatment removes contaminants in the sample which affect performance of biosorption. Three parameters were considered during the washing pre-treatment of the NW-PSMC. These included biosorbent concentration, immersion time and number of washing cycle. Details of experimental design to optimise the washing pre-treatment procedures are described in following sections.

3.8.1 Biosorbent Concentration

Effect of biosorbent concentration was evaluated in this study. Four beakers, each filled with 200 mL of UPW, were labelled as 10 g/L, 20 g/L, 50 g/L and 100 g/L. NW-PSMC

amounting to 2, 4, 10 and 20 g were added to the labelled flasks. The beaker with the NW-PSMC was then shaken and the resulting mixture allowed to settle for the next separation stage. Samples were left for an immersion time of 2 hours and filtered through Advantec 5C filter paper of pore size < 5 µm. The method described was adopted from Reddad *et al*. (2002) and Entezari and Soltani (2009). Finally, filtrates were analysed by ICP-OES. The biosorbent concentration corresponded to the maximum contaminants concentration leached out per gram of biosorbent was selected for subsequent experiments in order to minimise the cost of washing pre-treatment.

3.8.2 Immersion Time

Immersion times of 0.5, 1, 2 and 3 hours were investigated. The optimal biosorbent concentration obtained from Section 3.8.1 was added into 200 mL UPW. Samples were filtered after each immersion time. Later, filtrates were analysed by ICP-OES. The optimal immersion time was employed in number of washing cycle in order to minimise time of washing pre-treatment.

3.8.3 Number of Washing Cycle

Number of washing cycles was examined using the optimal biosorbent concentration and immersion time. The NW-PSMC corresponding to optimal biosorbent concentration was weighed and placed into the 2 L beaker with 1 L UPW. After two hours, settled samples were then filtered and their filtrates were analysed using ICP-OES. The washing technique was repeated for three subsequent cycles. The number of washing cycle at not significant contaminants removal with less than 0.1000 mg/L light metal and 0.0030 mg/L heavy metal was selected in order to minimise the time and cost of washing pre-treatment.

Percentage of contaminants removal (R_c) was calculated with the typical formula of:

$$Percentage\ of\ contaminants\ removal,\ R_c\% = \frac{C_w}{C_o}\ x\ 100\% \quad \text{(Equation 3.1)}$$

Where R_c = percentage removal of contaminants (%), C_o = the contaminants concentration for first washing cycle (mg/L), C_w = the final concentration of contaminants after washing cycle (mg/L).

All tests were performed in duplicates. Data for each contaminant concentration was averaged based on duplicate samples and standard error corresponded to ± 1 SD. Finally, biosorbent was dried in oven at 60 ^{o}C until a constant weight was obtained. Sample was kept in desiccators for subsequent analysis. This washed pre-treated biosorbent was referred to as *Pleurotus ostreatus* spent mushroom substrate compost (PSMC).

3.9 Evaluation of Washing Pre-treatment Performance

Washing pre-treatment performance was evaluated through Ni(II) biosorption efficiency as well as reproducibility of biosorbent preparation and repeatability of biosorption efficiency in batch mode. Experimental designs of these two parameters are described in Sections 3.9.1 and 3.9.2 respectively.

3.9.1 The Effect of Washing Pre-treatment on Ni(II) Biosorption

A comparison between the NW-PSMC and PSMC was conducted to evaluate the effect of washing pre-treatment on Ni(II) biosorption. Optimised conditions as described in Sections 3.8.1 through 3.8.3 were utilised in this study. The NW-PSMC ranging from 0.05 to 3.0 g was added into conical flasks containing 50 mL of 50 mg/L of Ni(II) solution. Then, samples were placed in an incubator shaker at 125 rpm and temperature

of 25 ± 1 ^{o}C. After an hour of biosorption process, samples were filtered through Advantec 5C filter paper with pore size < 5 µm and filtrates were analysed by ICP-OES. Similar experiment was repeated using PSMC. All samples were performed in duplicates and obtained data were presented in average with standard error of ± 1 SD.

Ni(II) biosorption performance was evaluated by percentage of biosorption (*B*) as shown in Equation 3.2.

$$\textit{Percentage of heavy metal biosorption,}\quad B\ \% = \frac{C_i - C_f}{C_i} x\ 100\%$$

(Equation 3.2)

Where B = percentage of biosorption (%), C_i = the initial heavy metal concentration (mg/L), C_e = the equilibrium concentration of heavy metal in solution (mg/L).

3.9.2 Reproducibility of Biosorbent and Repeatability of Ni(II) Biosorption

Sampling of R-PSMC was repeated four times (n = 5) in order to get different batches of biosorbent samples. Collected samples were processed and washed with optimised washing pre-treatment procedures (from Sections 3.8.1 through 3.8.3) before biosorption experiments were conducted. An amount of 0.7 g PSMC was weighed and added into 50 mL and 50 mg/L Ni(II) solution in conical flasks. Samples were incubated in an incubator shaker operating at 125 rpm and temperature of 25 ± 1 ^{o}C. After 90 minutes of biosorption process, samples were filtered and filtrates were analysed by ICP-OES. Reproducibility of biosorbent from different sampling batches and repeatability of Ni(II) biosorption efficiency were evaluated through average and standard deviation of experimental data. Samples were prepared and tested in duplicates.

3.10 Advanced Characterisation of Biosorbent

The methods adopted for advanced characterisation analysis are in compliance with specific instruments and their standard methods. Instruments used is described in Section 3.5 and shown in Table 3.5. Details of all biosorbent advanced characteristics analysis are provided in subsequent sections.

3.10.1 Brunauer Emmett Teller (BET) Surface Area Analysis

An amount of 0.2080 g PSMC sample was degassed in vacuum at 200 ^{o}C for at least 6 hours. Nitrogen adsorption/ desorption isotherms were measured using a BELSORP-MINI-II instrument at 77 K. The accuracy of the pressure sensors is 0.25 %. For each data point the sample was exposed to the vapour pressure for 900 seconds. Equilibrium pressure outside of the hysteresis loop was typically attained after about 1 minute. Inside the hysteresis loop, 900 seconds were needed to verify that the pressure is constant. The BET method was used in the calculation of surface area values (*aBET*) from the isotherm of nitrogen adsorption. The mean apparent diameter (*dBET*) was inferred from surface area assuming that PSMC sample has a smooth and spherical shape with a narrow granulometric distribution.

3.10.2 Elemental Analysis

Before analysis, 2.00 g of PSMC sample was dried for two hours at 363 K. Elemental analyses were accomplished in Leco TruSpec CHN and Leco CHNS-932. After correction for ash content, the percentage of elements were calculated by mass difference. Sample was analysed two times with a standard method. The average of CHNSO content in percentage and its absolute standard deviation were calculated.

3.10.3 Cellulose and Lignin Analysis

Chemical analysis of the PSMC for cellulose and lignin content were conducted by methods described in Table 3.7.

Table 3.7 Methodology for cellulose and lignin content analysis

Description	Test method	Unit	No. of specimen
Holocellulose content	Wise, Murphy, and d'Addieco (1946) Sample was treated with 1 % w/v of sodium chlorite and followed by glacial acetic acid in a water bath at 70 ^{o}C. The reaction was carried out for four hours with acetic acid and sodium chlorite added hourly. Then, the sample was filtered through a medium porosity filter glass previously weighed. Finally, sample was dried in a vacuum oven at 40 ^{o}C until a constant weight was recorded.	%	2
Alpha-cellulose content	TAPPI standard T203 cm-09: Alpha-, beta- and gamma-cellulose in pulp (2009)	%	2
Lignin content	TAPPI standard T222 om-02: Acid-insoluble lignin in wood and pulp (2002)	%	2

3.10.4 Scanning Electron Microscope/Energy Dispersive X-ray Spectroscopy (SEM/EDX) Analysis

Samples were prepared by dispersing dry powder of PSMC biosorbent on double sided conductive adhesive tape. Samples were coated with gold by arc discharge method with purpose to increase electric conductivity and to improve the quality of the images. A Leo 1455 VP scanning electron microscope (SEM), in combination with an Oxford Instruments INCA energy dispersive X-ray spectrometer (EDX) was used for sample mapping and analysis. The X-ray element maps were obtained at 20 kV. The secondary electrons (SE) was used to obtain morphology image of sample and back scattered electron (BSE) mode was applied for compositional image. White patches particles were analysed by EDX to ascertain presence of metal elements. A 80 µA beam current

at 20kV accelerating voltage, 10 mm specimen working distance and 15 mm EDX working distance were employed for point analyses. The entire sample was mapped and montaged in the range of 2 Gb range in order to identify the areas of interest. Areas of interest were then mapped at higher resolution of 1024 x 768 and 150 – 200 frames. Meanwhile, phases point analysed with the SEM. INCA software was used to determine the elemental composition of the biosorbent surface. Experiment was repeated with PSMC after heavy metal biosorption.

3.10.5 X-ray Photoelectron Spectrometer (XPS) Analysis

Sample of PSMC was pressed into a tablet of 10 mm diameter and 1 mm of thickness. The prepared PSMC sample was filled to the modified stub XPS sample holder. All XPS measurements were performed with a Kratos Axiz Ultra photoelectron spectrometer. Experiments were conducted in an analysis chamber with a base pressure in the 1 x 10^{-10} Pa range. The monochromatic Al Kα X-ray source was operated at 150 W (15 kV, 10 mA). The calibration on energy scale was referenced to Cu $2p_{3/2}$ and Ag $3d_{5/2}$ peaks at binding energies (BE) of 932.6 eV and 368.2 eV respectively. Charge compensation was provided by a coaxial charge neutralization system.

The analysis area for the 'slot' aperture was set at 2mm x 1mm. The C 1s, O 1s, N 1s, Ca 2p, Pb 4f, Cu 2p and Ni 2p spectra were acquired at a passed energy of 20 eV and 2 minutes per element of acquisition time. Quantitative XPS analysis was analysed with the Kratos VISION software version 2.1.2. The C 1s, O 1s, N 1s, Ca 2p, Pb 4f, Cu 2p and Ni 2p regions were deconvoluted using shirley components. Peaks of BEs were calibrated with reference to the adventitious C 1s photoelectron peak at 284.5 eV to substract the C 1s charging effect background. For semi-quantitative measurement, the atomic concentration and mass concentration of elements were calculated by peak areas

of wide scan spectra at 160 eV passing energy. Similar experiment procedures were conducted for PSMC after heavy metal biosorption samples.

3.10.6 Zeta Potential Analysis

The PSMC sample was ground into fine particle size by using benchtop platenary miller. The suspension was prepared in a polyethylene container by mixing 0.1 g of PSMC sample with 100 mL of UPW. After 10 minutes, the suspension was filtered slowly into the electrophoresis cell. After the voltage was applied, the pH was adjusted in the range of 2 to 9 by drop wise addition of HCl and NaOH before each measurement. The monitored zeta potential was measured by using Zetasizer Nano ZS, Malven with multi-purpose titrator MPT2. The titration conditions for the zeta potential were 0.25 M HCl, 0.5 M HCl and 0.25 M NaOH. Ten replicates for each titration point were conducted. The reliability of the pH measurements was controlled with the standard deviation of the values for less than 0.2.

3.10.7 Fourier Transform Infrared Spectrometry (FTIR) Analysis

The samples were placed onto the Universal Diamond Attenuated Total Reflectance (ATR) top plate and in direct contact with the ATR crystal. The analysis conditions used were 16 scans at a resolution of 4 cm^{-1} measured between 400 - 4000 cm^{-1}. Test was carried out for PSMC and PSMC after heavy metal biosorption samples to identify functional groups involve in biosorption process.

3.10.8 ^{13}C Solid State Nuclear Magnetic Resonance (^{13}C ssNMR) Analysis

Samples of PSMC biosorbent before and after heavy metal biosorption were wetted by using distilled water to increase the mobility of samples in order to obtain higher resolution in NMR anisotropic chemical shift spectra and to make sample condition

more realistic. The ratio of sample to UPW was 2:3 (40 % sample; 60 % UPW). Samples were filled in 4 mm MAS zirconium oxide rotors and sealed with a *Kel-f* cap. The samples were examined on a Bruker Advance 400 MHz spectrometer equipped with a double resonance probe. The spectrometer frequency was set at 100 MHz for ^{13}C nuclei and the ^{1}H proton decoupling field was 78 kHz. When sample was loaded in NMR instrument, wobbling process for tuning and matching of ^{13}C and ^{1}H was conducted.

Test substance of L-alanine and Adamantane were utilised for establishing the calibration for ^{13}C nuclei and ^{1}H proton respectively. Details of experiments are shown in Table 3.8. Cross polarization (CPMAS) and direct pulse saturation recovery (DP SatRec) parameters were extracted from the VCT data – T_{CH} (cross polarization rate constant), $T_1{}^{H}$ (^{1}H spin-locked spin-lattice relaxation time) by curve fitting the normalised signal intensity profiles. Normalised signal intensity of M-M_o over log time profile was plotted to evaluate ^{1}H through direct pulse inversion recovery (DP Invrec) experiments. For TORCHIA experimental result, the normalised signal intensity in log scale time was used to calculate T_1. Mathematical and statistical analysis on chemical shifts and T_1 relaxation time of each sample was carried out.

Table 3.8 Details of ^{13}C ssNMR experimental designs

Experimental design	CPMAS	DP SatRec	DP Invrec	TORCHIA
Software applied	TopSpin 2.0	TopSpin 2.0	TopSpin 3.0	TopSpin 3.0
MAS (kHz)	8	8	10	8
Nucleic	^{13}C	^{13}C	^{1}H	^{13}C
Test substance	L-alanine	L-alanine	Adamantane	L-alanine
90^{o} pulse length (μs)	3.2	3.2	3.2	3.2
Contact time (s)	0.00005-0.003	0.01-10	0.1-64, 0.00001-30	0.01-10
No. of scan	32000	20000	4000	1500
Pulse program	cf.cp	ch.satrect	sp, ch.invrec	cf_torchia
Reference	Appendix A1	Appendix A2	Appendix A3	Appendix A4

3.11 Optimisation of Biosorption Study

The optimum biosorption conditions were determined as a function of biosorbent concentration, initial pH, contact time, initial heavy metal concentration and temperature. Summary range of abiotic factors used in this study for heavy metal biosorption is presented in Table 3.9 while the biosorption procedure is shown in Figure 3.2.

Table 3.9 Summary of abiotic factors range for heavy metal biosorption

Abiotic factors	Range				
	Single heavy metal	Bi-heavy metal			Multi-heavy metal
		PbCu	PbNi	CuNi	PbCuNi
Biosorbent concentration, g	0.02–3.0	-	-	-	-
Initial pH	1-6	1-5.28	1-5.16	1-5.46	1-5.5
Contact time, min	0.5-120	0.5-120	0.5-120	0.5-120	0.5-120
Initial heavy metal concentration, mg/L	10-60	10-60	10-60	10-60	10-60
Temperature, ^{o}C	5-35	-	-	-	-

– Not applicable

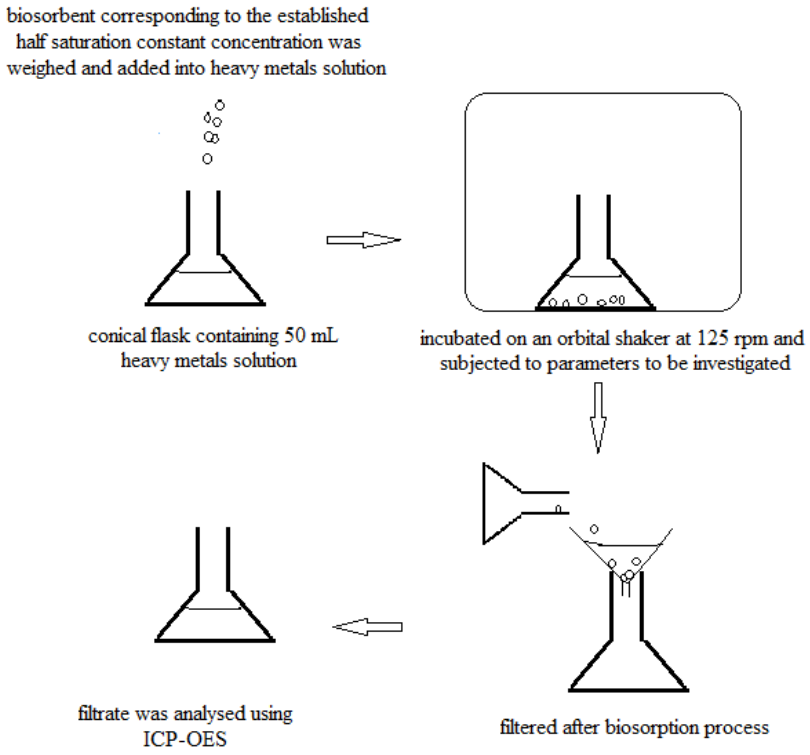

Figure 3.2 Schematic diagram of heavy metal biosorption procedure

All experiments were conducted in batch mode and samples were prepared in duplicates. The expressed values represented the average of the obtained ICP-OES analysis results and standard error bar of standard deviation corresponding to ± 1 SD. The experimental data was analysed by the Equation 3.2 in order to obtain the percentages of heavy metal biosorption (*B*).

Finally, pH of samples was measured using a metrohm pH meter fitted with a combine glass microelectrode. Details of five optimisation parameters of heavy metal biosorption process are discussed in subsequent sections.

3.11.1 Biosorbent Concentration

Biosorbent concentration that plays a prominent role as limiting factor in this study was investigated. A range of biosorbent concentration from 0.02 to 8.0 g was added into

conical flasks containing 50 mL and 50 mg/L heavy metal solution. The samples were placed in an incubator shaker operating at 125 rpm for 60 minutes at 25 ± 1 ^{o}C. After an hour of biosorption, samples were filtered through Advantec 5C filter paper with pore size < 5 µm. The filtrates were later analysed using ICP-OES.

Optimisation result for biosorbent concentration was fitted to Hanes-Woolf plot in order to determine the half saturation constant (K_m) (Hanes, 1932). It is based on the rearrangement of the Michaelis Menten equation as shown in Equation 3.3.

$$\frac{[S]}{r} = \frac{[S]}{r_{\max}} + \frac{K_m}{r_{\max}} \qquad \text{(Equation 3.3)}$$

Where S = the initial biosorbent concentration (g), r_{max} = the maximum reaction rate (mg/min), K_m = the half saturation constant (g).

The established half saturation constant concentration of biosorbent for each heavy metal was employed in subsequent experiments with the aim to reduce biosorbent usage and obtain result in shorter time.

3.11.2 Initial pH

Experiments were carried out at different initial pH values, in the range of pH 1 - 6 for heavy metal solutions. The samples were also examined at normal pH of heavy metal solution without prior pH adjustment. The initial pH of heavy metal solution was adjusted to the desired pH by using sodium hydroxide and hydrochloric acid. Samples corresponding to the amount of biosorbent utilised at the established half saturation constant concentration in Section 3.9.1 were weighed out and added into different conical flasks with initial pH measured in 50 mL and 50 mg/L heavy metal solutions. Through these steps, samples were agitated at 125 rpm for 60 minutes in an incubated

shaker of 25 ± 1 ^{o}C. Samples were separated by filtration; the filtrates were analysed using ICP-OES. The initial pH that gives the maximum heavy metal biosorption was selected for subsequent experiments.

3.11.3 Contact Time

The time dependence experiments were performed at the established half saturation constant concentration of biosorbent and optimal pH conditions (from Sections 3.9.1 and 3.9.2). The contact time for this study was 0.5 - 120 minutes. Initially, the biosorbent concentration at half saturation constant was weighed and added into 50 mL and 50 mg/L heavy metal solution in conical flasks. The experiments were conducted in an incubator shaker of 125 rpm, temperature at 25 ± 1 ^{o}C for 0.5 – 120 minute contact time. After that, the samples were filtered and filtrates were analysed using ICP-OES. The contact time at maximum biosorption percentage at saturation phase was used in subsequent experiments. This corresponded to the minimum time needed for maximum heavy metal biosorption.

3.11.4 Initial Heavy Metal Concentration

The effect of the initial heavy metal concentration on heavy metal biosorption by PSMC was examined. The established half saturation constant concentration of biosorbent, optimal initial pH and maximum contact time obtained from Sections 3.9.1 through 3.9.3 were utilized in this study. The weighed PSMC biosorbent corresponding to the established half saturation constant concentration was added into 50 mL of 10, 20, 30, 40, 50 and 60 mg/L heavy metal solutions. The conical flasks were placed on an incubator shaker at 125 rpm and temperature 25 ± 1 ^{o}C for a period of selected optimal contact time. After filtration, the filtrates were analysed by ICP-OES. The lowest initial

heavy metal concentration was selected for temperature experiments as biosorption equilibrium condition can be achieved in shorter time.

3.11.5 Temperature

Temperatures of 5, 15, 25 and 35 ± 1 ^{o}C were investigated. The established parameters of biosorbent concentration, initial pH, contact time and initial heavy metal concentration were used in this study. Samples were prepared by adding weighed biosorbent corresponding to the established half saturation constant concentration into conical flasks with 10 mg/L and 50 mL heavy metal solution. Experiments were carried out in an incubator shaker operating at 125 rpm under controlled temperature of 5, 15, 25 and 35 ± 1 ^{o}C. After selected contact time of biosorption process, samples were filtered and filtrates were examined by ICP-OES for heavy metal concentrations.

For bi-heavy metal and multi-heavy metal studies, initial heavy metal concentrations were prepared in 50 mg/L in total with ratio 1:1 and 1:1:1 respectively. The half saturation constant concentration of biosorbent established from single heavy metal experiments in Section 3.10.1 were employed in this study. The optimisation parameters procedures as discussed in Sections 3.10.2 through 3.10.4 were repeated in subsequent experiments to determine the selectivity of the biosorbent to heavy metal.

3.12 Evaluation of Existing Mathematical Models

Experimental data was evaluated by three types of existing mathematical models, namely, isotherms, kinetics and thermodynamic. Langmuir isotherm model, pseudo first-order and second-order kinetic models and thermodynamic model will be addressed in following sections.

3.12.1 Langmuir Isotherm Model

The initial heavy metal concentration experimental results were evaluated by Langmuir isotherm to determine the maximum heavy metal uptake (Langmuir, 1916 and 1917). The biosorption parameters were calculated from the isotherm using linearized Langmuir plot of C_e/q_e versus C_e (Equation 3.4).

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{b\ q_{\max}}$$

(Equation 3.4)

Where q_e = heavy metal uptake by biosorbent (mg/g), q_{max} = maximum heavy metal uptake (mg/g), C_e = heavy metal concentration at the equilibrium stage (mg/L), b = Langmuir constant (L/mg).

3.12.2 Pseudo First-order and Second-order Kinetic Models

The experimental data from contact time experiments were fitted to linearized pseudo first-order and second-order kinetic models as shown in Equation 3.5 and Equation 3.6 respectively (Benguella and Benaissa, 2002).

$$\log(q_e - q_t) = \log q_e - \frac{k_1\ t}{2.303}$$

(Equation 3.5)

$$\frac{t}{q_t} = \frac{1}{2\ k_2\ q_e^{\ 2}} + \frac{t}{q_e}$$

(Equation 3.6)

Where q_e = heavy metal uptake by biosorbent at equilibrium (mg/g), q_t = heavy metal uptake by biosorbent at time (mg/g), t = time (min), k_1 = rate constant of pseudo second-order (1/min), k_2 = rate constant of pseudo second-order (g/mg/min).

3.12.3 Thermodynamic Model

Results obtained from temperature optimisation studies were evaluated. Thermodynamic parameters such as change in Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) were determined using the following equations (Reddy and Dunn, 1986; Khan and Singh, 1987):

$$K_c = \frac{C_b}{C_e}$$

(Equation 3.7)

$$\log K_c = -\frac{\Delta H}{2.303RT} + \frac{\Delta S}{2.303R}$$

(Equation 3.8)

$$\Delta G = \Delta H - T\Delta S$$

(Equation 3.9)

Where K_c = the distribution coefficient (L/g), C_b = heavy metal concentration in biosorbent (mg/L), C_e = heavy metal concentration in solution at equilibrium (mg/L), T = temperature (K), R = the gas constant (J/(K.mol)), ΔG = free energy (kJ/mol). ΔH and ΔS were obtained from the slope and intercept of the plots of log K_c versus 1/T.

3.13 Application of Biosorbent

In this study, application of biosorbent was evaluated via biosorbent performance in automobile wastewater and recovery of heavy metal from biosorbent. Details of experiments are described in following sections.

3.13.1 Biosorbent Performance in Treatment of Automobile Wastewater Containing Ni(II)

Sampling of wastewater containing Ni(II) from automobile industry was conducted five times (n = 5). The selected operating conditions for Ni(II) biosorption was applied to evaluate the biosorbent performance in treatment of automobile wastewater. The PSMC

biosorbent corresponding to the established Ni(II) half saturation constant concentration was weighed and added into 50 mL of collected wastewater samples. Samples were shaken at 125 rpm and temperature of 25 ± 1 ^{o}C for 10 minutes. After selected contact time of biosorption process, samples were filtered and analysed using ICP-OES. Finally, biosorption efficiency in treatment of automobile wastewater was evaluated via Equation 3.2 and compared with results obtained from Ni(II) biosorption in synthetic solutions.

3.13.2 Recovery of Heavy Metal from Biosorbent

Recovery of heavy metal from biosorbent was investigated using 0.1 M HNO_3 as elution solution. First, experiments using batch mode single heavy metal biosorption process was conducted with selected operating conditions from Sections 3.10.1 through 3.10.3. The amount of biosorbent corresponding to the half saturation constant concentration was weighed and added into 50 mL and 50 mg/L heavy metal concentration in centrifuge tubes. Samples were incubated in incubator shaker at 125 rpm and temperature of 25 ^{o}C for selected biosorption contact time. After heavy metal biosorption process, samples were centrifuged at 5000 rpm for 10 minutes. Supernatants were collected and analysed using ICP-OES. The biosorbent samples were washed intensively with 20 mL UPW for three times. Next, 50 mL of 0.1M HNO_3 was added into centrifuge tubes containing biosorbent. Samples were placed in an orbital incubator shaker at 125 rpm and temperature of 25 ± 1 ^{o}C for 10 minutes to allow for desorption process. Samples were centrifuged again and supernatants were collected for ICP-OES analysis. Finally, biosorbent samples were washed with 20 mL UPW and dried in an oven until a constant weight was recorded. The results were analysed by Equation 3.10 and Equation 3.11 in order to calculate the recovery percentage of heavy metal from biosorbent and percentage weight loss of biosorbent.

$$Percentage\ of\ heavy\ metal\ recovery,\ C\ \% = \frac{C_b - C_d}{C_b} x\ 100\%$$

(Equation 3.10)

$$Percentage\ of\ biosorbent\ weight\ loss,\ W\ \% = \frac{W_b - W_d}{W_b} x\ 100\%$$

(Equation 3.11)

Where C_b = the heavy metal concentration after biosorption (mg/L), C_d = the concentration of heavy metal after desorption (mg/L), W_b = the initial weight of biosorbent before biosorption (mg/L), C_d = the weight of biosorbent after desorption (mg/L).

3.14 Artificial Neural Network (ANN) Modelling

MATLAB version 7.5.0.342 (R2007b) software was used to predict the percentage of Ni(II) biosorption, uptake of Ni(II) by PSMC biosorbent and effluent quality. The ANN modelling included ANN structure development, ANN structure optimisation and sensitivity analysis. Detail descriptions of ANN modelling are described in subsequent sections.

3.14.1 ANN Structure Development

Experimental data from biosorption optimisation study in synthetic Ni(II) solutions was categorized into two different data sets, namely training data set (50%) and testing data set (50%), in order to avoid producing bias results. Training and testing data set consisted of 36 experimental sets each. Four variables were selected for input data, namely, biosorbent concentration, initial pH, contact time and initial Ni(II) concentration. Meanwhile, percentage of Ni(II) biosorption, Ni(II) uptake of biosorbent and effluent quality were the three output variables for the model. All input and output data were normalized within the range of 0 to 1 to ensure that values from different

parameters are within the same range. Typically, tangent and linear transfer functions were used for input and output layer which are bounded by 0 to 1 and -1 to 1, respectively. A single hidden layer feed forward networks and back propagation training algorithm was systematically trained using the four input and three output variables for adjusting the network weights. The Levenberg-Marquardt algorithm is the fastest training algorithm for network of moderate size; therefore, it was selected in the present study. A similar architecture and training algorithm has been published previously by Taib *et al*. (1996) and Brook *et al*. (1997).

3.14.2 ANN Structure Optimisation

The epochs between displays of 10, ratio to increase learning rate of 0.001, maximum number of epochs to train of 1000 and performance goal of 0.00001 were set based on empirical approach. With the above fixed parameters, optimal step sizes taken in weight space were function of the hidden layer, learning rate and momentum rate.

3.14.2.1Hidden layer

The developed ANN structure was trained for 1 to 25 hidden layers with fixed parameters of 0.9 learning rate and 0.7 momentum rate. After training session, the training errors of mean square error (m.s.e) and sum square error (s.s.e) were recorded. The hidden layer with minimum m.s.e and s.s.e values were selected for subsequent experiments.

3.14.2.2Learning rate

The training of ANN structure was performed at the selected hidden layer as outlined in Section 3.14.2.1. The learning rate of 0.1 to 1.2 was investigated with the fixed momentum rate of 0.7. The obtained m.s.e and s.s.e from training results were

documented and compared. The optimal condition at minimum m.s.e and s.s.e values were applied in following momentum rate experiments.

3.14.2.3Momentum rate

The selected hidden layer and learning rate from Section 3.14.2.1 to 3.14.2.2 were utilised in this study. Momentum rate of 0.1 through 1.2 was used in this study. Results based on m.s.e and s.s.e value were compared. The minimum m.s.e and s.s.e momentum rate was selected in order to develop the optimised ANN model. Finally, the ANN structure with the minimum m.s.e and s.s.e were selected for the ANN model and used in sensitivity analysis.

3.14.3 Sensitivity Analysis

The developed ANN model was tested with testing data set. Performance validation was based on m.s.e and s.s.e. Experimental data was compared to prediction data for both training and testing data sets to evaluate the effectiveness of developed ANN model.

3.15 Concluding Remarks

The apparatus and procedures adopted in this study described in Chapter 3 were developed to determined advanced characterisation of biosorbent and ANN model with multiple output for Ni(II) biosorption process. Furthermore, new approaches of washing pre-treatment in biosorbent preparation and the half saturation constant concentration of biosorbent in heavy metal biosorption were successfully developed and evaluated in this study. Field work sampling, experimental laboratory work and mathematical analysis including modelling were conducted to evaluate viability and potential of PSMC as biosorbent for heavy metal biosorption. Results of activities described in this chapter are presented in Chapter 4.