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Structures, Energies, and Bonding Analysis of Monoaurated Complexes with N-Heterocyclic Carbene and Analogues

T.A.N. NGUYEN[1]*, T.P.L. HUYNH[1], T.X.P. VO[1], T.H. TRAN[1], D.S. TRAN[2],
T.H. DANG[3] AND T.Q. DUONG[4]

In this work, we computationally investigated from quantum chemical calculations (DFT) at the BP86 level with the various basis sets def2-SVP, def2-TZVPP, and TZ2P+, chemical bonding issues of the recently described carbene-analogues gold(I) complexes AuCl-NHE_{Me} (Au1-NHE) with E = C – Pb. The optimized structures and the metal-ligand bond dissociation energy (BDE) were calculated, and the nature of the E→Au bond was studied with charge and energy decomposition methods. The equilibrium structures of the system showed that there were major differences in the bonded orientation from the ligands NHC-NHPb to gold(I) complex between the lighter and the heavier homologues. The BDEs results showed that the metal-carbene analogues bonds were very strong bonds and the strongest bond was calculated for Au1-NHC which had the bond strength D_e = 79.2 kcal/mol. Bonding analysis of Au1-NHE showed that NHE ligands exhibited donor-acceptor bonds with the σ lone pair electrons of NHE donated into the vacant orbital of the acceptor fragment (AuCl). The EDA-NOCV results indicated that the ligand NHE in Au1-NHE complexes were strong σ-donors and very weak π donor and the bond order in complexes was Au1-NHC > Au1-NHSi > Au1-NHGe > Au1-NHSn > Au1-NHPb. We also realised that the gold-ligand bond was characterized by a π back-donation component from the Au to the ligand. All investigated complexes in this study were suitable targets for synthesis and gave a challenge in designing Au nano-crystals of narrow size distribution from gold(I) complexes that carried versatile N-heterocyclic carbene-analogues NHE.

Key words: N-heterocyclic carbene ligands; bond dissociation energy; EDA-NOCV; gold; DFT calculations

The first direct synthesis of metal complexes with N-heterocyclic carbenes (NHCs) as ligand was pendently presented by Hans Werner Wanzlick (Wanzlick 1968) and Karl Öfele (1968). After that the break through result of the isolation of stable carbenes was reported by Arduengo *et al*. (1991). It has been known that NHCs have emerged as an essential class of ligands in inorganic and organometallic chemistry (Bourissou *et al*. 2000; Hahn & Jahnke 2008; Herrmann 2002). In the report of Jacobsen (Jacobsen *et al*. 2009) showed that NHCs display particular properties across the wide family of neutral ligands used in catalysis due to strong σ-donating character. Furthermore, ligand NHCs exhibit a special

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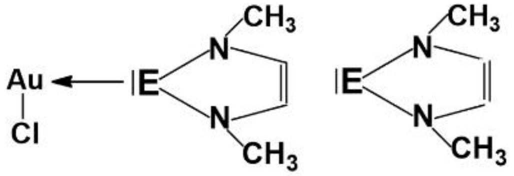
[1] Department of Chemistry, Hue University of Sciences, Hue University, 77 Nguyen Hue, Hue, Vietnam
[2] Department of Chemistry, Quang Binh University, 312 Ly Thuong Kiet, Dong Hoi, Quang Binh, Vietnam
[3] HCMC University of Food Industry, 140 Le Trong Tan, Tan Phu, Ho Chi Minh, Vietnam
[4] Department of Chemistry, Hue University of Education, Hue University, 34 Le Loi, Hue, Vietnam
* Corresponding author (e-mail: ainhungnguyen.chem@gmail.com)

geometry associated with a flexible structure allowing fine-turning of their steric properties (Poater *et al.* 2009). In addition to this, NHCs can be very popular used for a large variety of reactions of high synthetic interest and these two-coordinate forms of carbon with two unpaired electrons have been considered as "new" ligands for bioactive coordination compounds (Hermann *et al.* 2002; Nemcsok *et al.* 2004). It has been noted that NHCs ligands can be stabilized by two nitrogens and form stable complexes with transition metals (Ag, Au) and with main-group elements (Nemcsok *et al.* 2004). Although the first stable transition metal carbene complex was investigated in 1964 (Fischer & Maasböl 1964), but after a long time, the metal-ligand bonding in complexes of mixed carbene-halogen complexes (NHC-TMX with TM = Cu, Ag, Au and X = F – I) was published for using a charge decomposition analysis which was noticed for the first time by Frenking and Boehme (1998) and group 11 elements (Cu, Ag, Au) called as coinage metals, have aroused intense interest (Zhu *et al.* 2012). The chemical bonding between NHCs and group 11 metals have been investigated theoretically (Nemcsok *et al.* 2004; Hu *et al.* 2004). Moreover, theoretical studies of the electronic structure of transition metal complexes with NHCs ligand have been recently carried out by other group (Schwarz *et al.* 2000; Weskamp *et al.* 1999; Lee *et al.* 2004). The fact was that, the type of NHC ligands that have been developed by Arduengo *et al.* [1991] have found recent use in the synthesis of molecular gold(I) fluoride and chloride complexes. Particularly in recent years, it has been known that gold could from stable coordination complexes with NHC ligand (Marion & Nolan 2008; Nolan 2011; Zhu *et al.* 2012). The nature of the Au-NHC binding has been presented from the structures and properties of the complexes (Nemcsok *et al.* 2004). In this study, we want to choose NHC_{Me} and to extend to the heavier homologues in order to give insight into the structures and bonding situation using NBO and energy decomposition analysis (EDA) with the set of orbitals — the natural orbitals for chemical valence (NOCV) methods. The main purpose of this study was to investigate in details the bond strength of Au-E bond; the nature of the Au-E bond in AuCl-ligands; and the differences in the Au-ligand bonding from the carbene to plumbylene complexes. *Scheme 1* shows the overview of the compounds investigated in the presented work and the schematic representation of a donor-acceptor bonding in Fischer-type carbene complex is shown in *Scheme 2*. The C atom in NHC_{Me} ligand retains one lone pairs which is formally formed from a carbene ligand in a triplet state to a triplet AuCl fragment. To understand the gold-chloride and NHE ligands interactions in complexes, we have carried out density functional theory calculations. We investigated the bonding situation in complexes and the electronic structure of the molecules was analyzed with charge- and energy-decomposition methods.

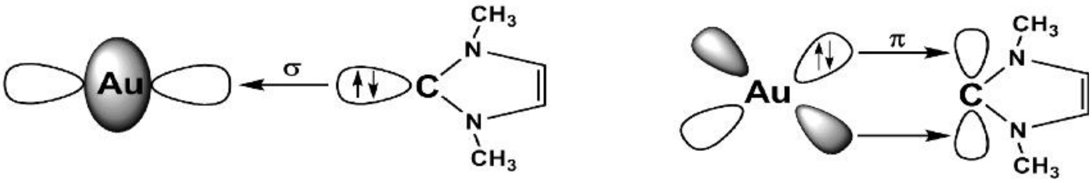


(a) (b)

Scheme 1. *Overview of the compounds investigated in the present work: (a) Complexes [AuCl-{NHE_{Me}}] (Au1-NHE) and (b) Ligand NHE_{Me} (NHE) with E = C, Si, Ge, Sn, Pb.*

COMPUTATIONAL DETAILS AND THEORETICAL ASPECTS

The geometries of the gold(I) carbene-analogues complexes [AuCl-{NHC_{Me}}]−[AuCl-{NHPb_{Me}}] (Au1-NHC − Au1-NHPb) were carried out at the gradient corrected DFT level of theory using Beck's exchange functional (Becke 1998) in conjunction with Perdew's correlation functional (BP86) (Perdew 1986). The calculations were carried out using



Scheme 2. *Schematic representation of donor-acceptor bonding in Fischer-type of AuCl complex that carries NHC ligand.*

the program package Gaussian03 (Frisch *et al.* 2004) optimized together with Turbomole 6.01 (Ahlrichs *et al.* 1989). All geometries were fully optimized without any symmetry constraints. A *triple zeta* valence basis set (def2-SVP) (Schäfer *et al.* 1992) was used for all of the main group elements and the relativistic of effective core potentials (ECPs) (Weigend & Ahlrichs *et al.* 2005) were applied for the heavier group-14 atoms Sn, Pb, and atom Au. The nature of the stationary points was checked by frequency calculations at the same level of theory (BP86/def2-SVP). All the structures of the complexes were verified as minimum by confirming that their respective Hessians were real on the BP86/def2-SVP level. The calculation of the bond dissociation energies (BDEs) and the charge analysis with Wiberg bond indices (WBI) as well as the natural partial charges at the BP86/def2-TZVPP (Snijders *et al.* 1981) //BP86/def2-SVP level of theory were carried out by using NBO 3.1 partitioning method in Gaussian03. Next, the bonding analysis was considered by using the Ziegler-Rauk-type energy decomposition analysis (EDA) (Ziegler & Rauk 1977) and natural orbital for chemical valence (NOCV). All complexes on the BP86/def2-SVP optimized structures were re-optimized by using the BP86/TZ2P+ of core functional/basis set combination as implemented in ADF 2013.01 (Velde *et al.* 2001). An auxiliary set of *s*, *p*, *d*, *f*, and *g* STOs was used to fit the molecular densities and to represent the Coulomb and exchange potentials accurately in each SCF cycle (Krijn & Baerends 1984). Relativistic effects were taken by means of the zeroth-order regular approximation (ZORA) Hamiltonian (Velde *et al.* 2001; Lenthe *et al.* 1993; Lenthe *et al.* 1996) with a small frozen core. The nature of the Au-E bonds in Au1-NHC−Au1-NHPb were investigated at BP86/TZ2P+ with the EDA-NOCV (Mitoraj & Michalak 2007a; Mitoraj & Michalak 2007b; Mitoraj *et al.* 2009) method which combines the EDA (Nemcsok *et al.* 2004) with the NOCV (Mitoraj & Michalak 2007b; Mitoraj *et al.* 2009) under C1 symmetric geometries (without symmetry).

Herein, we want to present a detailed theoretical aspect about the EDA-NOCV method. The EDA gave very well-defined energy terms for the chemical bonds in molecules. In the EDA developed independently by different groups (Morokuma 1971; Ziegler & Rauk 1979), and the recently introduced EDA-NOCV, the bond dissociation energy, D_e, of a molecule was divided into the instantaneous interaction energy ΔE_{int} and the preparation energy ΔE_{prep}:

$$\Delta E\ (= -D_e) = \Delta E_{int} + \Delta E_{prep} \qquad (1)$$

The preparation energy ΔE_{prep} was the energy which was required to promote the fragments from their equilibrium geometries in the electronic ground state to the geometries and electronic reference state which they had in the molecule. The interaction energy ΔE_{int} could be further divided into three main components:

$$\Delta E_{int} = \Delta E_{elstat} + \Delta E_{Pauli} + \Delta E_{orb} \qquad (2)$$

where, ΔE_{elstat} is the quasiclassical electrostatic interaction energy between the fragments, calculated by means of the frozen electron density distribution of the fragments in the geometry of the molecules. ΔE_{Pauli} was referred to the repulsive interactions between the fragments which were caused by the fact that two electrons with the same spin could not occupy the same region in space, and could be calculated by enforcing the Kohn–Sham determinant on the superimposed fragments to obey the Pauli principle by anti-symmetrisation and renormalisation. The stabilising orbital interaction term ΔE_{orb} was calculated in the final step of the energy partitioning analysis when the Kohn–Sham orbitals relaxed to their optimal form. The EDA-NOCV method combined the charge (NOCV) and energy (EDA) partitioning schemes to decompose the deformation density which was associated with the bond formation, $\Delta\rho$, into different components of the chemical bond. Furthermore, the EDA-NOCV calculations also provided pair wise energy contributions for each pair or interacting orbitals to the total bond energy. NOCV is defined as the eigenvector of the valence operator, ν, given by *Equation 4*:

$$\mathrm{v}\ \psi_i = \upsilon\ \psi_i \tag{4}$$

In the EDA-NOCV scheme the orbital interaction term, ΔE_{orb}, is given by *Equation 5*:

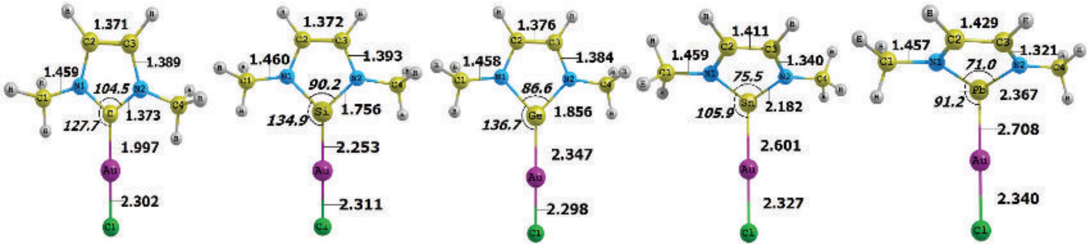
$$\Delta E_{\mathrm{orb}} = \sum_{k=1}^{N/2} \Delta E_k^{\mathrm{orb}} = \sum_{k=1}^{N/2} \upsilon_k \left[-F_{-k,-k}^{\mathrm{TS}} + F_{k,k}^{\mathrm{TS}} \right] \tag{5}$$

in which $F^{TS}_{-k,-k}$ and $F^{TS}_{k,k}$ were diagonal transition-state Kohn–Sham matrix elements corresponding to NOCVs with the eigenvalues $-\upsilon_k$ and υ_k, respectively. The ΔE_k^{orb} term of a particular type of bond was assigned by visual inspection of the shape of the deformation density, $\Delta\rho_k$. The EDA-NOCV scheme thus provided information about the strength of orbital interactions in terms of both, charge ($\Delta\rho_{orb}$) and energy contributions (ΔE_{orb}) in chemical bonds.

RESULTS AND DISCUSSION

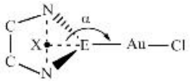
Structures and Energies

The theoretically predicted geometries of Au1-NHC−Au1-NHPb with bond length, bond angle, and bending angle are shown in *Figure 1* together with *Table 1*. Complexes Au1-NHC−Au1-NHPb clearly feature η^1 coordinated AuCl at the central E atom (E = C − Pb). To the best of our knowledge, there was no experimental geometries for gold(I) complex that carried $\mathrm{NHE_{Me}}$. Note that the theoretical study with geometries and bond dissociation energies of less bulky N-heterocyclic carbene, silylene, and germylene complexes of MCl (M = Cu, Ag, Au) have been investigated by Boehme and Frenking (1998) for the first time in the recent past. Moreover, we have found somewhere else that the theoretical as well as the experimental geometries of related carbene complexes where the substituents at nitrogen was R = hydrogen, benzyl, benzoyl, were in good agreement with our calculated values (Boehme & Frenking 1998; Bovio *et al.* 1993). The calculated Au-C bond length of Au1-NHC gives the shortest value (1.997 Å) and the theoretically predicted Au-E bond lengths of complexes Au1-NHC−Au1-NHPb in this study increased from 1.997 to 2.708 Å. This could be easily explained by the increasing radii of the group-14 atoms. The calculated equilibrium structures of complexes Au1-NHC−Au1-NHPb in *Table 1* show that lighter ligands NHE (E = C – Ge) were bonded in a head-on way to the metal fragment AuCl in which the bending angle was 180°. A comparison of the bending angle of the theoretical structures of $\mathrm{Au1\text{-}NHC_H}$ − $\mathrm{Au1\text{-}NHGe_H}$ indicates that our calculated values are quite similar (Boehme & Frenking 1998). In contrast to that the bending angle of Au1-NHE



Aul-NHC Aul-NHSi Aul-NHGe Aul-NHSn Aul-NHPb

Figure 1. Optimized geometries of complexes Au1-NHC−Au1-NHPb at the BP86/def2-SVP level. Bond lengths are given in Å; angles in degrees. The bending angle, α, is the angle X-E-Au where X is the mid-point between the N-N distance:



became much more acute when E was heavier (bending angles α of Au1-NHSn = 110.3° and Au1-NHPb = 91.5°). We want to discuss the changes in the geometries of free ligands and AuCl in complexes. *Table 1* shows that the bond lengths E-N in the complexes increased from C-N to Pb-N and those were shorter than in the free ligands in which the calculated values for the free ligands $NHC_{Me} – NHPb_{Me}$ have recently been discussed by us (Nguyen *et al.* 2015). The increase of Au-Cl distances from the lighter to the heavier complexes exhibits the same trend compared with the values in the $AuCl-NHE_H$ (E = C – Pb) complexes (Boehme & Frenking 1998).

Table 1 also shows the calculated BDEs for the $Au-NHE_{Me}$ bonds. There was a significant decrease from the carbene complex Au1-NHC (D_e = 79.2 kcal/mol) to the silylene Au1-NHSi complex (D_e = 67.0 kcal/mol) and continuous decrease for the BDEs of the heavier group-14 ligands (51.9 – 42.7 kcal/mol). The calculations suggest that the NHC_{Me} ligand in Au1-NHC is the strongest bonded while the heavier homologues Au1-NHE where E = Si, Ge, Sn, Pb have weaker bonds which are not much different compared with the BDEs of the complexes in the previous studies (Boehme & Frenking 1998; Nguyen & Frenking 2012). The trend of the theoretically predicted AuCl-carbene and analogues bond energy in this study was significantly higher than the calculated values for the borane-NHE_{Me} complexes (D_e= 59.8 – 13.8 kcal/mol) (Nguyen *et al.* 2015), the classical Fischer complex $(CO)_5W-CH(OH)$ (D_e = 75.0 kcal/mol) (Vyboishchikov & Frenking 1998) as well as the $(CO)_5W$-carbene (D_e = 54.4 kcal/mol) and analogues (D_e= 44.3 – 25.5 kcal/mol) {$(CO)_5W$-NHE with E = C – Pb} (Nguyen & Frenking 2012). This was quite suitable because the metal-NHE_{Me} interactions of NHE_{Me}-AuCl had small NHE_{Me}←AuCl π-back-donation in complexes. From this, it follows that the monoaurated donor-acceptor complexes with carbene, silylene, and germylene ligands could have very strong bonds and the appearance of a small contribution in free ligands←AuCl π-back-donation in complexes would be further explained in bonding analysis.

Analysis of the Bonding Situation

The bonding situation in the complexes Au1-NHC−Au1-NHPb was analyzed using charge- and energy-decomposition methods. *Table 2* shows the results of the NBO partitioning scheme and the Wiberg bond indices as well as the natural partial charges. The calculated partial charges showed that the metal fragment AuCl in the complexes carried always a negative charge which increases from Au1-NHC (–0.31 e) to Au1-NHPb (–0.53 e).

Table 1: *Bond length (Å), bond angle (°), and bending angle (α°) for the optimized neutral molecules of Au1-NHC – Au1-NHPb calculated at the BP86/def2-SVP level, and calculated bond dissociation energy, D_e (kcal/mol) for the dissociation of one molecule of AuCl from Au1-NHC to Au1-NHPb at the BP86/def2-TZVPP//BP86/def2-SVP level of theory.*

Molecule	Bonding (Å)	Bonding angle (°)	Bending angle (α°)	D_e (kcal/mol)
Au1-NHC	C-Au = 1.997 C-N = 1.373 Au-Cl = 2.302	N1CN2 = 104.5 N1CAu = 127.7	180.0	79.2
Au1-NHSi	Si-Au = 2.253 Si-N = 1.756 Au-Cl = 2.311	N1SiN2 = 90.2 N1SiAu = 134.9	180.0	67.0
Au1-NHGe	Ge-Au = 2.347 Ge-N = 1.856 Au-Cl = 2.298	N1GeN2 = 86.6 N1GeAu = 136.7	180.0	51.9
Au1-NHSn	Sn-Au = 2.601 Sn-N = 2.182 Au-Cl = 2.327	N1SnN2 = 75.5 N1SnAu = 105.9	110.3	44.2
Au1-NHPb	Pb-Au = 2.708 Pb-N = 2.367 Au-Cl = 2.340	N1PbN2 = 71.0 N1PbAu = 91.2	91.5	42.7

Table 2. NBO results with Wiberg bond indices (WBI) and natural population analysis (NPA) at the BP86/def2-TZVPP// BP86/def2-SVP level for complexes Au1-NHC – Au1-NHPb. The partial charges, q, are given in electrons [e].

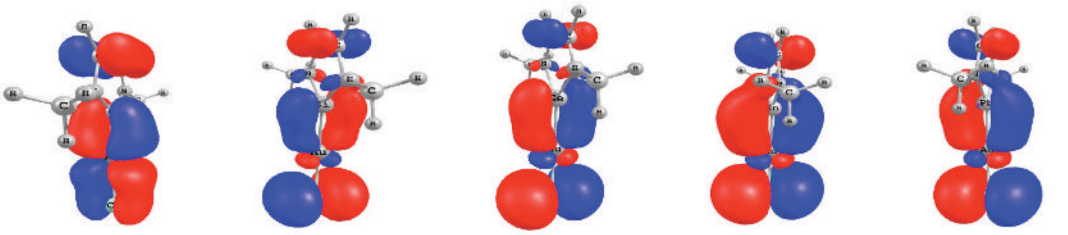
Molecule	Bond	WBI	*q*[AuCl]	Atom	NPA (*q*)
Au1-NHC	Au-C	0.70	–0.31	Au	0.22
	C-N1	1.25		C	0.13
	C-N2	1.25		N	–0.31
Au1-NHSi	Au-Si	0.88	–0.43	Au	0.10
	Si-N1	0.82		Si	1.16
	Si-N2	0.82		N	–0.71
Au1-NHGe	Au-Ge	0.75	–0.37	Au	0.14
	Ge-N1	0.80		Ge	1.07
	Ge-N2	0.80		N	–0.68
Au1-NHSn	Au-Sn	0.62	–0.47	Au	0.04
	Sn -N1	0.61		Sn	0.86
	Sn -N2	0.61		N	–0.56
Au1-NHPb	Au-Pb	0.61	–0.53	Au	–0.01
	Pb-N1	0.49		Pb	0.74
	Pb-N2	0.49		N	–0.49

The amount of charge donation to the AuCl fragment is always smaller than the donation to other transition metal moieties such as $W(CO)_5$ and $Mo(CO)_4$ that carry the similar NHE_{Me} ligands in the complexes which have been calculated in the previous papers (Nguyen & Frenking 2012; Nguyen *et al*. 2014a; Nguyen *et al*. 2014b) in which the more negative charges in transition metal fragments $W(CO)_5$ and $Mo(CO)_4$ are between –0.47 and –0.77 e from the lighter to the heavier homologues.

The Wiberg bond orders for the Au-E bond in Au1-NHC was 0.70 and increased in the Au1-NHSi (0.88) and then decreased from Au1-NHGe to Au1-NHPb which were from 0.75 to 0.61. The bond order for the E-N bond became clearly larger in the complexes Au1-NHC−Au1-NHPb compared with the free ligands NHC_{Me} – $NHPb_{Me}$. This is in agreement with the change in the E-N bonds which become shorter in the complexes than in the free ligands (Nguyen 2015; Nguyen *et al.* 2014b). The natural population analysis carried out for complexes found that the electrostatic charges of carbon atom in the NHC_{Me} fragment of Au1-NHC complex was nearly neutral whereas Si and Ge carried large positive charges which were 1.16 and 1.07 e and then slightly decreased in the heavier homologues (Sn = 0.86 e and Pb = 0.74 e).

As mentioned in the computational methods, all complexes were considered under without any symmetry constraints meant the molecules had C1 symmetry. So there were no genuine σ and π orbitals because there was no mirror plane in the molecular structure. Although the lighter complexes Au1-NHE exhibited the end-on bonded in the NHE_{Me} ligands (E = C – Ge) whereas the heavier ligands $NHSn_{Me}$ and $NHPb_{Me}$ were bonded side-on to the metal fragment AuCl. In order to consider the strength of the π donation NHE_{Me}→AuCl which might be expected from the σ- and π lone-pair orbital of the ligand NHE_{Me} into the second vacant coordination side of metal fragment AuCl, we had to visually keep the shapes of Au1-NHSn and Au1-NHPb in one plane to identify σ- and π-type molecular orbitals. *Figure 2* shows two occupied molecular orbitals and orbital energies of σ-type and π-type MOs from Au1-NHC–Au1-NHPb at the BP86/TZVPP level. The energy levels of the π-type donor orbitals of complexes were higher lying than the σ-type donor orbitals. The orbital energy values were particularly large in the Au1-NHC in both σ- and π-type MOs and decreased in the silylene and germylene as well as in the heavier analogues. Especially, the shape of the molecular orbitals which indicated that NHE_{Me}→AuCl not only had significant σ donation but also exhibited a bit π donation in complexes. We can explain that the π donation in complexes due to the strong N→E π donation at the ring of the NHE_{Me} ligands.

We showed the frontier orbitals with the plot of the energy levels of the energetically highest lying σ and π orbital of the isolated NHE ligands (*Figure 3*) in order to know whether the ligands had an occupied π-orbital in the E center atom. The HOMO of NHE ligand had π symmetry, except for NHC, in which the HOMO has σ symmetry whereas the HOMO-1 and HOMO-2 had π symmetry. The σ orbitals of NHE ligand were uniformly lone-pair molecular orbitals (MOs), but the π orbitals were delocalized over the NHE ring atoms. *Figure 3* also shows that the energy level of the π orbital increased, whereas that of the σ orbital decreased as atom E became heavier. The trend of the energy levels of the energetically highest-lying σ and π orbitals of NHE ligand rationalize the preference of the heavier ligands NHSn to NHPb for side-on co-ordination to the metal, in which the σ-donation takes place through the π orbital of the ligand (Nguyen & Frenking 2012). The end-on coordination of the lighter homologues NHC to NHGe could be explained by various factors that also influence the bending angle α of the ligands (*Table 1*).



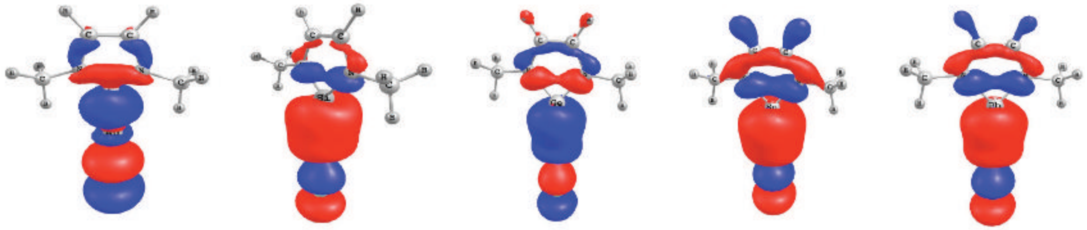
Aul-NHC (π)
HOMO-6
–7.157 (eV)

Aul-NHSi (π)
HOMO-2
–6.150 (eV)

Aul-NHGe (π)
HOMO-2
–6.123 (eV)

Aul-NHSn (π)
HOMO-2
–5.660 (eV)

Aul-NHPb (π)
HOMO-2
–5.361 (eV)



Aul-NHC (σ)
HOMO-9
–8.136 (eV)

Aul-NHSi (σ)
HOMO-5
–7.460 (eV)

Aul-NHGe (σ)
HOMO-7
–7.674 (eV)

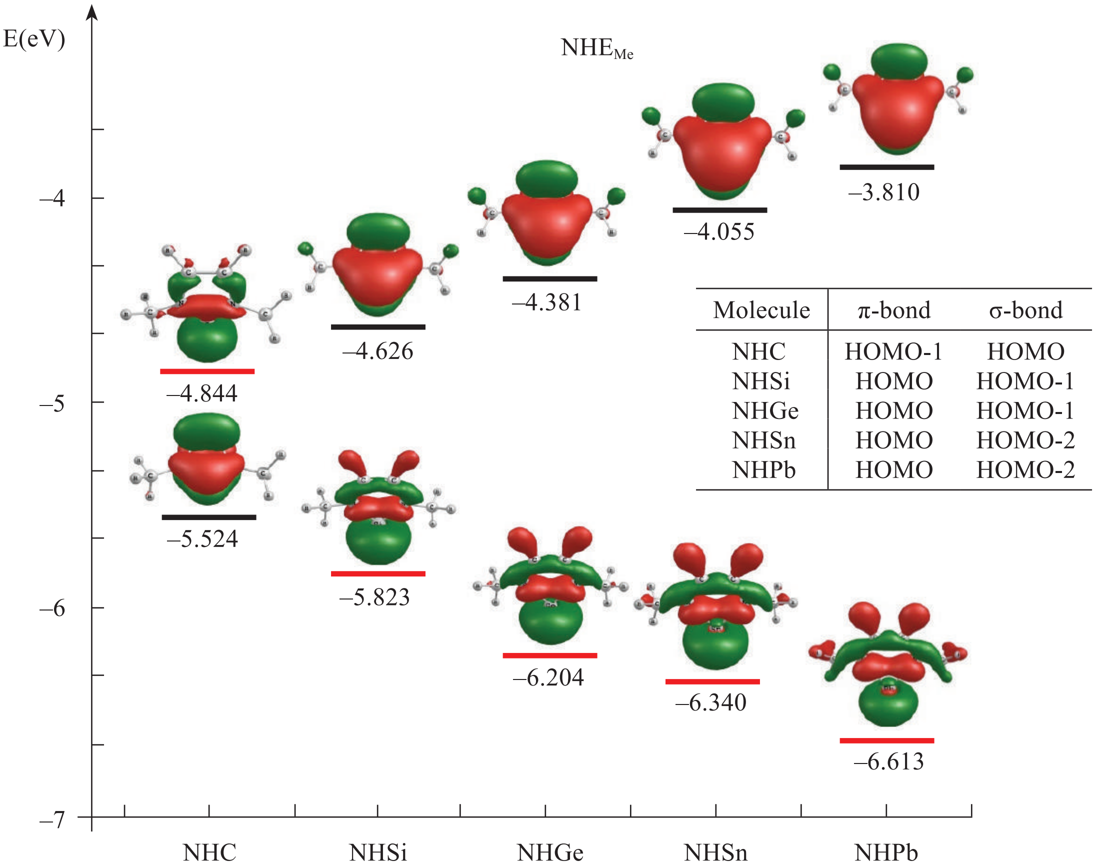
Aul-NHSn (σ)
HOMO-6
–7.320 (eV)

Aul-NHPb (σ)
HOMO-9
–7.810 (eV)

Figure 2. Molecular orbitals and orbital energies of σ-type and π-type MOs from Au1-NHC–Au1-NHPb at the BP86/TZVPP level. Orbital energies are given in eV.

We also want to point out the orbitals at the Au side carried a little $NHE_{Me} \leftarrow AuCl$ back-donation and mainly exhibited $NHE_{Me} \rightarrow AuCl$ σ-donation. We suggested the scheme illustration showing the mixing of the empty *s* and occupied d_z^2 orbitals of Au(I) which was graphically shown in *Figure 4a*. Note that the Au cation had an s^0d^{10} electron configuration but the *s* orbital was mostly filled in AuCl due to the ionic $Au^+{-}Cl^-$ bond and the lowest lying empty orbitals were at the Au might be *p* orbitals. Although in the gold(I) complex, the relativistic effects were responsible for the very small charge transfer from Au to Cl but when the $AuCl\text{-}NHE_{Me}$ was formed, there was no further charge transfer from the Au atom to chlorine. From this it could be asserted that there was the mixing of the valence s orbital with occupied d_z^2 orbital of the Au. The competition for the empty Au *s* orbital between donation from chlorine and the carbene-analogues σ-lone pair might lead to the longer Au-Cl bonds in the complexes (*Figure 1*).

Furthermore, the three filled orbitals arose from the E atom to Au and to Cl (E−Au−Cl) σ-interactions which were illustrated in *Figure 4b*. The highest energy had d_z^2 symmetry along the Au-Cl axis which could be considered as σ-antibonding character toward chlorine. The middle level of energy showed an anti-state combination of the Cl p_z and an sp_2 hybrid on the E atom and also revealed that there was no bonding toward Au. The lowest energy of E−Au−Cl σ-bonding orbitals exhibited a constructive overlap of the Au(I) $5d_z^2$ orbital that carried the σ-symmetry on Cl and E atoms. We could point out that the covalent part of the metal bonding in $AuCl\text{-}NHE_{Me}$ had the patent of the familiar 6-electron-3 center interactions with the fully important d_z^2 orbital which significantly contributed to the Au-Cl



Molecule	π-bond	σ-bond
NHC	HOMO-1	HOMO
NHSi	HOMO	HOMO-1
NHGe	HOMO	HOMO-1
NHSn	HOMO	HOMO-2
NHPb	HOMO	HOMO-2

Figure 3. Plot of the energy levels of the energetically highest lying σ and π orbital of ligands NHE (E = C − Pb).

and AuCl-NHE$_{Me}$ bonding (*Figure 4b*). Note that the transition metal complexes MCl with M = Au, Ag, Cu that carry the less bulky NHC ligand has been recently described by Nemcsok *et al*. (2004) using EDA method. In this study, we used the EDA-NOCV calculations in order to give a thorough insight into the nature of the metal-ligand bonding in Au1-NHE. This led to a donor-acceptor description of the Au-E bond in the system. *Table 3* showed the results of EDA-NOCV when considering NHC$_{Me}$ as the donor fragments and AuCl as the acceptor fragment. *Table 3* shows that EDA-NOCV results at the BP86/TZ2P+ level for compound Au1-NHC–Au1-NHPb using the moieties [AuCl] and [NHE$_{Me}$] as interacting fragments. The Au-E bond dissociation energies trend in Au1-NHE decreased from the lighter to the heavier homologues (Au1-NHC: D_e = 78.1 kcal/mol; Au1-NHPb: D_e = 42.6 kcal/mol).

The trend of the bond dissociations energies (BDEs) D_e for the Au-E bond in Au1-NHE system was Au1-NHC > Au1-NHSi > Au1-NHGe > Au1-NHSn > Au1-NHPb. The decrease of the BDEs from the lighter to heavier adduct was determined by the intrinsic strength of the Au-ligand bonds ΔE_{int}. The carbene adduct had a smaller preparation energy of the interacting fragments (ΔE_{prep} = 1.2 kcal/mol) and stays nearly the same in the heavier homologues (ΔE_{prep} = 2.0 – 2.5 kcal/mol) and the largest

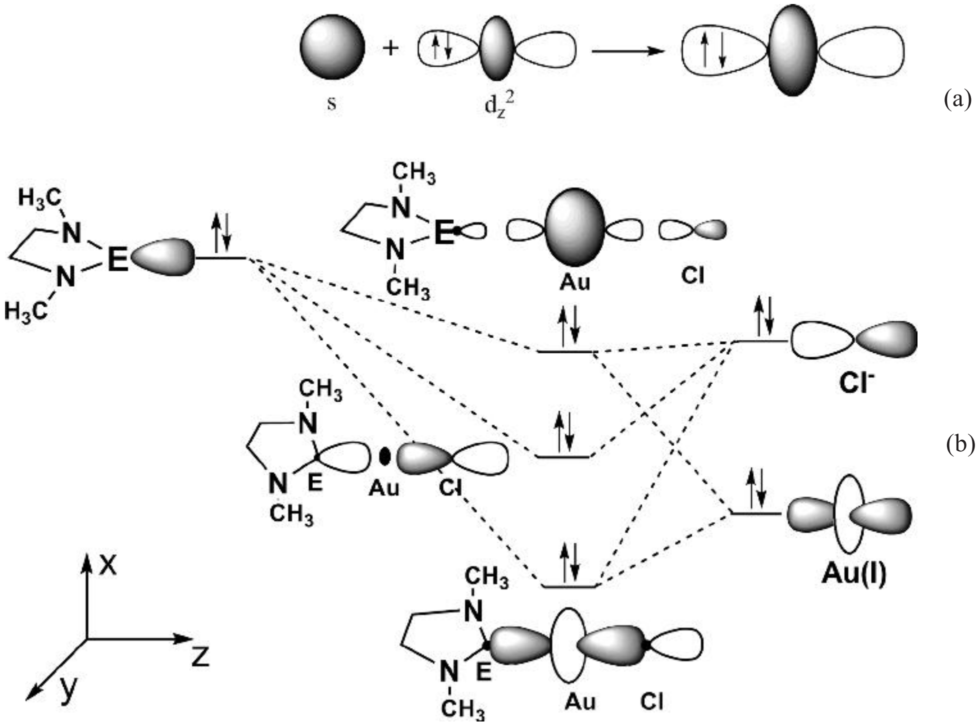


Figure 4. (a) Scheme illustration showing the mixing of the empty s and occupied d_z^2 orbitals of Au(I); (b) Qualitative diagram of the interaction of the Au$^+$ d_z^2 orbital with Cl$^-$ and σ-donor orbital of E atom with E = C − Pb.

Table 3. EDA-NOCV results at the BP86/TZ2P+level for compound Au1-NHC–Au1-NHPb using the moieties [AuCl] and [NHE$_{Me}$] as interacting fragments. The complexes are analyzed with C1 symmetry. Energy values in kcal/mol.

Compound	Au1–NHC	Au1–NHSi	Au1–NHGe	Au1–NHSn	Au1–NHPb
Fragment	[AuCl] [NHC$_{Me}$]	[AuCl] [NHSi$_{Me}$]	[AuCl] [NHGe$_{Me}$]	[AuCl] [NHSn$_{Me}$]	[AuCl] [NHPb$_{Me}$]
ΔE_{int}	–79.3	–68.5	–52.2	–47.2	–50.0
ΔE_{Pauli}	212.1	186.9	124.9	95.5	84.8
ΔE_{elstat}[a]	–219.3 (75.3 %)	–182.0 (71.3 %)	–118.5 (66.9%)	–87.2 (61.1 %)	–78.3 (58.1 %)
ΔE_{orb}[a]	–72.1 (24.7 %)	–73.3 (28.7 %)	–58.6 (33.1 %)	–55.5 (38.9 %)	–56.5 (41.9 %)
ΔE_{σ}[b]	–51.3 (71.2 %)	–50.0 (68.2 %)	–42.6 (72.8 %)	–45.4 (81.7 %)	–49.0 (86.7 %)
ΔE_{π}[b]	–17.0 (23.5 %)	–20.8 (28.4 %)	–13.7 (23.4 %)	–7.9 (14.3 %)	–4.9 (8.7 %)
ΔE_{rest}[b]	–3.8 (5.3 %)	–2.5 (3.4 %)	–2.3 (3.8 %)	–2.2 (4.0 %)	–2.6 (4.6 %)
ΔE_{prep}	1.2	2.4	2.0	2.5	7.4
ΔE (= $-D_e$)	–78.1 (79.2)[c]	–66.0 (67.0)[c]	–50.2 (51.9)[c]	–44.7 (44.2)[c]	–42.6 (42.7)[c]

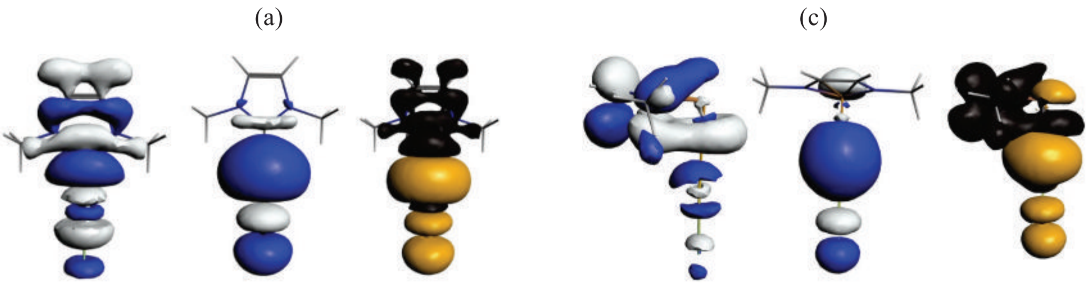
[a] The values in parentheses are the percentage contributions to the total attractive interaction ΔE_{elstat}+ ΔE_{orb}.

[b] The values in parentheses are the percentage contributions to the total orbital interaction ΔE_{orb}.

[c] The values in parentheses give the dissociation energy at the BP86/def2-TZVPP//BP86/def-SVP level.

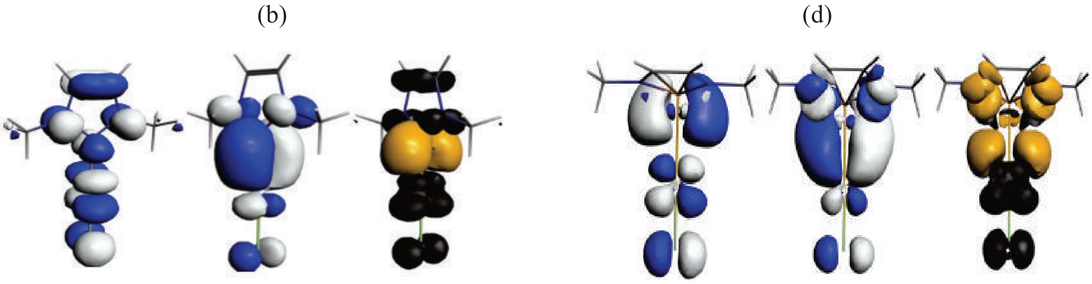
value preparation energy was 7.4 kcal/mol which gave the smallest BDE for Au1-NHPb. Inspection of the three main terms which contributed to the interaction energy ΔE_{int} indicated that the Pauli repulsion ΔE_{Pauli} and the electrostatic attraction ΔE_{elstat} were smaller for the heavier group-14 atoms Au1-NHSi–Au1-NHPb than for Au1-NHC**.** The largest contributions to the ΔE_{int} values of the system always came from the electrostatic interaction term ΔE_{elstat} which contributed more than 60% in complexes except for the less than that in Au1-NHPb (58.1%). In contract to this, the orbital term contributed < 30% in the lighter complexes which were 24.7% and 28.7% for Au1-NHC and Au1-NHSi. The strength of the total orbital interactions ΔE_{orb} decreased from the lighter to the heavier homologues**.** Note that the most important information which was provided by the EDA-NOCV calculations concerned the strength of the σ and π bonding. *Table 3* also shows that the contribution of ΔE_σ to ΔE_{orb} was rather large for all complexes where the values were between 68.2% – 86.7%. For all complexes holds that the contribution of ΔE_π to ΔE_{orb} is significantly smaller than in other transition metal complexes of tungsten pentacarbonyl and teracarbonyl complexes [W(CO)$_5$-NHE] (with E = C – Pb) (Nguyen & Frenking 2012). Thus, the EDA-NOCV calculations showed that the Au-E bonding in the complexes Au1-NHC–Au1-NHPb had a small contribution which might come from NHE$_{Me}$→AuCl π-donation and NHE$_{Me}$→AuCl π-back-donation. We continue determining the charge transfer between the donor and acceptor fragments by plotting of the pairs of orbitals, the associated deformation densities, and stabilization energies. The fact was that, the plots of the pairs of orbitals ψ_k/ψ_{-k} that yield the NOCVs provided the largest contributions to the σ- and π-orbital terms ΔE_σ and ΔE_π in Au1-NHE (E = C, Si) and the associated deformation densities $\Delta\rho$ and stabilization energies were shown in *Figure 5*. The shape of pairs for Au1-NHSi and Au1-NHGe were quite similar with the carbene complex, while the shape of pairs for the heavier specie shape of pairs for Au1-NHSn possessed the same shapes compared with the shape of pairs for Au1-NHPb, and there for were not shown in *Figure 5*.

Note that the white/blue colours in the small figures for ψ_k/ψ_{-k} indicated the sign of the orbitals, and the black/yellow colours in the deformation density $\Delta\rho$ designate charge depletion, and the yellow areas point to charge accumulation. The charge flow $\Delta\rho$ occurred in the direction from black to yellow. *Figure 5(a)* shows that the σ- type interaction was clearly from the donating NHC$_{Me}$ fragment to the accepting AuCl fragment which was the deformation density $\Delta\rho_1$ which showed that stabilization of energy was –42.0 kcal/mol. The shapes of the NOCV pairs ψ_2/ψ_{-2} and the deformation density $\Delta\rho_2$ showed that stabilization of –11.1 kcal/mol could be assigned to ClAu←NHC$_{Me}$ π-donation while the stabilization of also came from relaxation of the acceptor fragment AuCl in Au1-NHC. *Figure 5(c,d)* shows significantly different EDA-NOCV results for Au1-NHPb because of the surprising structure of the plumbylene ligand which was bonded through its π-electron density. Note that the structures and orbitals pairs of the lighter homologues Au1-NHE with E = C, Si, Ge had head-on modes between the ligands and metal fragment, whereas the heavier species Au1-NHE with E = Sn, Pb exhibited a side-on bonded ligands to the AuCl fragment. *Figure 5(c)* clearly shows that the σ-type interaction had the direction of the charge flow of ClAu←NHPb$_{Me}$. The deformation density ψ_1 exhibited an area of charge donation (black area) at the NHPb$_{Me}$ moiety associated with the deformation density $\Delta\rho_1$ and stabilization energy was –46.1 kcal/mol. *Figures 5(d)* show that the very weak π-type orbital interactions in Au1-NHPb came from typical π-back-donation ClAu→NHPb$_{Me}$ with the charge flow ψ_2/ψ_{-2} which indicated stabilization at –2.9 kcal/mol. Thus, the bonding in Au1-NHE complexes exhibited the typical feature in terms of strong σ-donation and weak π-back-donation. From



Aul-NHC (σ) Aul-NHPb (σ)

$\psi_{-1}(-0.56)$ $\psi_{1}(0.56)$ $\Delta\rho_{1}(\Delta E = -42.0)$ $\psi_{-1}(-0.81)$ $\psi_{1}(0.81)$ $\Delta\rho_{1}(\Delta E = -46.1)$



Aul-NHC (π) Aul-NHPb (π)

$\psi_{-2}(-0.36)$ $\psi_{2}(0.36)$ $\Delta\rho_{2}(\Delta E = -11.1)$ $\psi_{-2}(-0.26)$ $\psi_{2}(0.26)$ $\Delta\rho_{2}(\Delta E = -2.9)$

Figure 5. Most important NOCV pairs of orbitals Ψ_{-k}, Ψ_k with their eigenvalues $-\upsilon_k$, υ_k given in parentheses, and the associated deformation densities $\Delta\rho_k$ and orbital stabilization energies ΔE for the complexes Au1-NHC and Au1-NHPb. The charge flow in the deformation densities is from the black→yellow region. (a) σ-NOCV of Au1-NHC; (b) π-NOCV of Au1-NHC; (c) σ-NOCV Au1-NHPb; (d) π-NOCV of Au1-NHPb. Energy values in kcal/mol.

the above results, it could be asserted that the decrease in the donation ClAu←NHE$_{\mathrm{Me}}$ which was manifested in the calculated values for ΔE_σ and in the electrostatic attraction, ΔE_{elstat}, provided a rationale for the weaker bonding of the heavier atoms E. The π-interactions in [ClAu-NHE$_{\mathrm{Me}}$] were due to very weak π-back-donation and were also irrelevant for the bond strength. The ligand←Au π-back-donation in the complexes was very small and the Au-ligand bonds had strong ionic character which came from the electrostatic attraction between the positively charged Au atom and the σ-electron pair of the E donor atom.

CONCLUSIONS

In summary, the full isolable carbene analogues-stablized gold(I) chloride was structurally characterized in this work. DFT calculations found that the equilibrium structures of the Au1-NHE system showed major differences in the bonded orientation from the ligands NHC to NHPb of gold(I) complex between the lighter and the heavier homologues. The calculated structures of carbene-analogues complexes Au1-NHE of the lighter complexes Au1-NHE possessed end-on-bonded NHE$_{\mathrm{Me}}$ ligands (E = C–Ge) with the bending angles, α, were 180°. In contrast, the heavier species

Au1-NHSn and Au1-NHPb exhibit side-on-bonded ligands which the bending angle, α, became more acute (110.3° for Au1-NHSn and 91.5° for Au1-NHPb). The trend of the bond dissociation energy of Au-ligands in Au1-NHE show for the ligands NHE order is: Au1-NHC > Au1-NHSi > Au1-NHGe > Au1-NHSn > Au1-NHPb. Bonding analysis of Au1-NHE showed that NHE ligands exhibited donor- acceptor bonds with the σ lone pair electrons of NHE donated into the vacant orbital of the acceptor fragment (AuCl). The EDA-NOCV results indicated that the ligand NHE in Au1-NHE complexes were strong σ-donors and very weak π donors. The Au-NHE bonds were mainly held together by electrostatic energy, which contributed >60% of the binding interactions for the lighter homologues (Au1-NHC − Au1-NHGe). The NOCV pairs of the bonding showed small π-back donation from the Au to the NHE ligands.

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Methanolysis of Crude Jatropha Oil using Heterogeneous Catalyst from the Seashells and Eggshells as Green Biodiesel

A. N. R. REDDY[1], A. S. AHMED[1]*, M. D. ISLAM[2] AND S. HAMDAN[1]

In this work, heterogeneous calcium oxide catalysts gleaned from *Polymedosa expansa* and eggshell were investigated for the transesterification of crude jatropha oil with methanol, to access their prospective performance in biodiesel production as an alternative green energy resource. The best yield of biodiesel achieved was 96% in 1 h for *Step 1* using 0.01:1 ratio of acid catalyst to oil and 0.6:1 ratio of alcohol to oil ratio, together with 2 h of *Step 2* using 0.02:1 ratio with base catalyst CaO, derived from *P. expansa*, to oil ratio and 5:1 ratio of alcohol to oil. The properties of jatropha biodiesel were analyzed and found to have calorific value of 35.43 MJ/kg, density value of 895 kg/m^3 and flash point of 167. The biodiesel was blended with mineral diesel from B0 to B50 for a diesel engine performance test. B20 indicated comparable characteristics with pure mineral diesel, like lowest fuel consumption rate, specific fuel consumption rate, highest brake horsepower and mechanical efficiency.

Key words: Biodiesel; jatropha; heterogeneous catalyst; transesterification; alternative green energy resource

Due to increase in the prices of petroleum, gradual depletion of the world's petroleum reserves and impact of environmental pollution caused by combustion of fossil fuel, the search for substantial alternative energy resources such as biodiesel has gained importance (Lippke *et al.* 2011). As reported in the role of biodiesels beyond 2020 (Cluzel 2013) biodiesel has imperative role in the energy sector. Compared to the conventional petroleum diesel, biodiesel is renewable, non-toxic, non-flammable, have low pollutant emissions and safe for use in all conventional diesel engines which has the same performance and engine durability. Srithar *et al.* (2014) and many researchers reported biodiesel can be used alone or can be blended with any ratio of mineral diesel to produce biodiesel blend. The blending of biodiesel with petroleum diesel depicts similar characteristics with lower hazardous exhaust emissions compare to the emission of fossil fuel (Koh *et al.* 2011).

Biodiesel is a green energy resource, compromises mono alkyl ester of long fatty acids derived from biologically produced oils or fats including vegetable oils, animal fats and microalgae oils (Qien *et al.* 2010). Initially many researchers and some developing countries are using edible oils such as rapeseed, soybean, palm, sunflower, coconut, and linseed oils as feedstock for commercial produce of biodiesel (Demirbas 2009; Gui *et al.* 2008).

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[1] Department of Mechanical Engineering, Faculty of Engineering, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[2] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
* Corresponding author (e-mail: aasaleh@feng.unimas.my)

The use of edible oils for biodiesel production is banned in few countries like china since it's not feasible due to limited availability of per capita agriculture land resources. Hence there is a potential demand and promotion for the non-edible oils like crude jatropha oil (CJO) as feed stock in biodiesel produce (Yang *et al.* 2010; Deng *et al.* 2010). The current interest is developing a biofuel with international specifications, without affecting the food demand (Mofijur *et al.* 2012). Jatropha has been chosen as feedstock due to its adaptability in tropical and sub-tropical climates in marginal and non-agriculture areas. Apart from that, the JCL seeds are found to be toxic to human and animals (Leung *et al.* 2010). *Jatropha Curcas Linnaeus* (JCL) comes from Euphorbiaceous family. It is a fast growing tree has a life span of about or more than 50 years (Nahar *et al.* 2011). *Jatropha Curcas Linnaeus* contains oil in the seed which is 27% to 40% of oil.

There are many methods to produce biodiesel from vegetable oils or animal fats like direct use or blending, pyrolysis, micro-emulsion and transesterification (Meher *et al.* 2013; Bobade *et al.* 2013). Among these, the most common method is transesterification of triglycerides using methanol fatty acid methyl esters (FAME). Also, transesterification is discovered to be the most efficient way to reduce the viscosity of vegetable oils to meet the biodiesel standards like *EN 14214*. In transesterification, triglycerides in vegetable oil will react with alcohol to form a mixture of and fatty acid alkyl esters. (Chung *et al.* 2008) reported, in transesterification, based on the amount of free fatty acids (FFA), both acidic-catalysts and basic-catalysts can be used. Since 3% to 40% FFAs present in acid oils homogeneous catalysts are not recommendable. Marchetti *et al.* (2007) and Gubitz *et al.* (1999) reported that the homogeneous catalyst and the FFA will interact to produce soap. This makes the amount of available catalyst for the transesterification reaction to be reduced and also complicates the down streaming separation and the biodiesel purification further, the rate of transesterification reaction by alkaline catalysts is much faster than that by acid catalysts.

The main focus of the present work was to synthesize heterogeneous base catalysts from the sea shells and egg shells for biodiesel produce from CJO. A comprehensive study on the efficiency of biodiesel conversion from CJO to jatropha biodiesel (JB) by using two-step transesterification to optimize the reaction process and found the best yield of biodiesel was carried out. The properties of biodiesel produced were analyzed through Fourier Transform Infrared Spectrometer (FT-IR), bomb calorimeter, density meter, flash point tester and flue gas analyzer. Subsequently a comparative analytical approach on comparable characteristics of biodiesel blends with pure mineral diesel, such as lowest fuel consumption rate, specific fuel consumption rate, highest brake horsepower and mechanical efficiency were analyzed.

MATERIALS AND METHODOLOGY

Material and Equipment

The research was conducted in the Faculty of Engineering, Universiti Malaysia Sarawak. Material used for biodiesel production were CJO, methanol (MeOH) >99% pure), sulphuric acid (H_2SO_4) 95%–97%, Desterilized water/pipe water, sodium hydroxide, NaOH, potassium hydroxide (KOH), calcium oxide (CaO) from *P. expansa* and eggshell. Equipment used are magnetic stirrer cum heater, orbital shaker, electronic scale and furnace.

Catalyst Preparation

Polymedosa expansa, whose scientific name Solander 1786 belongs to 23375 Corbiculidae families and locally it is called as Lokan (Bachok *et al.* 2003). *P. expansa* has large rounded, thick-shelled bivalve reaching about 110 mm. Exterior has sculpture of distinct concentric growth lines, often developed as ridges with strong and wide hinge and in white or with thick olive-brown periostracum colour. Interior colour is white.

P. expansa shells were collected from a local villager's stall. The edible portion attached to the shells and other impurities were removed, they were rinsed thoroughly with tap water followed by double rising with distilled water. The *P. expansa* shells were dried in hot air oven at for 24 h. The *P. expansa* shells were tested for calcium, one seashell was dissolved in 10% HCl to obtain a large amount of effervescence and a colourless solution. If this solution was added to a sodium carbonate and/or sodium sulphate solution a white precipitate could be formed:

$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2$

$CaCl_2 + Na_2CO_3 \rightarrow 2NaCl + CaCO_3\downarrow$

$CaCl_2 + Na_2SO_4 \rightarrow 2NaCl + CaCO_3 \downarrow$

The dried *P. expansa* shells were ground in an agate mortar until they became a form of powder. Mustakimah *et al.* (2012), Azimah *et al.* (2012) and Said *et al.* (2014) reported the calcination of the seashells using standard process. The shell powder was calcinated in an open furnace at 900°C for 2 h. At 850 °C, the calcium carbonate of *P. expansa* shell powder decomposed to calcium oxide and carbon dioxide. Calcium oxide is a white crystalline solid with a melting point of 2572°C.

Eggshells were collected from UNIMAS (Students Pavilion). Buasri *et al*. (2013) reported on the calcination of eggshells. The raw eggshells were calcined at temperatures between 300°C to 900°C, for 1, 3 and 5 h. The proper calcination conditions were: 900°C and 1 h, yielding calcium oxide with a purity of 99.06% w/w. The calcium carbonate of the rhombohedral form ($CaCO_3$) transformed completely into the calcium oxide or lime of the face-centered cubic form (CaO) at 900°C.

Free Fatty Acids Measurement

Titration was adopted to determine the FFA content in crude jatropha oil. It is expressed as the amount of KOH in mg required to neutralize 1 g of fatty acid methyl ester. KOH 0.1 M was diluted into ethanol (Azhari *et al.* 2008; Bojan, *et al.* 2012). The solid KOH was fully dissolved in the ethanol by constant stirring. A weight of 2 ± 0.5 g of jatropha oil and a 50 ml solvent mixture (95% ethanol + diethyl ether, 1:1 v/v) were mixed in a conical flask and heated on the heater with magnetic stirrer. Few drops of phenolphthalein were added to the mixture and titrated. The mixture with the KOH solution was stirred continuously for 10 s until the colour of the solution turned pink. The process was repeated three times to increase the accuracy. If the FFA was more than 4%, acid pretreatment was required to be carried out to reduce the FFA value in first step.

Transesterification

Since the FFA value determined was greater than 4%, acid pretreatment is required. Thus, biodiesel conversion by using two-step transesterification is carried out (Ismail *et al.* 2014).

Pretreatment *(Step 1)*

Filtered 25 ml CJO was heated over 100°C to remove the water content. Mixture of sulphuric acid (catalyst) and methanol (alcohol) were prepared according to the catalyst to oil ratio, alcohol to oil ratio by volume and heated to 50°C. Oil sample was added to the catalyst mixture and heated to 50°C to 60°C by using magnetic stirrer for 1 h then it was allowed to settle down for 1 h. Methanol-water fraction was removed. The remaining oil was washed by using de-sterilized water. It was allowed to settle for 30 min to remove the water at the lower layer using the separation funnel. The pretreated oil was heated to 100 with magnetic stirrer to remove the water content.

The pretreated oil was for second step based catalyzed transesterification (Bojan *et al.* 2012).

Transesterification by Base Catalyst *(Step 2)*

The optimum condition obtained which has the lowest acid value was selected to proceed with second step transesterification. The pretreated oil was heated to 50°C to make it turn into a clearer fluid. A mixture of CaO from *P. expansa* (catalyst) and methanol (alcohol) according to the catalyst to oil ratio and alcohol to oil ratio by volume was prepared and heated up to 50°C. The oil sample and the catalyst mixture were allowed to react using magnetic stirrer for 2 h at 60°C. The mixture was shacked for 2 h using orbital Shaker at room temperature at 250 r.p.m. speed and allowed the mixture to settle for 24 h. The glycerol stocked at lower layer and the methanol-water fractions at the upper layer were separated. The remaining biodiesel was washed by using de-sterilized water and allowed 30 min for removing the water at the lower layer in the separation funnel. The filtered biodiesel produced was heated to 100°C with a magnetic stirrer, to remove the water content in order to get pure biodiesel. The process was repeated with catalyst calcium oxide (CaO) extracted also from eggshells.

Biodiesel Properties Analysis

The biodiesel produced was tested by using FT-IR analysis, bomb calorimeter, density meter, flash point tester and flue gas analyzer to compare its properties with diesel and CJO. Engine performance test was conducted by using Jatropha Biodiesel blends to determine the optimum ratio.

RESULTS AND DISCUSSION

Acid Value

From *Table 1*, the acid value obtained was 29.466. The acid value obtained is 29.466. The acid value was too high so it was not suitable to run one-step esterification. Thus, two-step transesterification was conducted where *Step 1* acid pretreatment to reduce the high free fatty acid content in the CJO to less than 4%.

Catalyst to Oil Ratio

Figure 1 shows the different proportion of acid catalyst which is from 1% to 6% to oil ratio by volume, 1% of sulphuric acid had shown the best result which the FFA value of the Jatropha oil sample was reduced to 1%. Thus, it is suitable for alkali catalyzed transesterification to be carried out.

Figure 2 shows the concentration of the catalyst increases, biodiesel yield also increases. However, it reached optimum condition during 2% (w/w). Further increases in the concentration of catalyst result in lower yield as more soap formation. Among the catalysts used, Calcium oxide (CaO) from *P. expansa* had shown the highest yield which was 96% with the concentration of catalyst at 2% to oil ratio (w/w).

Methanol to Oil Ratio

After the FFA value was determined and qualified for alkali catalyzed transesterification process, different ratio of alcohol to oil ratio were tested to identify the optimum proportion of methyl ester yield produced. From *Figure 3*,

Table 1. Acid value analysis.

Experiment	Weight (g)	Volume (ml)	Molarity (mol/l)	Acid value
1	2.706	14	0.1	29.024
2	2.952	15.7	0.1	29.836
3	2.773	14.6	0.1	29.537
Average				29.466

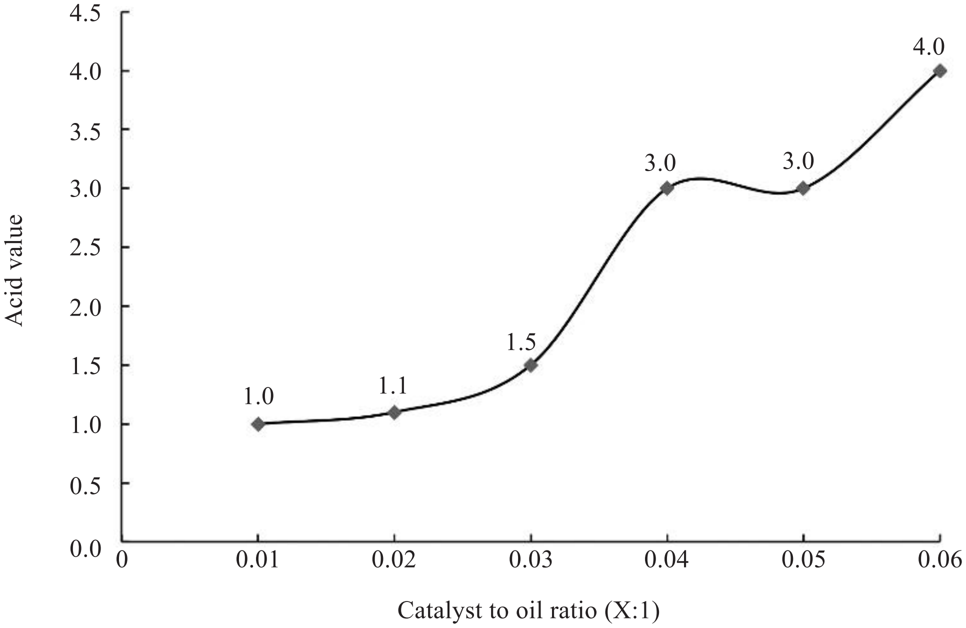

Figure 1. Acid value by different proportion of catalyst to oil ratio for Step 1 *transesterification.*

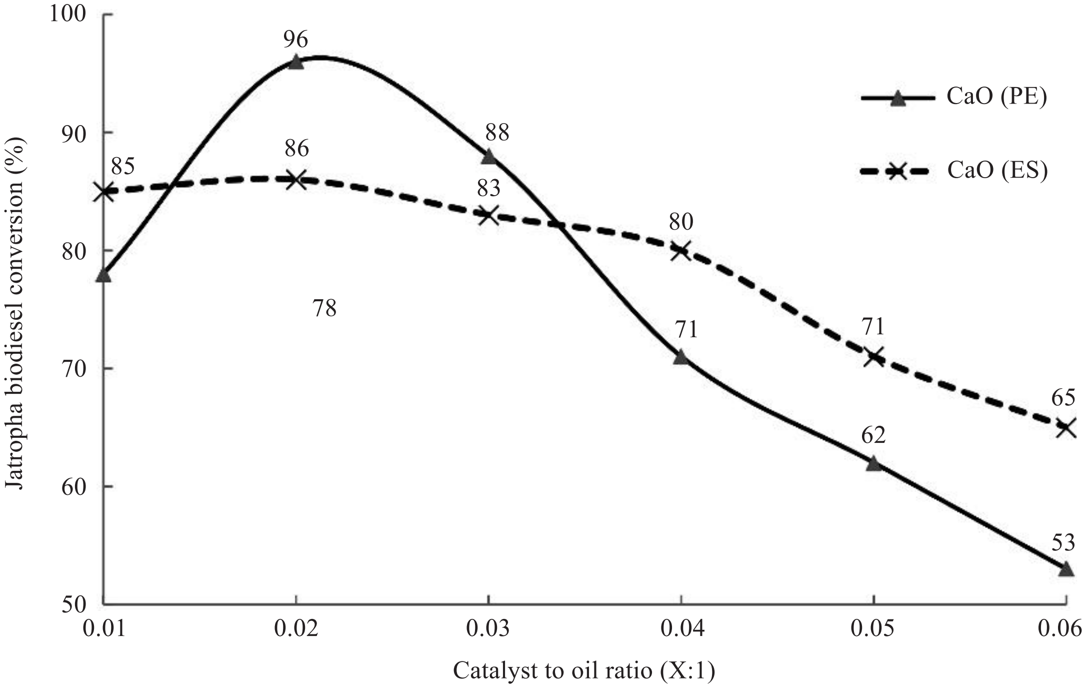

Figure 2. Biodiesel conversion by changing catalyst to oil ratio for Step 2.

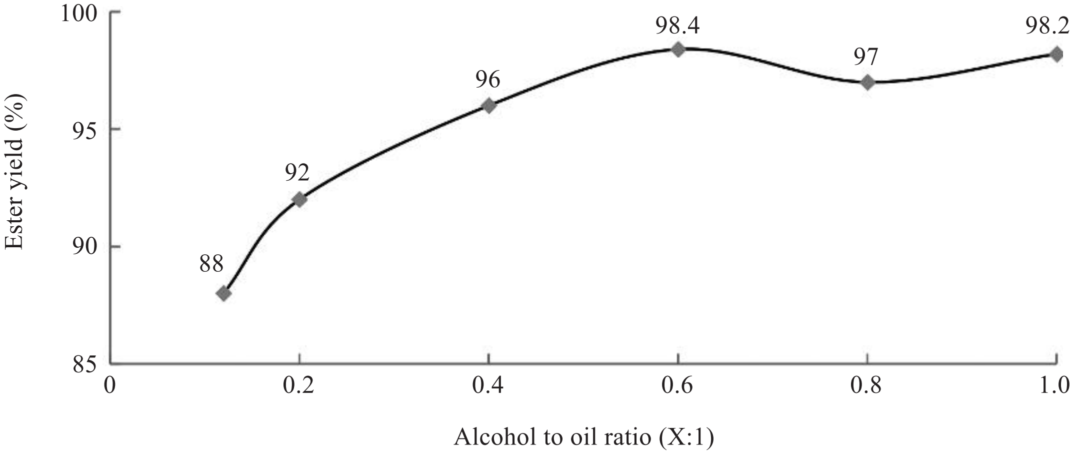

Figure 3. Ester yield by different proportion alcohol to oil ratio for Step 1 *transesterification.*

it was found that, 60% of methanol to oil ratio by volume showed the highest ester yield which was 98.4%.

As shown in *Figure 4*, the amount of alcohol increased; the biodiesel yield also increased. It reached its optimum condition during alcohol to oil ratio 5:1. Further increasing of methanol led to lower conversion. This was due reverse reaction as the amount of alcohol was too high. Among the catalysts used, the optimum ratio obtained for methanol to oil was 5 to 1 and CaO from *P. expansa* catalyst had the highest yield at 92%. Thus, CaO from *P. expansa* were the best catalyst among the catalysts used. From the experiments carried out it proven that the optimum ratio of methanol to oil ratio was 5:1, the optimum concentration of catalyst to oil ratio was 2% which gave the highest JB conversion at 96%.

Equipment Type

Figure 5 shows that the Orbital Shaker (OS) converted a higher yield which averaged 92% compared to Magnetic Stirrer (MS) cum heater, i.e. 74.33%. OS showed a better and more complete mixing process for biodiesel conversion. Also, it provided more balanced mixing compared to MS which contributed to higher yield or conversion efficiency. Hence, OS was a more reliable equipment to be used in biodiesel production.

Reaction Time

Figure 6 shows that during *Step 1* esterification, the optimum reaction time obtained was 1 h. Further increase in reaction time did not contribute to a higher percentage of conversion. This was due to esterification which is a reversible process where the ester yield will be converted back to crude jatropha oil if it exceeds the optimum reaction time.

The optimum reaction time for *Step 2* transesterification was 2 h with a conversion of 96% as shown in the *Figure 7*. The rate of conversion to biodiesel increased with the reaction time until it reached 2 h. However, further increase in reaction time exceeded the optimum condition of biodiesel yield; the yield decreased with the reversible reaction and soap formation.

Analysis of Jatropha Biodiesel Properties

Spectrum analysis. The spectrum for the biodiesel produced from two different heterogeneous catalysts derived from *P. expansa*

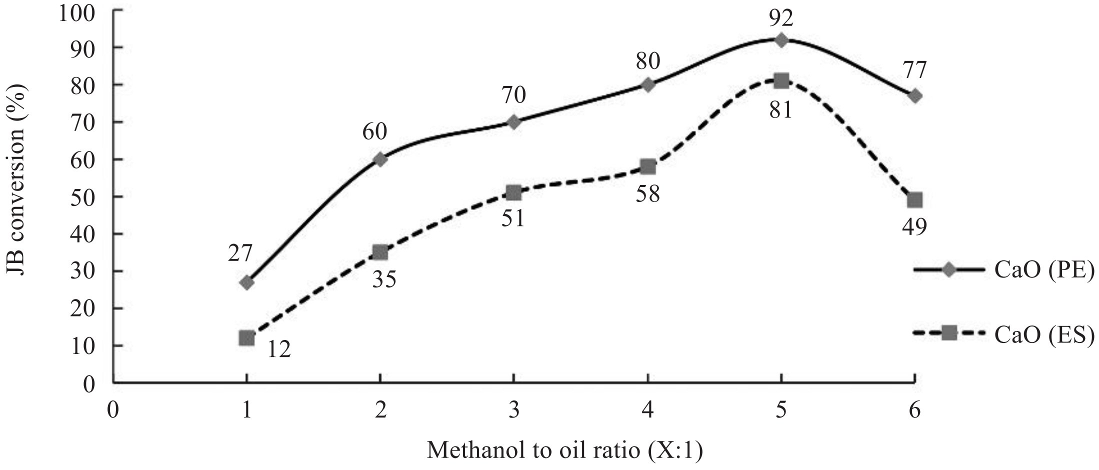

Figure 4. Biodiesel conversion by changing methanol to oil ratio for Step 2.

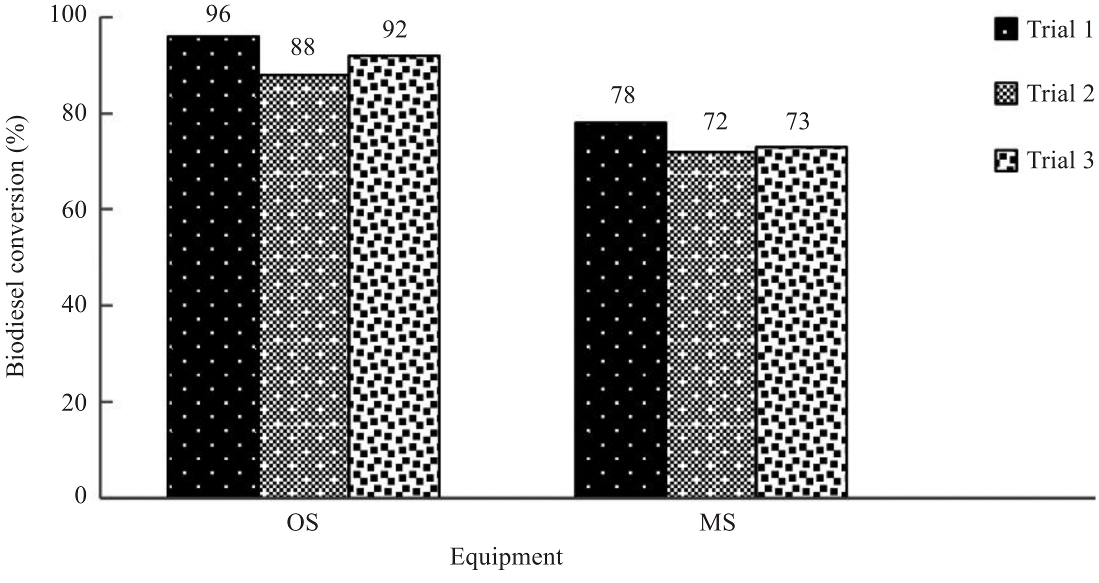

Figure 5. Biodiesel conversion by changing the types of equipment.

and eggshells was of same pattern. In *Figure 8*, the main difference of spectrum of biodiesel produced from mineral diesel was at 1700 cm^{-1} – 1750 cm^{-1} due to the presence of aldehyde or ester carbonyl or C=O group. The presence of the spectrum proved that the specimen was a fatty acid methyl ester or biodiesel where the absorption bands were only present for biodiesel. Besides that, JB also showed absorption spectrums in between 1000 cm^{-1} – 1320 cm^{-1} due to the presence of ester carbon oxygen group or C-O group.

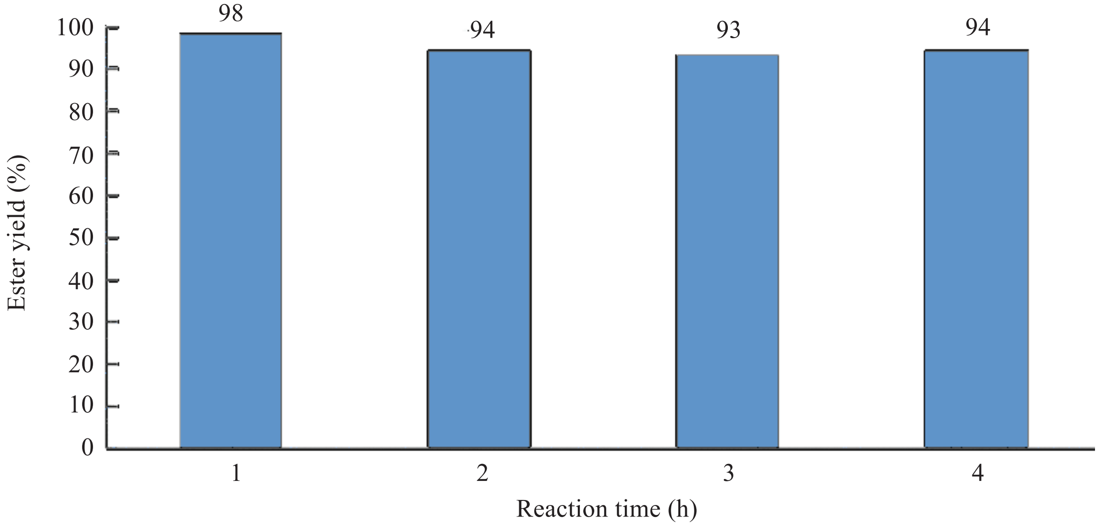

Figure 6. Reaction time to ester yield for Step 1.

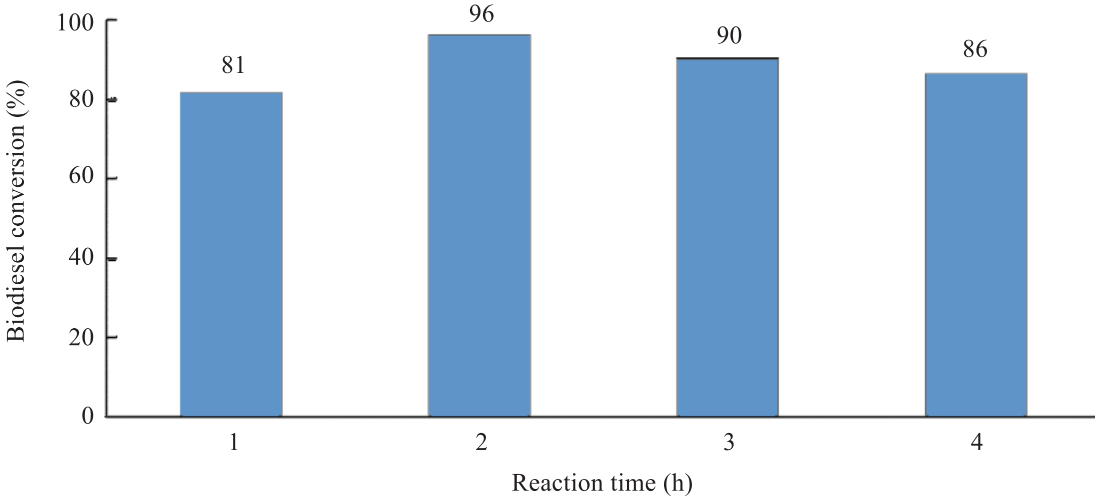

Figure 7. Biodiesel conversion to reaction time for Step 2.

Calorific Value

Caloric value is a measure of the energy produced when the fuel is burnt completely in order to determine the suitability of biodiesel as an alternative to diesel fuel. From *Figure 9*, diesel showed that the highest caloric value which was 43.6475 MJ/kg. This was followed by CJO, 39.4381 MJ/kg. JB had the lowest caloric value which was 35.4284 MJ/kg. The caloric value of the JB was lower than diesel because of the oxygen content in biodiesel which was higher. Higher oxgen content improves the combustion process and decreases its oxidation potential. So, the

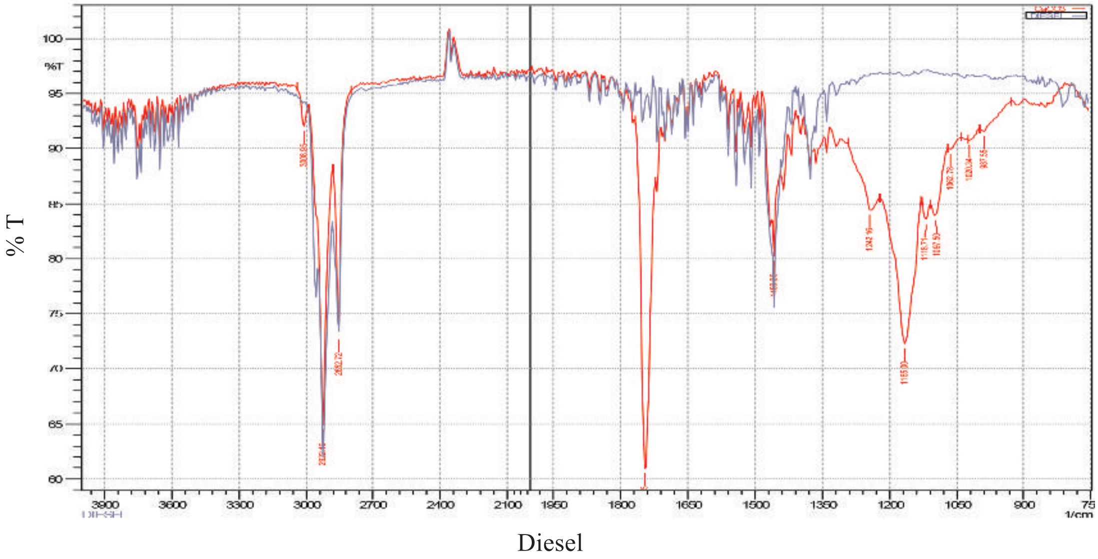

Figure 8. Infrared spectra of FAME (red) and diesel (blue).

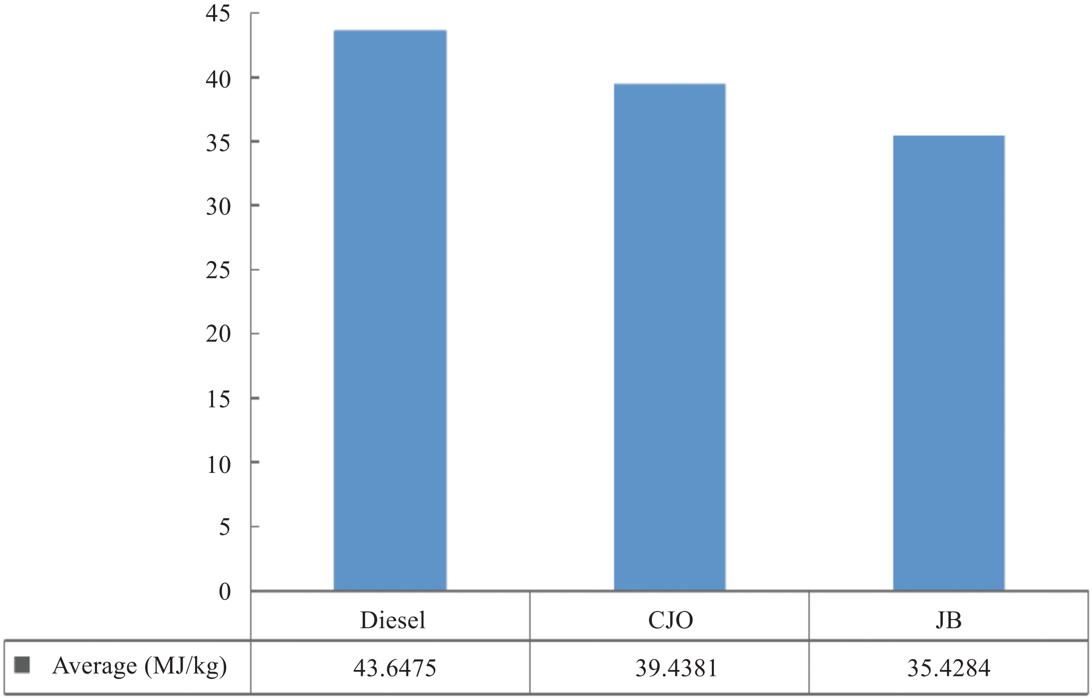

Figure 9. Analysis of calorific value.

decrease of hydrogen and carbon content is the source of thermal energy. Thus more complete combustion leads to less emission of carbon monoxide.

Density Analysis

Density influences the efficiency of the fuel atomization for airless combustion system. It effects the break-up of fuel injected into the cylinder. The biodiesel produced from vegetable or fats are slightly denser and less compressible than the diesel fuel (Leung *et al.* 2010). The density measured is displayed in the *Figure 10*. The results shown is in the acceptable density range in accordance to the biodiesel standard *DIN EN 14214* which is in between 860 – 900 kg/m^3. Thus, the biodiesel produced was comparable as the mineral diesel.

Flash Point

Flash point is the temperature at which the fuel will start to burn when it comes in contact with fire. *Figure 11* showed flash point of diesel at 68, CJO at 200 and JB at 167. The flash point of JB was higher than diesel as JB was having volatile impurity which was very important for the engine's starting and warming. Flash point is a crucial parameter from the point of safety. This is because higher flash point demostrates safely during transport, handling and storage (Leung *et al.* 2010).

Emissions

In accordance to *Figure 12*, carbon monoxide (CO), Sulphur dioxide (SO_2), nitrogen oxide (NO) and nitrogen dioxide (NO_2) produced in JB was lower than diesel. This was a result of a more complete combustion in JB as it was having a higher ketone number compared to petrol diesel. This is due to its long chain fatty acids with 2–3 double bonds, not aromatic and contains 10%–11% oxygen by weight (Koh *et al.* 2011). A decrease in CO emission was due to more oxygen content in biodiesel compared to diesel. Meanwhile, a decrease in SO_2, NO

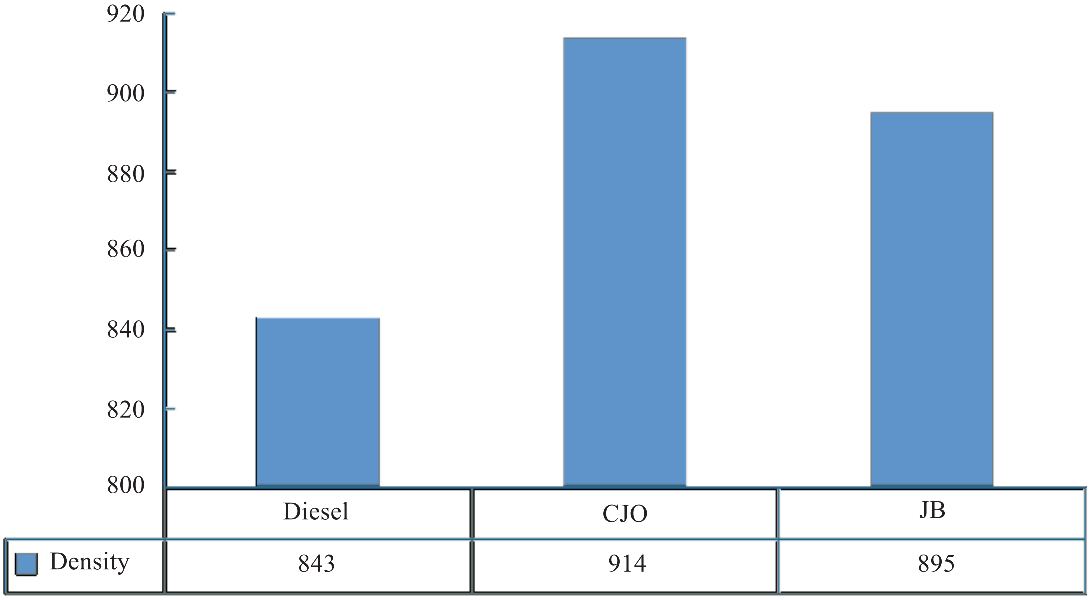

Figure 10. Density of diesel, CJO and JB.

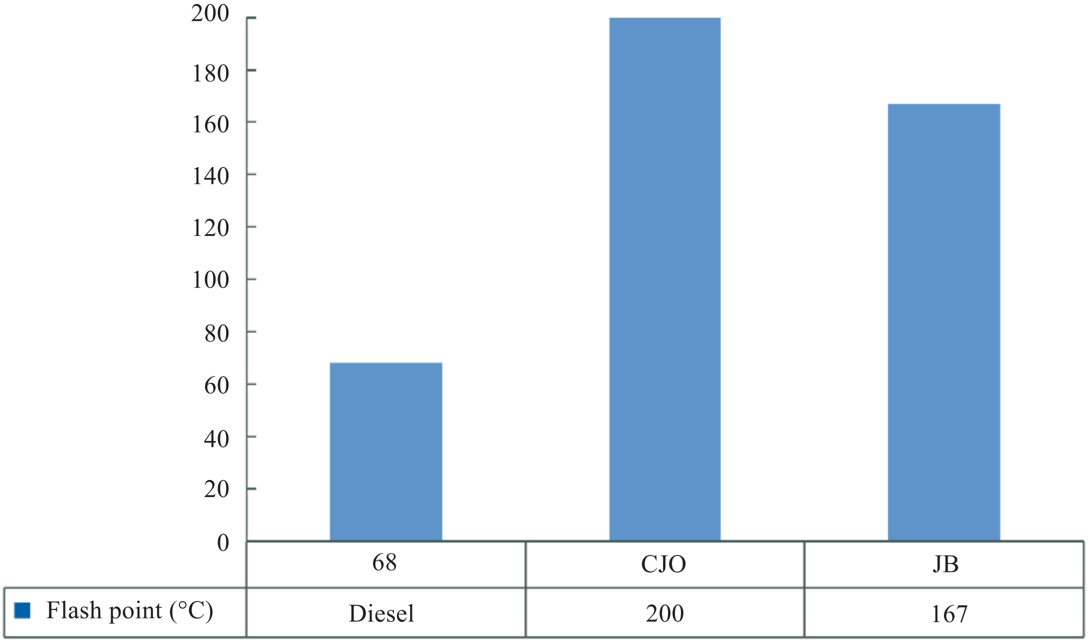

Figure 11. Flash point of diesel, CJO and JB.

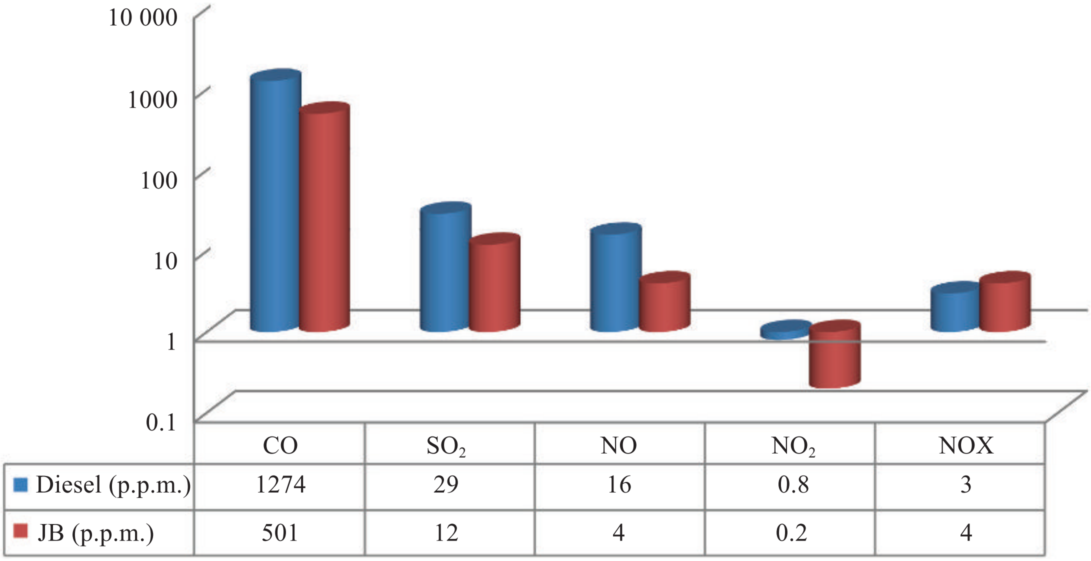

Figure 12. Emission analysis of mineral diesel and JB.

and NO_2 was due to better fuel properties of JB produced. A complete combustion led to higher combustion temperature which resulted in higher NO_x formation. Other than that, high viscosity caused a bigger droplet size and shorter ignition delay might lead to the rise of the NO_x emission.

Diesel Engine Performance

Diesel engine performance testing was performed to study the fuel consumption, specific fuel consumption, brake horsepower and mechanical efficiency (Edin *et al*. 2013). The diesel engine used was model TNM-TDE-700. The experiments were carried out by preparing the JB blends by mixing mineral diesel and JB was prepared for 20 ml for each sample. Six samples were prepared namely B0, B10, B20, B30, B40 and B50. B0 consists of 0% of JB and 100% of mineral diesel. The load used was set to 120 N and the speed was set to maximum throughout the experiment. The results were presented in Table 2 and *Figure 13*.

Fuel consumption rate is the ratio of distance travelled over the volume of the fuel burned. It is used to measure the fuel economy as lower consumption rate represents that the fuel is more economic. B0 or pure diesel showed the lowest fuel consumption at 0.3210 ml/s. B10 to B50 were having higher fuel consumption rate due to lower calorific value of JB. Thus, JB required more fuel in order to produce the same amount of energy. Among the JB, B20 showed the lowest fuel consumption rate which was 0.3235 ml/s that was very near to B0. Thus, B20 was the optimum blending ratio.

Specific fuel consumption is a measure of fuel efficiency of any prime mover that burns fuel and produces rotational or shaft power. The specific fuel consumption rate of B0 or pure diesel was lower than the other JB blends as pure diesel had higher energy content. B20 showed a similar value to B0 which meant B20 was comparable to B0 and it showed a potential to be a substitute to diesel.

Brake horsepower is the actual or useful horsepower of an engine and its power is measured using dynamometer. Brake horse power of B0 or pure diesel was the highest while as the percentage of JB blends increased it showed a reduction in brake power. However, B20 showed an increment which was 41.5420 kW. The decreasing is a result of lower caloric values and higher viscosities as both factors

Table 2. Diesel engine performance study calculation for B0 to B50.

Engine performance	JB Blends					
	B0	B10	B20	B30	B40	B50
Torque (N.m)	36	36	36	36	36	36
Fuel consumption rate (ml/s)	0.3210	0.3309	0.3235	0.3381	0.3389	0.3424
Engine power output (kW)	1.3500	1.3410	1.3500	1.3261	1.3112	1.3024
Specific fuel consumption (mL/kW)	14.8148	14.9142	14.8148	15.0818	15.2532	15.3563
Brake horsepower	41.5579	41.5342	41.5420	41.5027	41.4946	41.4709
Indicated horsepower (kW)	57.8	57.8	57.8	57.8	57.8	57.8
Mechanical efficiency, n (%)	71.8994	71.8584	71.8720	71.8039	71.7900	71.7490

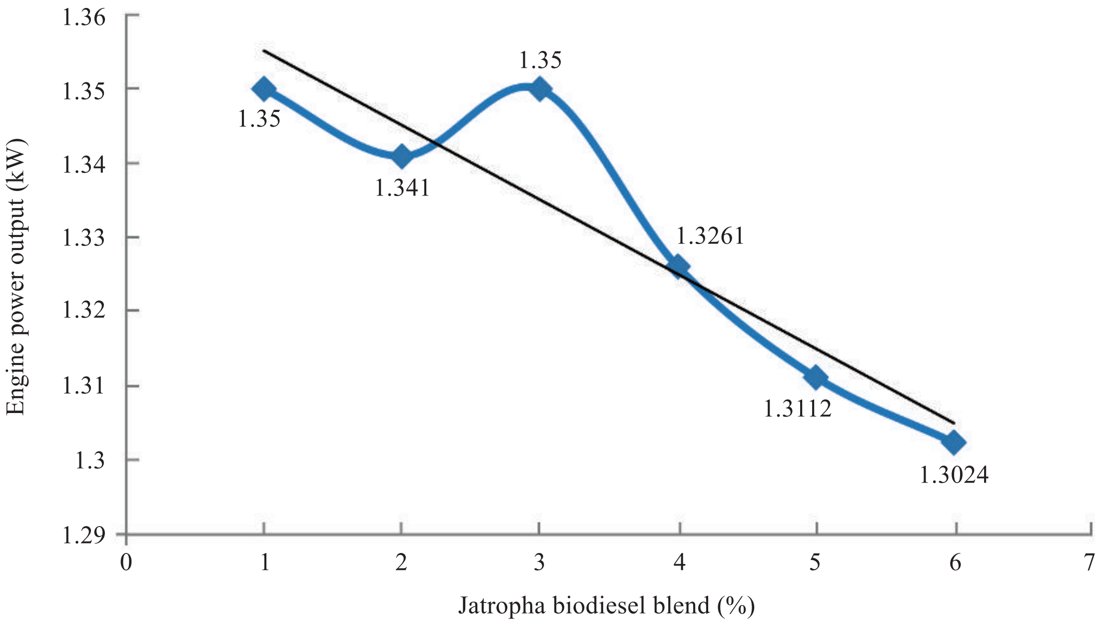

Figure 13. Mechanical efficiency versus jatropha biodiesel blends.

have an effect on combustion. An uneven combustion characteristic of biodiesel also contributes to decline in the percentage of break power.

Mechanical efficiency refers to the measurement of the effectiveness of the diesel engine in transforming the energy and power that is input to the diesel engine into output mechanical work or in other words to measure the engine performance. B0 had the highest energy efficiency which was 71.8994% compare to the other JB blends. As percentage of JB blends increased, the mechanical efficiency decreased except for B20 which showed an increment. The results showed that B20 was the optimum blend and performed as comparable to diesel.

CONCLUSIONS

In this study, a heterogeneous catalyst from seashells and eggshells was synthesized and used for biodiesel production. During *Step 1*, acid pretreatment process the identified optimum catalyst to oil ratio was 0.01:1 and the optimum methanol to oil ratio was 0.6:1. On the other hand, for *Step 2*, base-catalyzed process the optimum to oil ratio was 0.02:1 and the optimum methanol to oil ratio was 5:1. Among the catalysts used, CaO from *P. expansa* performed the best. The optimum reaction time for *Step 1* was 1 h while for *Step 2* it was 2 h. For equipment, orbital shaker showed a higher conversion rate compared to magnetic stirrer cum heater due to its better mixing rate. The highest rate of conversion of the JB yield was 96%.

For FT-IR analysis, the main differences in the spectrum of JB produced from mineral diesel were strong infrared absorption at 1700 cm^{-1}–1750 cm^{-1} due to the presence of aldehyde, C=O group. The presence of C=O group confirmed the specimen to be biodiesel or fatty acid methyl esters. Other functional groups such as C-O, C-H were also present. For calorific value analysis, JB had the lowest calorific value among CJO and diesel. This was due to higher oxygen content and led to more complete combustion in JB. So, the hydrogen and carbon content decreased which was the source of the thermal energy. For density analysis, the JB produced had a density of 895 kg/m^3 which was higher than the mineral diesel and lower than CJO. The biodiesel produced was comparable to mineral diesel as it fell in the acceptance density range of biodiesel standard *DIN EN 14214* which is 860 kg/m^3 – 900 kg/m^3. For flash point analysis, JB had a flash point of 167. It is safer and cheaper for transport, handling as well as for storage compared to mineral diesel which had a flash point of 68. From the diesel engine performance testing conducted, it was found that JB of B20 was the optimum ratio as it had the lowest fuel consumption rate, highest brake horsepower and highest mechanical efficiency among the JB blends. JB showed a similar characteristics of mineral diesel. Therefore, it had the potential to act as a substitute for diesel.

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Decision-making Processes for a Do-not-resuscitate Poisoned Pediatric Patient Admitted to the Department of Emergency and Medical Services — A Case Study

R. (III) P. DIOSO*

A team of healthcare professionals in the Department of Emergency and Medical Services demonstrated decision-making processes for a poisoned pediatric patient with a Do-not-resuscitate (DNR) order. At the same time, clinical guidelines for poison resuscitations were critically applied. Lastly, the case examined and evaluated emergency care, management, treatment and diagnostic laboratory tests for pediatric poisoned patients.

Ideas on pertinent literatures of decision-making models, clinical guidelines on poison resuscitation and DNR policies were already known by the majority. In addition, ideas on the benefits such as becoming enlightened on how to make sound judgments in an emergency situation, particularly addressing issues on how to prioritize patients waiting in the emergency departments were featured.

Poison resuscitations were decided upon by using the descriptive, normative and prescriptive clinical decision-making models in a fast-paced environment. But the most important outcome however, was the recognition of client/relative satisfaction from hospital services — the demonstration of a sound decision-making that is legally, physiologically and financially in tandem with patient needs.

Key words: Clinical decision-making; poison; Department of Emergency and Medical Services; emergency; control

The case aims to apply the processes of decision-making to a Do-not-resuscitate (DNR) pediatric patient admitted to the Department of Emergency Medical Services (DEMS) of a state-run hospital located in a low-income country. It also aims to examine and evaluate the case of a 16-year-old female intoxicated pediatric patient to provide her with emergency care, management, diagnostic investigations and treatments. The descriptive, normative and prescriptive models of decision-making (Shaban 2005) are demonstrated and therefore concludes with a sound decision.

Ethics of justice were considered and this case study maintained anonymity of patients, healthcare professionals and hospital's name. The purpose is to make a world view on how a sound decision-making is demonstrated in a fast-paced environment.

BACKGROUND OF THE STUDY

The state-run hospital used for this study admits more than 180 000 patients per year with over 1000+ bed capacity — admitting an estimated 500+ patients per day (Philippine Statistical

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Lincoln University College Malaysia, Block A Lot 1, Mayang Plaza, 1 Jalan SS 26/1, Taman Mayang Jaya, 47301 Petaling Jaya, selangor, Malaysia

* Corresponding author (e-mail: duke@lincoln.edu.my)

System, 2005). This is why the emergency department imposes a policy of 24-hours maximum waiting time as a driving force to ensure that all healthcare professionals work efficiently and effectively.

According to the Department of Health (DOH) (2005a), the issue on patient waiting time has become a worldwide problem. Due to the fact that in most countries the finances of patients are limited, thus it affects the hospitals' system of prioritizing those who needs immediate admission with free healthcare services (Sachs 2012; Kobusingye *et al*. 2005).

In addition, child protection and rights to have access to immediate treatment affects decisions on rationing hospital resources especially if the child's prognosis of surviving is poor, i.e. children who are comatose for two days due to poisoning requiring a free-of-charge use of mechanical ventilation (Lowry 2008; Aggarwal *et al*. 2004).

Incidence of child poisoning according to the World Health Organization (WHO) in 2004, are found on regions from Africa (4: 100 000), America (0.3:100 000), Southeast Asia (1.7:100 000), Europe (2:100 000), Eastern Mediterranean (1.6:100 000) and Western Pacific (1.8:100 000) per 100 000 population. *Table 1* shows the incidence of fatal child poisoning rates by using regions' and countries' income level.

The Case

A 16-year-old 50 kilogram female patient was admitted to the DEMS diagnosed with non-accidental, self-induced poisoning. According to the DEM guideline, a pediatric poisoning is always classified as emergent.

A statement taken from patient's relatives by the duty nurse at the DEMS triage admission desk showed that the patient took a liquid poison while alone at home in an apparent suicidal attempt. Baseline demographic data such as financial status or indigence were gathered by the social workers and documented it on the patient's record. Since the patient was indigent, being classified as belonging to a 'low income family', the hospital allowed her with a privilege to avail of free medical services (DOH 2005a).

The demographic data was documented and the consent from the relatives for patient's immediate care was secured.

WHO (2008) reiterates the importance of explaining procedures to relatives for emergency cases especially on pediatric patients ages 17 and below. Therefore, poison resuscitations were explained (*Tables 3* and *4*) before transferring her to the acute care unit.

Upon Transfer to the Acute Care Unit

An initial stabilization of circulation, airway and breathing was initiated by primarily connecting her to a cardiac monitor. It was then found that the patient's heart rate was 50 beats per minute and respiration was shallow; therefore the nurse-in-charge provided oxygen on facemask at high flow level to stabilize her oxygen circulation. In addition, an immediate assessment using a Glasgow coma scaling device was advised in order to monitor her

Table 1. Incidence of child poisoning on middle- and low-income regions and countries per 100 000 population.

Africa	America	Southeast Asia	Europe	Eastern Mediterranean	Western Pacific
4	0.3	1.7	2	1.6	1.8

level of consciousness (Stewart-Amidei 2009), found to be decreasing — a guideline from the American Heart Association (AHA) (2000/2010).

In response to the decreasing level of consciousness, an initial venous and arterial blood were extracted for investigations, which include sugars, creatinine, urea, electrolytes, hemoglobin, hematocrit, platelets, white blood cells and blood gases to investigate why the heart rate was decreasing and the breathing was shallow.

By intuition, charcoal resuscitation was then explained to the relatives by the team of healthcare professionals and gave out a written English pamphlet to give them a clear mental

Table 2. Written pamphlet used by clinical experts to explain the use of charcoal.

Position statement of single-dose and multiple-dose activated charcoal

Single-dose Activated Charcoal

- Sodium sulphate, sodium chloride and soap suds enema are used to avoid adverse reaction of charcoal resuscitation. This will reduce the incidence of bowel obstruction;
- Benefit from activated charcoal is more likely to occur if administered within one hour;
- There is the potential for some benefit to reduce morbidity and mortality of activated charcoal if administered after one hour of ingestion;
- The optimal dose is unknown but recommended to benefit: Babies up to 1 year of age: 10–25 gram or 0.5–1.0 gram/kilogram — Children 2 to 17 years of age: 25–50 grams or 0.5–1.0 gram/kilogram —Adolescents and adults: 25–100 grams;
- Activated charcoal has no benefits if the patient has an unprotected airway, if its use increases the risk of aspiration;
- The most common complication of activated charcoal is aspiration or direct instillation of activated charcoal into the lungs; and
- Activated charcoal may not benefit for some ingestants, including heavy metals, metal salts (lithium and iron), alcohols, cyanide and other rapid-acting medications.

Multiple-dose Activated Charcoal

- There are benefits demonstrating that multiple-dose activated charcoal reduces morbidity and mortality in the poisoned patient better than single-dose activated charcoal;
- Sodium sulphate, sodium chloride and soap suds enema are used to avoid adverse reaction of charcoal resuscitation. This will reduce the incidence of bowel obstruction;
- Multiple-dose activated charcoal benefits life-threatening ingestions of carbamazepine, dapsone, phenobarbital, quinine, theophylline, chlorox and alcohol;
- The first dose is recommended to benefit: Babies up to 1 year of age: 10–25 gram or 0.5–1.0 gram/kilogram — Children 2 to 17 years of age: 25–50 grams or 0.5–1.0 gram/kg — Adolescents and adults: 25–100 grams and given every 8 hours interval;
- Multiple-dose activated charcoal is contraindicated if the patient has an unprotected airway, if its use increases the risk of aspiration or if any anatomical or medical conditions exist that may compromise by its benefits; and
- Rarely, aspiration, constipation and bowel obstruction can occur.

picture of the planned resuscitation process.

Table 2 enumerates written position statements for single-dose and multiple-dose activated charcoal in a form of pamphlet.

When no bowel sounds were heard after auscultation of the abdomen of the patient, a nasogastric tube (size French 12), was then inserted to the patient and a *nothing by mouth* was ordered by the physicians in the DEMS in order to start the charcoal resuscitation.

The nurse-in-charge gave a single-dose of charcoal resuscitation followed by an insertion

Table 3. Clinical features and associated poisons.
(Lowry 2008; Weerasuriya *et al*. 2012; Aggarwal *et al*. 2004)

Clinical features	Associated poisons
Non-reactive pupils	Chloroform, alcohol, cyanide, arsenic, organophosphates, carbamates, phosphorus and kerosene.
Odour of the breath	Chloroform, alcohol, digitalis, cyanide, arsenic, organophosphates, phosphorus and kerosene.
Hypertension	Amphetamine, cocaine, and antipsychotic (mao inhibitor).
Tachycardia	Marijuana, phencyclidine, alcohol, nicotine, antihistamine, antipsychotic, and antidepressant.
Hypotension, bradypnoea and bradycardia	Antidepressants (severe cases), barbiturates, narcotics, benzodiazepines, cyanides, nicotinics, organophosphates, alcohols and chloroforms.
Hypotension and tachycardia	Aluminium phosphides, antipsychotics, caffeines, cyanides, disulphiram-ethanols, and tricyclic antidepressants.
Hyperthermia	Amoxapines, amphetamines, antidepressants, cocaines, lithiums, phencyclidines, anticholinergics, salicylates and antihistamines.
Hypothermia	Antidepressants, ethanols, benzodiazepines, narcotics, barbiturates, phenothiazines, alcohols and chloroforms.
Tachypnoea and respiratory acidosis	Amphetamines, atropines, cocaines, salicylates, carbon monoxides, cyanides, paracetamols and amatoxin mushrooms.
Bradypnea, loss of bowel sounds and metabolic alkalosis	Antidepressants, antipsychotic agents, barbiturates, ethanols, benzodiazepines, chlorinated hydrocarbons, narcotics, nicotines, organophosphates, cobra salivas, antidepressants, antihistamines, organophosphates, barbiturates, lithiums, cyanides, narcotics and carbon monoxides
Seizures	Antidepressants (amoxapine and maprotiline), antipsychotics, antihistamines, chlorinated hydrocarbons, organophosphates, cyanide, leads and other heavy metals, lithiums, narcotics, sympathomimetics
Meiosis (Constricted pupils)	Barbiturates, phenothiazines, ethanols, narcotics, nicotines, organophosphates, chlorinated hydrocarbons, narcotics, and cobra salivas
Mydriasis (Dilated pupils)	Amphetamines, caffeine, cocaines, nicotines, antidepressants, antihistamines, atropines, methaemoglobinaemias, alcohols and chloroforms

of continuous bladder drainage and taken urine samples for the presence of metabolites (Weerasuriya *et al*. 2012).

As a standard procedure, after charcoal resuscitation, the patient was placed on hourly monitoring to assess clinical features associated with poisons (*Table 3*).

Healthcare professionals involved in the care, management and treatment of the patient were guided by the active and passive complementary steps in resuscitation for poisoned patients (*Table 4*).

Table 4 highlights the algorithm or guideline on poison resuscitation that is according to the WHO (2008), to be always available on emergency departments worldwide.

Few hours passed, the nurse-in-charge for the patient noticed that she was having episodes of difficulties in breathing with blood oxygen level of 70%. Immediately, the DEMS' physician-in-charge in the acute care unit intubated the patient with an endotracheal tube (size 6.5) as a form of active resuscitation in response to a decreasing blood oxygen level.

After intubation, the patient was connected to a mechanical ventilator by the physician-in-charge, to replace the facemask removed. Mechanical ventilator's settings were done according to patient's 50 kilogram weight

Table 4. Five complementary steps on both active and passive resuscitation for poisoned patients in the emergency department.
(AHA 2000; Aggarwal *et al*. 2004; WHO 2008)

Steps	Activities as clinical guidelines
Step 1: Initial stabilisation	• On arrival of a patient with poisoning, the initial priorities are the maintenance of circulation, airway and breathing whether it is in an emergent or urgent case. • If the patient has an altered level of consciousness, it is considered to be in the category of emergent. Another emergent resuscitation is intravenous fluid fast drip due to fluids loss. • Before infusing intravenous fluids, blood should be withdrawn for investigations, which include sugars, ureas, electrolytes and arterial blood gases.
Step 2: Diagnosis of type of poison	• For a non-emergent or non-urgent category, this step can be done first to complement with step 1. This is also known as hourly monitoring.
Step 3: Nonspecific therapy	• The activities done here are the gastric lavage; gastric emptying, enhancing fecal excretion, urine output manipulation, bowel irrigation and use of activated charcoal after nasogastric tube insertion.
Step 4: Specific therapy	• Urine output manipulation. • Mechanical ventilation or oxygen therapy. • Cardioversion or defibrillation.
Step 5: Supportive therapy	• Antidotes are largely used for passive resuscitation. The aim is to preserve the vital organ functions until poison is eliminated from the body and the patient resumes normal physiological functions.

having her tidal volume (500 milliliters of air), required fraction of inspired oxygen (100%), documented respiratory rate per minute (20 breaths per minute) and positive end expiratory pressure of 5 cm water, that was based on the guidelines for poisoned patients (AHA 2000).

When the patient's abnormal heart rhythm persisted, as shown on the heart monitor while on mechanical ventilation, healthcare professionals then referred the patient to the DEMS physician-in-charge for a choice of defibrillation or cardioversion as a form of active resuscitation (AHA 2010) in response to a premature ventricular contraction.

In response to an abnormal heart rhythm and hypotension the patient was then referred (Mace *et al*. 2008) to the toxicologist of the DEMS. She was then given an intravenous fluid for fluid resuscitation as ordered, in response to a low blood pressure.

As the patient's pupils became non-reactive, an antidote was given (AHA 2000) by the DEMS physician before referred to the Intensive Care Unit (ICU) for admission. However, there were no vacancies specifically for indigent patients as for the moment; therefore, the patient had to wait at the DEMS until further vacancies in ICUs were endorsed to the nurse-in-charge.

Hours, passed, an arterial blood gas result of the patient was interpreted by the DEMS physician and was noted to be a respiratory acidosis. In response to the arterial blood gas findings, the patient was positioned to moderate high back rest.

At the end of the 8-hour shift, the nurse-in-charge of the patient gave a report to the attending nurse in the succeeding shift for continuity of care and for further treatment and management. The endorsement of the patient's transfer to ICU was discussed by the team of nurses since it is more often a 90% chance of survival compared to a regular ward transfer which is 10% being practiced for intoxicated/poisoned patients with unstable vital signs.

After 24 Hours

Hours passed but the patient did not improve from her comatose status, without spontaneous respiration. Her previous 70% partial oxygenation result after giving active and passive form of resuscitation was complicated by a loss of bowel sound and abnormal heart rhythm.

Again, a 70% partial blood oxygenation result was recorded but with an improved pupilary reaction that sluggishly reacts to light. The non-spontaneous eye movement on Glasgow coma scale instigated the experts on poison control to respond by placing the patient on seizure precaution with body temperature monitoring every hour that may mask seizure episodes if chills occur (AHA 2000).

A second arterial blood gas assessment of the patient in response to a respiratory acidosis was again ordered. The result changed to a metabolic alkalosis (decrease in bicarbonate arterial blood level). A bicarbonate 50 MEQs drug was given intravenously by the DEMS physicians as one time dose (AHA 2010) in response to a metabolic alkalosis result.

Clinical experts provided rigorous resuscitation with antidotes, such as pralidoxime and Atropine in response to an unimproved heart rhythm noted through cardiac monitor.

The patient was given 1 litre of normal saline solution intravenously in response to low urine output noted every hour for 24 hours. *Table 5* summarizes the care plan while the drug study was further explained in the Appendix.

Table 5. Care plan.

<table>
<tr><th>Assessment upon admission</th><th>Intervention</th><th>Assessment after 24 hours</th><th>Intervention</th></tr>
<tr>
<td>Decreasing level of consciousness with Glasgow coma scale result as follows:
<table>
<tr><th colspan="2">Glasgow coma scale result</th></tr>
<tr><td>Eye movement</td><td>No response</td></tr>
<tr><td>Verbal response</td><td>No response</td></tr>
<tr><td>Motor response</td><td>Flexes to pain</td></tr>
<tr><td>Orientation to:
1. Time
2. Place
3. Person</td><td>No response</td></tr>
</table>
</td>
<td>

Triage officers assessed the demography of the patient for social service referral and acquired consent from the relatives for primary care to resuscitate the patient with antidotes.
The nurse-in-charge put the patient on critical hourly monitoring to analyse clinical features associated with poison.
Blood extraction was done by the nurse-in-charge for sugars, urea, and electrolytes investigation.
Heart rate, blood pressure, pupilary reactions, saturated partial oxygenation, bowel sounds and respiratory rate were monitored hourly.
Placed on seizure precautions.

</td>
<td>

Level of consciousness did not improve.

<table>
<tr><th colspan="2">Glasgow coma scale result</th></tr>
<tr><td>Eye movement</td><td>No response</td></tr>
<tr><td>Verbal response</td><td>No response</td></tr>
<tr><td>Motor response</td><td>Flexes to pain</td></tr>
<tr><td>Orientation to:
1. Time
2. Place
3. Person</td><td>No response</td></tr>
</table>
<table>
<tr><th colspan="2">Blood investigation results</th></tr>
<tr><td>Blood</td><td>Results</td></tr>
<tr><td>Sodium</td><td>114</td></tr>
<tr><td>Potassium</td><td>3.0</td></tr>
<tr><td>Calcium</td><td>5.0</td></tr>
<tr><td>Chlorine</td><td>120</td></tr>
<tr><td>Blood Urea N2</td><td>3.9</td></tr>
<tr><td>Creatinine</td><td>1.1</td></tr>
<tr><td>Fasting sugar</td><td>110</td></tr>
<tr><td>Hemoglobin</td><td>12</td></tr>
<tr><td>Hematocrit</td><td>45%</td></tr>
<tr><td>White blood cells</td><td>15000</td></tr>
</table>
</td>
<td>

Placed on moderate high back rest
Maintained on nothing by mouth
Plan for ICU admission
Seizure precautions were ordered.
Monitored temperature to ensure that hypotension and chills will not mask seizure episodes.

</td>
</tr>
</table>

Table 5 (Cont.). Care plan.

Assessment upon admission	Intervention	Assessment after 24 hours	Intervention
Heart rate = 50 beats per minute	• The nurse-in-charge connected the patient to a cardiac monitor. • Primarily gave oxygen on facemask at high flow level. • Atropine sulphate IV (preferred) >12 yrs. 0.4–2.0 mg q/15’; <12 yrs. 0.05 mg/kg q/15	• Heart rate = varying from 50 to 70 beats per minute	• Blood pressure monitoring every hour • Pralidoxime >12 yrs. 1–2 gm/minute IV (No more than 0.2 gm/minute) <12 yrs. 20–50 mg/kg. Repeat in 1–2 hours, then in 10–12 intervals
No bowel sounds heard	• The nurse-in-charge inserted nasogastric tube and started charcoal resuscitation	• A few bowel sounds heard	• Lavaged again with activated charcoal in isotonic saline as multiple dose
70% saturated partial oxygenation result with fast respiration	• The team of nurses collaborated with physicians to intubate the patient. • Clinical experts removed oxygen on facemask and replaced it with a mechanical ventilator machine. Mechanical ventilator set up Tidal Volume = 500 ml of air Fraction of inspired oxygen = 100% Respiratory rate = 20 breaths per minute Positive end expiratory pressure = 5 cm H_20 • Bedside chest X-ray requested.	• 80% saturated partial oxygenation result with regular respiration rhythm and clear breath sounds • Bedside chest x-ray result shows complete lung expansion with no edema noted	• Monitored respiratory rate and sound every hour

Table 5 (Cont.). Care plan

<table>
<tr><th>Assessment upon admission</th><th>Intervention</th><th>Assessment after 24 hours</th><th>Intervention</th></tr>
<tr>
<td>Low blood pressure and abnormal heart rhythm</td>
<td>The nurse-in-charge then referred the patient to a DEMS physician for a choice of defibrillation or cardioversion.
The nurse-in-charge gave the patient intravenous fluid resuscitation at slow drip.</td>
<td>Low blood pressure and abnormal heart rhythm</td>
<td>Intravenous fluid therapy of at least 1 liter for 6 hours
Monitored heart rate and rhythm every hour</td>
</tr>
<tr>
<td>Non-reactive pupils with arterial blood gas result showing respiratory acidosis
<table>
<tr><th colspan="2">Arterial blood gas results</th></tr>
<tr><td>Arterial Hydrogen</td><td>Decreased</td></tr>
<tr><td>Arterial CO_2</td><td>Elevated</td></tr>
<tr><td>Arterial bicarbonate</td><td>Normal</td></tr>
<tr><td>Arterial oxygen</td><td>70%</td></tr>
</table></td>
<td>The nurse-in-charge referred the patient to the physician for an initial analysis of her baseline blood oxygenation and for ICU admission.
Urine sample was investigated for presence of metabolites
Clinical experts inserted continuous bladder drainage</td>
<td>Slow reactive pupils with arterial blood gas result showing metabolic alkalosis
<table>
<tr><th colspan="2">Arterial blood gas results</th></tr>
<tr><td>Arterial Hydrogen</td><td>Increased</td></tr>
<tr><td>Arterial CO_2</td><td>Normal</td></tr>
<tr><td>Arterial bicarbonate</td><td>Decreased</td></tr>
<tr><td>Arterial oxygen</td><td>70%</td></tr>
</table></td>
<td>Bicarbonate 50 MEQs was given intravenously as one time dose
Monitored urine output every hour</td>
</tr>
</table>

After 72 Hours

The condition of the patient prompted the attending physician to recommend DNR to the relatives and to the nurses-in-charge. A discussion was however made by the physician with the relatives whether to admit the patient who has been waiting in the DEMS for 48 hours or proceed with the signing of DNR consent to be effective immediately.

Probabilities were presented to the relatives during the discussion using the *home against advice* method (50% practised), omission of medical treatment while confined in the hospital (5% practised) and the provision of passive resuscitation without active resuscitation (45% practised) in the acute care division of the DEMS. Relatives' decision (Say & Thomson 2003) are also important to be considered. *Figure 1* illustrates the decision tree divided into percentages recommendable for the pediatric indigent patient.

The DNR consent was signed. The poisoned DNR pediatric overstaying patient was restrained from receiving active resuscitation that is 45% practiced in clinical settings (WHO 2008). Clinical experts however, still intuitively recommended to the team of healthcare professionals that the indigent overstaying DNR pediatric patient should be monitored intensively in the acute care division of the DEMS department despite of her DNR status.

While the patient was being hourly monitored with all the assessment cues directly linked to poison clinical features, it was noticed by healthcare professionals, that the heart rate, heart rhythm and level of consciousness were accelerating and evidence of defecation was seen.

At early 72 hours in the DEMS, the overstaying DNR pediatric patient's Glasgow coma scale result (9/15) improved. The poison was excreted out from her body as evidence by her bowel movement and urine output (Youngner *et al*. 1985; Aggarwal *et al*. 2004).

On her late 72nd hour in the DEMS, the patient was referred back to the physician by healthcare professionals to cancel the DNR decision and proceed with the plan of admitting the patient to the ICU using the social service for pediatrics for financial support.

However, in order to determine the diagnosed nicotinic effects of organophosphates and carbamates poisoning the blood levels of the patient, pralidoxime was again administered intravenously as one time dose (AHA 2000; Aggarwal *et al*. 2004) by the nurse-in-charge.

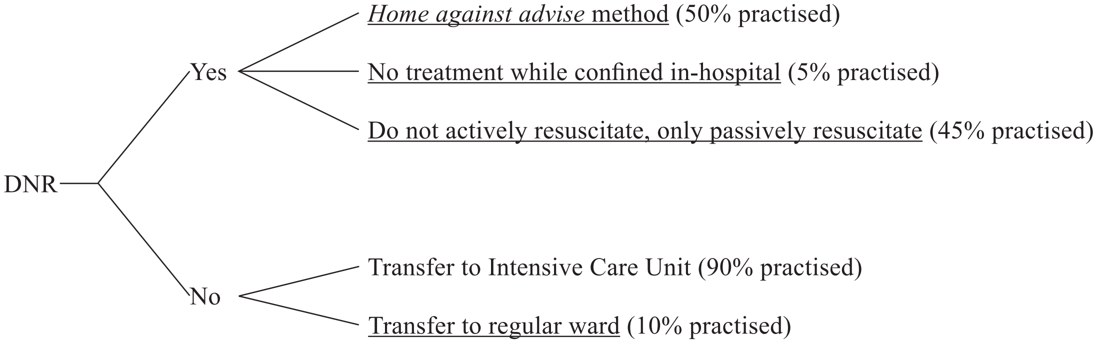

Figure 1. The decision tree divided into probabilities.

This will bind and cleave phosphate-esters between organophosphates ingested as poison by the patient to its acetylcholinesterase thus detoxifies her completely (AHA 2000; Aggarwal *et al*. 2004; WHO 2008).

Figure 2 explains the pathophysiology of the disease process analyzed on the patient's case and discusses how hidden signs and symptoms occurred termed as the pseudo-allergic reactions.

METHODOLOGY

This section examines and evaluates how the three basic models of decision-making — the descriptive, prescriptive and normative — were applied.

A descriptive decision is characterized by understanding how individuals make judgments and decisions focusing on the actual conditions, contexts, ecologies and environments in which they are made (Shaban 2005). An advantage of the descriptive model is the adequacy in supporting assumptions made about decision-making processes with relevant examples from a suitable period of observation (Shaban 2005). An example is intuition (Offredy & Meerabeau 2005; Bell *et al*. 1995).

Normative models in decision-making process (Offredy 1998; Shaban 2005) are characterized by rational, logical and scientific procedures supported by clear or probable evidences (Harrison 1996). Statistical analyses with decision trees of large-scale experimental and survey research which is representative of a target population where the findings apply are information sources in a normative decision (Bell *et al*. 1995). Advantages herewith enable decision-makers to predict and explain the outcomes of decisions and minimize judgment errors (Thompson & Dowding 2002) especially when patients or relatives are key decision makers (Say & Thomson 2003).

Lastly, prescriptive models use information processing theory as a prescriptive tool to assist practitioners in enhancing decision tasks to analyse sources, principles and findings of previous research or clinical guidelines with algorithms (Shaban 2005).

Table 6 summarizes the three decision-making models.

The Prescriptive Decision-making: Information Processing Theory Applied

Its characteristic uses framework or information and facilitating more effective decision-making as its advantage (Bell *et al*. 1995).

Information Processing Theory Step One: Cue Acquisition

Using clinical guidelines the cues were acquired. Biophysiologic instruments used to acquire cues were the Glasgow coma scale, electrocardiograms, pulse oxymeter, urine collection, blood pressure auscultation, and venous and arterial blood interpreting machines (Stewar-Amidei 2009; AHA 2010). Other important cues acquired are her pupilary reactions, urine outputs, and bowel sounds (Mace *et al*. 2008; AHA 2000) to support the cues investigated through biophysiologic instrumentations.

Information Processing Theory Step Two: Hypothesis Generation

Healthcare professionals caring, treating and managing the poisoned pediatric patient hypothesized that she has ingested organophosphate and carbamate poisons found in insecticides. It was also hypothesized by the clinical experts that the patient had a prolonged paralysis of the muscles such as the diaphragm and heart. Therefore, clinical experts hypothesized that she needs to be monitored her intensively.

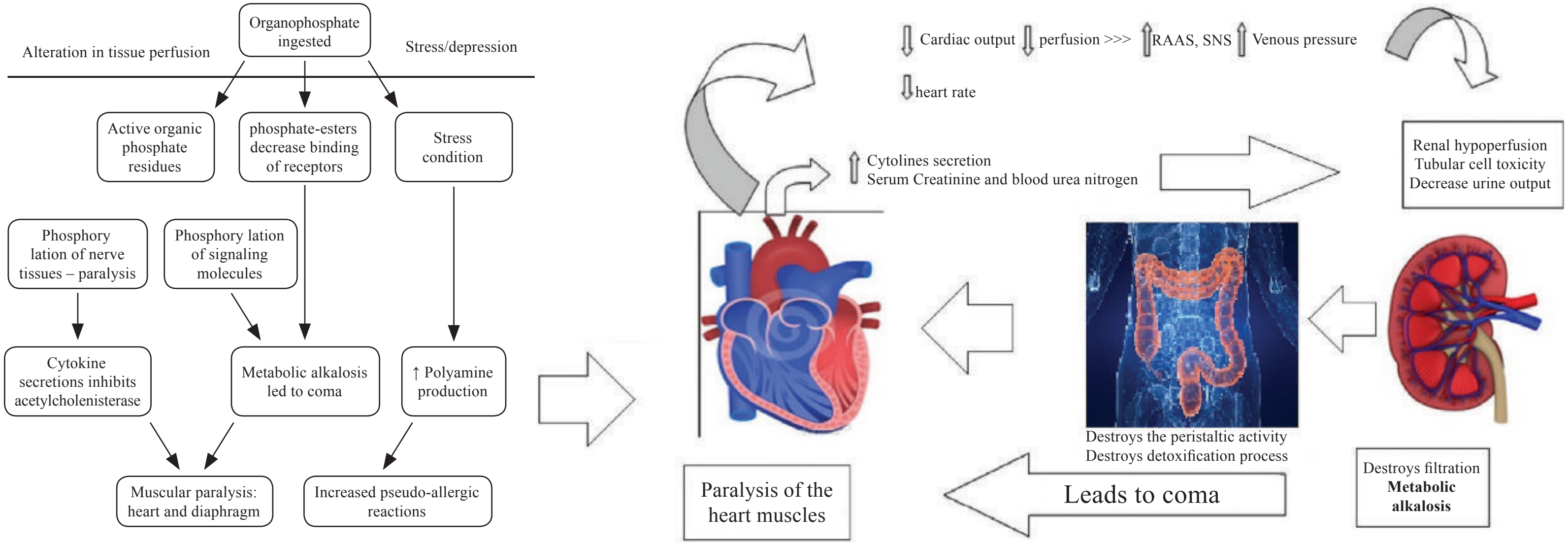

Figure 2. Altered physiology.

Table 6. Summary of three decision-making models.
(Shaban 2005; Thompson & Dowding, 2002; Tanner *et al*. 1987; Offredy 1998)

Normative model	
Characteristics	Rational, logical, scientific, evidence based decisions.
Information sources	Statistical analysis of large-scale experimental and survey research which is representative of a target population where the findings can be applied.
Examples	Decision trees.
Advantages	Enable decision-makers to predict and explain the outcomes of decisions.
Prescriptive model	
Characteristics	Frameworks or information processing designed to enhance specific decision tasks.
Information sources	Principles and findings of previous scientific research (associated with normative models).
Examples	Cue acquisition, hypothesis generation, interpretation of cues, and hypothesis generation — information processing theory.
Advantage	Facilitating more effective decision-making.
Descriptive model	
Characteristics	Understanding how individuals make judgments and decisions focusing on the actual conditions, contexts, ecologies, and environments in which they are made.
Information sources	Observation, description and analysis
Examples	“Think Aloud”, humanistic intuition and pattern recognition
Advantage	Adequacy in supporting assumptions made about decision-making processes with relevant examples from a suitable period of observation.

As the patient prognosis of survival did not improve after 24 hours the DEMS physicians hypothesized that a DNR should be recommended to the relatives.

Information Processing Theory Step Three: Interpretation of Cues

The patient was interpreted to be experiencing muscular paralysis — including the diaphragm and heart muscles — due to the manifested slow and shallow breathing and hypotension. In addition, it was also interpreted that the acetylcholinesterase was destroyed, hence the neurotransmitter called acetylcholine could not be broken down or deactivated leading to an over stimulation of the parasympathetic nervous system. The results were both muscarinic and nicotinic effects. That is why she was given treatments such as atropine and pralidoxime while activated charcoals were given via nasogastric tube as antidotes (refer to *Appendix 1*).

Information Processing Theory Step Four: Hypothesis Evaluation

In this step, DEMS physicians (especially the poison specialists) caring, treating and managing the patient recommended DNR. This was due to the deteriorating condition of the patient during the first 24 hours from admission. In addition to this specific hospital's local policy of 24-hour overstay for patients in the DEMS, a slow reactive pupil for 48 hours was evaluated to be insufficient to recommend a free medical service usually granted by the hospital insurance agency.

The Normative Decision-making: Decision Trees

Patients' relatives were presented with options in a form of decision trees (Say & Thomson 2003). The decision tree found on *Figure 1* was used for this case based on the clinical guidelines in outlining options. However, the physicians still instigated that DNR be advised because the prescriptive decision-making process was the norm that is always done in the DEMS.

Descriptive Decision Making: Intuition

Intuitively, during the first 24 hours in the acute care unit of the DEMS, the collaborating healthcare professionals descriptively decided that the patient could be placed in the ICU due to a recognized pattern of complex masked assessment cues or hidden signs and symptoms (Phaneuf 2008) from poisoned patients undergoing active resuscitation.

In a descriptive decision-making, *intuition* (Offredy & Meerabeau 2005) enumerates probabilities during a discussion. Probabilities are easier to use when collaborating with other experts to intuitively enumerate tasks to measure clinical reasoning (McAllister *et al*. 2009). In addition to Brien and co-researcher's (2011) and Benner and Tanner's (1987) study on how to trust an intution, Hams (2000) also said that during discussions with the relatives and the clinical experts, patterns of previously experienced events must be intuitively considered in order to validate an intuition. Banning (2007) supported this method of decision-making as a role of learning from experiences.

Intuitions are done descriptively according to Banning (2007) by starting with an intuitive hypothesis and perception.

This links to the interpretation of cues from the information processing theory (Tanner *et al*. 1987) of the prescriptive model, that the patient is manifesting hidden signs and symptoms also known as complex masked assessment cues (Phaneuf 2008; Stewart-Amidei 2009).

Thereby DNR was chosen at an early stage of consultation (after 24 hours) but was rejected at the late 72 hours of the patient in the DEMS.

It is usually during the late stage of consultation where intuitive hypothesis generation is trusted. That is why the patient was decided to be admitted to the ICU after 72 hours.

Banning (2007) said that this process of decision-making, as an intuitive perception is also done by recognizing patterns from past experiences. *Figure 3* shows the process of trusting an intuition.

DISCUSSION

This section discusses the policy on DNR and the analysis on poison control as a clinical guideline that affects the decision-making.

The Poison Control Guideline Affecting the Decision-making

Poison control guideline affects decision-making in providing a more effective resuscitation especially in a fast-paced environment (Lowry 2008; Bronstein *et al*. 2006). Clinical features of poisoned victims can be delayed, which is also known as complex masked assessment cues or hidden signs and symptoms, hence decision-making must not be in a rush and done with good clinical judgment (Phaneuf 2008).

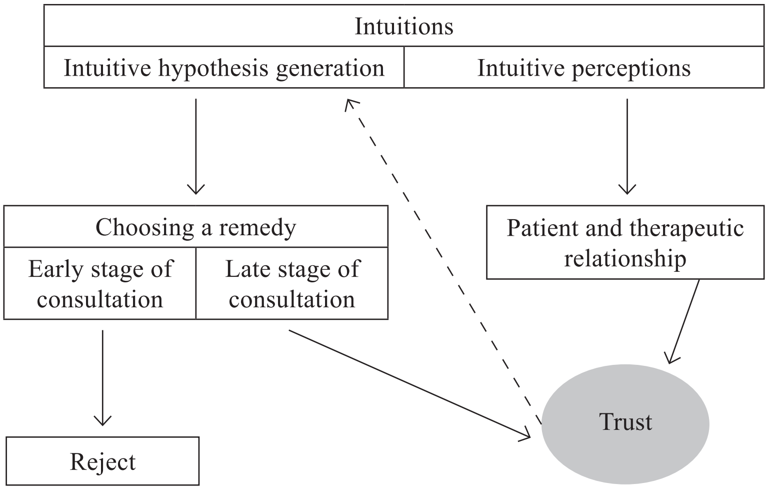

Figure 3. How to use an intuition.

There is a potential for decision-makers to commit errors if poison control guidelines were not critically analyzed. It was also expected that healthcare professionals must neither be too prescriptive nor descriptive in receiving orders that are found on clinical guidelines since a sound decision-making should not be affected by such. On the contrary, it does affect decision-making especially in the emergency department where every healthcare professionals and clinical experts rushes to make decisions. That is why in a fast paced environment, decision-making must use all the three processes to avoid errors. Therefore, rushing a decision affects a sound decision-making.

According to AHA (2000) and Aggarwal *et al*. (2004), acute poisoning was a common medical emergency that needs intensive care, management and treatment that are expensive. This again affected decision-making. Clinical guidelines could help in rationing expensive resources such as mechanical ventilators, heart monitor, drug antidotes and catheters to patients. But instead, the poison clinical guideline was used in a fast-paced environment to control wasting expensive resources by not giving priority to indigent patients who could not afford to pay for the replenishments of such.

That was why relatives/significant others responsible for a comatose patient chooses DNR especially if they lacked funds and insurance agencies denied their application for financial support.

The Policy on Do-not-resuscitate Affecting the Decision-making

A DNR option affects decision-making of healthcare professionals to patients of minor age especially when consent was signed to initiate a passive form of euthanasia that was unsoundly decided upon by the relatives. This is the omission of certain kinds of medical care, management and treatment (DOH 2005a) which was approved by the United Nations during the 1960s (Tolentino 1973) and has been used in this specific hospital (5% practised). Furthermore, research shows that most middle-income and low-income countries practise this system by omitting certain kinds of medical care that are used to sustain a patient's life (Kobusingye *et al*. 2005). This is the most common form of passive euthanasia (Tolentino 1973) — supported by a written consent (White 2010; Mace *et al*. 2008; Lin *et al*. 1999) voluntarily signed by either the patient or by the relatives/significant others.

The act of discharging is another method of executing a DNR (DOH 2005a) affects decision-making because it is consented and signed by relatives/significant others taking responsibility to bring home their patient against medical advice. In addition, this is against the rules of medical ethics on a patient with an existing heartbeat (DOH 2005a). This policy includes a criteria that the patient should still be in the state of 'being alive' before being discharged (Tolentino 1973). This form of passive euthanasia is more often used in this hospital (50% practised) and was formulated to 'avoid potential future litigation for negligence to treat when required' (Lin *et al*. 1999). Furthermore, it also affects the decision-making, because if the patient dies in the hospital after a discharge against medical advice is signed, negligence to treat maybe charged against healthcare professionals especially if a DNR decision is proven to be an error (DOH 2005a). Other research that supports the DNR local policy using the act of discharging says that death should occur outside hospital boundaries (Olsen *et al*. 1993). And according to the Philippine Nurses' Association (PNA) (1990), the patient must still be alive before she reaches her own home to give her a dignified death.

All these factors discussed affect the decision-making. There are three questions recommended for clinical decision-makers to consider who would in the future encounter the same or similar case.

RECOMMENDATIONS

(1) *Was the relative's or others of significant whose preference of care, management and treatment considered and respected?* This question was important to be asked because it addressed the financial barriers in decision-making thus, could affects the decision.

(2) *Was the decision to have benefits could outweigh the harm?* This question was also as important as with *Question 1* since it addresses the impact of a decision in reality.

(3) *How was the practise of autonomy in decision-making applied in this case?* This question addressed the legal issues affecting the decision-making.

The Financial Barrier in Decision-making — Addressing Question 1

It was recommended that the clients' or relatives' or others significant, whose preference of treatment, care and management would be prioritized.

However, hospital policies in high- middle- and low-income countries do not show equal distribution of free healthcare services and health insurances' assistance if the patient has poor prognosis of surviving (Taylor & Xiaoyun 2012; Kobusingye *et al*. 2005).

It is indeed true, that the patients in public hospitals of middle- and low-income countries who are below poverty line (Pagaduan–Lopez 1991) experiences similar situations among patients on high-income countries (Sachs 2012). Providing free health services is government's perennial problem on, high-, middle, and low-income countries (Ranson & Bennett 2009; Chen & Lit 2003; DOH 2005a). Hospitalization is expensive. Rentals for mechanical ventilators in the ICU are even more expensive (Reeves 1997).

The lesser the chances of survival connote the lesser the priority for accessing free healthcare services (Kobusingye *et al*. 2005). This financial need pushes relatives or significant others of indigent patients in government hospitals to make preferences based on their available funds (Lin *et al*. 1999;

Chen & Lit 2003). This financial problem also makes autonomous decision-makers — the relatives and significant others — to push themselves to the less expensive form of care, management and treatment, or sometimes agree with DNR (PNA 1990; Reeves 1997).

Nevertheless, it is recommended that the final decision or preference would still come from the client or relatives/others who were significant (whether manipulated or not).

The Impact of Beneficence Versus Non-malefesance on the reality of decision-making — Addressing *Question 2*

It was recommended that somehow, physicians could not prematurely recommend DNR especially when urgency of the situation in a fast paced environment deemed it necessary.

However, on government-owned hospitals in middle-, high- or low-income countries, for the benefit of the hospital that lacks bed capacity it is recommended to find ways on how to prioritize admissible patients that will somehow regain the quality of life (Kobusingye *et al.* 2005; DOH 2005b). While some physicians discuss DNR decision prematurely in a fast-paced environment such as the DEMS, it was seen as beneficial for others who also needed to be admitted. A DNR order is a reality that is always done in the DEMS (Weerasuriya *et al.* 2012). The benefit of prematurely deciding to send the patient home with poor prognosis of survival offered an advantage to other patients who were waiting to be admitted and had more chances of surviving.

While it is recommended to send patients home because of limited bed capacity, there is however a debatable reality impacting children's rights to be prioritized for hospital admissions whether they have a poor prognosis of surviving (Bass 2003; De Gendt *et al.* 2007; Miles & Burke 1996).

The Legal Issues Affecting Autonomy in Decision-making — Addressing *Question 3*

As highlighted by the National Consent Advisory Group (NCAG) (2012), it is recommended that children who are minors aged 12-years old to 17-years old must have the priority for access in most hospitals as a part of the universal child protection act. In support to this act, most senate health committee disapproves proposals of passive euthanasia for pediatrics (Pagaduan-Lopez 1991; Tolentino 1973) (Lin *et al.* 1999; Jevon 1999).

These legal issues are subject to affect an autonomous decision-maker who will be deemed to answer against the universal anti-medical malpractice law (Jevon 1999; Lin *et al.* 1999). It is recommended that decision makers in the emergency must uphold this law because it values human life settings (NCAG 2012; McClain & Perkins 2002).

CONCLUSION

It was therefore concluded that in this case study, information processing theory recommended DNR. Secondly, decision trees with options that affected the DNR decision had considered probabilities of patient survival. That is why intuition had made an impact on the decision-making process on this case that the patient was only experiencing complex masked assessment cues or hidden signs and symptoms, so the DNR decision was cancelled.

This case did not directly suggest the use of intuition, but concluded that decision-making on the DEMS started with critical awareness of the local policies in tandem with clinical guidelines.

Humanistic intuition and pattern recognition in a CDM process may resolve complex problems such as lacking of funds (Harris & Davies 2007), for indigent patients.

But these intuitions required extreme caution such as the recognition of patterns of previously encountered similar events especially in an emergency context to avoid error in decision-making.

Information processing in this case study, on the other hand, limited perspectives to only one side of the problem using clinical guidelines or algorithms (Manias & Street 2001). The decision was hence, detrimental to the patient. Therefore, in order to demonstrate a comprehensive range of sound judgment, the decision-maker must be in control emotionally, psychologically and intellectually (Lloyd *et al*. 2011) without rushing into a decision using clinical guidelines.

Problem solving using the normative decision-making in this case was somehow better if it was not affected primarily by clinical guidelines. This process used decision trees as options presented to patients and/or relatives significant to others. Broadening perspective using options was a key to decision-making. This system created a mental framework by cautiously considering all options, especially in a fast-paced environment, thereby turned decisions away from frame prescriptive blindness on algorithms and clinical guidelines.

Finally, deciding on patients' waiting time in the emergency departments worldwide also considers seeing the wider picture (Harris & Davies 2007; Howard 2011) — the legal, economical and physiological issues — using the decision-making processes. These considerations reflect sound decision-making if demonstrated well by their healthcare employees (Manias & Street 2001). Therefore, by looking on all sides of the problem before plunging in to a decision, the results of customer/client service satisfaction would be evident or effective enough to maintain a good image of a hospital and its healthcare employees.

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APPENDIX 1. DRUGS SPECIFICALLY GIVEN TO THE PATIENT.

Drug Study					
Drug name	Dosage	Classification	Indication	Action	Side effects
Activated charcoal	100 grams given trice	Poison antidote	Organophosphate poisoning	Binds and absorbs ingested toxins	Decreases peristalsis and palpitation with slow heart rate were noted
Pralidoxime	1 gram given twice	Poison antidote	Reversing paralysis of the respiratory muscles	Binds to organophosphate-inactivated acetyl-cholinesterase	Increased muscle stiffness was noted
Atropine	1 mg given once	Anticholinergic	Organophosphate poisoning that led to alterations in pupillary reactions	Atropine blocks the *action* of chemical called acetylcholine	Dryness of mouth and dysrhythmias were noted
Sodium bicarbonate	50 Milli-equivalents given once	Electrolyte / Alkalinizer	Metabolic alkalosis	Improves renal perfusion and decreases cytokine excretions specifically the ureas and creatinines	Slow breathing was noted

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Short Communication:
Sulphur Levels and Fuel Quality in Peninsular Malaysia

M. RAMALINGAM* AND A. AHMAD FUAD

This paper presents the investigation on sulphur levels in fuels collected from different petrol stations in Peninsular Malaysia. Diesel and gasoline samples were analysed for sulphur using X-ray fluorescence spectrometry technique. In general, diesel concentrations of sulphur were only slightly lower than the Deprtment of Environment's current sulphur limit of 500 p.p.m. across 2010–2014 (the range was 289–698 p.p.m., 374–410 p.p.m., 319–436 p.p.m., 447–605 p.p.m. and 300–477 p.p.m. for 2010, 2011, 2012, 2013 and 2014, respectively). Sulphur concentrations were relatively low in gasoline samples: the mean figures were 95, 77, 96, 74, 240 and 125 for 2009, 2010, 2011, 2012, 2013 and 2014, respectively when compared with the sulphur limit of 500 p.p.m.. The importance of determining sulphur concentration in fuel oils was critical in the efforts to determine if the vehicles used quality fuels that comply with *Euro 2* Standards; and also was relevant in meeting *Euro 4* Standards requirement of 50 p.p.m. sulphur that was proposed to be adopted in the future.

Key words: Sulphur; diesel; gasoline; *Euro Standards*; fuel quality; regulation; Department of Environmen Malaysia; ASTM Standards

Sulphur occurs naturally in crude oil and is found in diesel and gasoline fuels. When fuel is combusted, the sulphur in vehicle fuel causes the release of environmentally harmful compounds such as sulphur oxides and sulphate particles that can contribute to decreased air quality and thus have negative environmental and health effects (Blumberg *et al*. 2003; Alberta Ministry of Transportation 2006). The main environmental concerns related to sulphur emissions are acid rain and the formation of particulate matter (International Marine Organisation 2013). Environmental impacts of acid rain include the acidification of aquatic systems, increasing soil acidity and damage to vegetation (Blumberg *et al*. 2003; Alberta Ministry of Transportation 2006). PM emissions can lead to reduced visibility and also can cause effect to human health (Blumberg *et al*. 2003; Alberta Ministry of Transportation 2006).

Developed countries have several air pollution control programme that have shown to be effective for cleaner air, including adoption of cleaner fuels. Most of these countries also have adopted standards which have ultra-low sulphur content. For example, India, and Thailand adopted *Euro 2 Standards* way back in 2001 and 2002, respectively (Asian Development Bank 2006). Conversely, Hong Kong moved a step forward and adopted *Euro 3 Standards* in 2001, while Singapore, South Korea and Taiwan have migrated to *Euro 4 Standards* (Asian Development Bank 2006). Similar to other countries, the Malaysia government has also enacted the introduction of sulphur-free fuel (with a maximum tolerable level of 500 parts per million (p.p.m.) sulphur) in 2007, which coincides with the *Euro 2 Standards*. The related regulation is *Environmental Quality (Control of Petrol and Diesel Properties) Regulations 2007* (Environmental Quality Act

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Department of Chemistry Malaysia, Jalan Sultan, Petaling Jaya, 46661 Selangor, Malaysia
* Corresponding author (e-mail: malar@kimia.gov.my)

Malaysia 1974). However, it was only adopted in Malaysia in 2009 (Asian Development Bank 2006). Since then, all oil companies in Malaysia were required to upgrade their fuel quality to comply with the *Euro 2 Standards*. Formerly, the sulphur limit was 3000 p.p.m. for diesel and 1500 p.p.m. for gasoline (Asian Development Bank 2006).

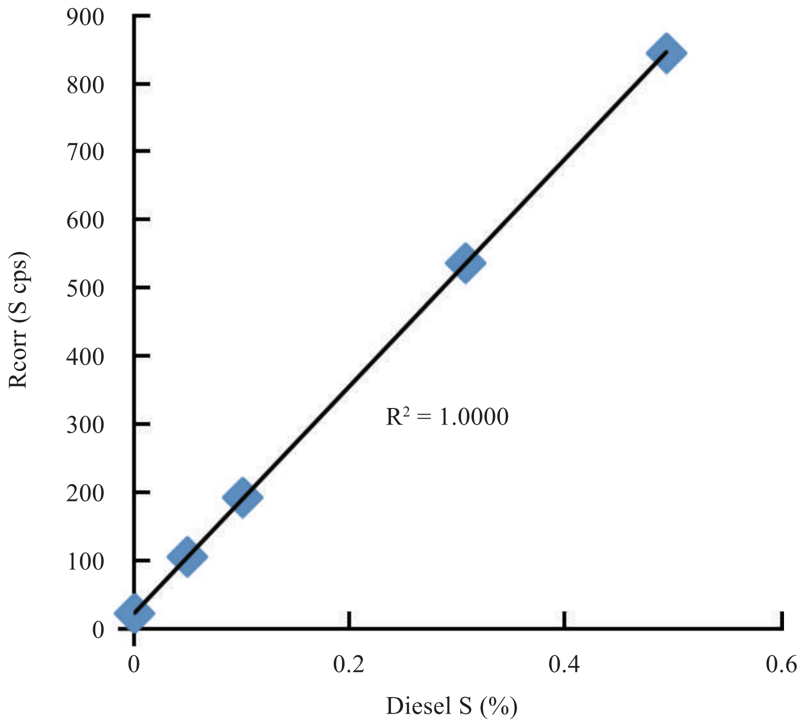
In Malaysia, sulphur in diesel and gasoline is measured and controlled for environmental and regulatory reasons. Low sulphur fuel is essential for cleaner environment and better engines (Blumberg *et al*. 2003; Zhang *et al*. 2009a; Zhang *et al*. 2009b). The Malaysia government, as such, recommends lower sulphur to assure the vehicles use fuels that comply with the *Euro 2 Standards* levels. The vehicle growth and modern emission reduction technology, which demand an adequate supply of low sulphur fuel, with levels of 50 p.p.m. or 10 p.p.m., however, incited Malaysia to move toward *Euro 4 Standards* implementation, the next step up in fuel quality. The *Euro 4 Standards* restricts sulphur content to a maximum of 50 p.p.m. in fuels. The use of high quality fuel, as part of clean fuel initiative, is expected to improve the air quality and allow for improvements in the emissions control technologies. Unfortunately, the new proposal has been delayed many times and is still underway. On the contrary, the European Union mandated the process of sulphur reduction in fuels to 10 p.p.m. by 2009 (European Commission 2003).

In Malaysia, there is little known about the levels of sulphur in diesel and gasoline. Determining the concentrations of sulphur in these fuels can be useful and vital because as well as being able to provide valuable information about the current situation of sulphur levels at petrol service stations, it may also provide some information about the fuel quality used before and after the adoption of *Euro 2 Standards*. Also the efficiency of the current regulation in controlling the sulphur levels in diesel and gasoline can be assessed. This study, therefore aimed to determine sulphur concentrations in diesel and gasoline using X-ray fluorescence spectrometry technique.

MATERIAL AND METHOD

The diesel and gasoline samples were provided by the Department of Environment Malaysia (DOE). These fuels are routine samples collected from different petrol stations located in Peninsular Malaysia from 2006 to 2014. The energy dispersive X-ray fluorescence (EDXRF) spectrometry method was used for analyzing sulphur concentration in the samples obtained from 2006 to 2009. For samples 2010 onwards, the analysis was performed using a PANalytical Axios wavelength diffractrometer X-ray fluorescence (WDXRF) spectrometer. All analysis was carried out at the Department of Chemistry Malaysia using validated methods, with reference to the American Society for Testing Materials Standards, *ASTM D4294-98 (EDXRF)* and *ASTM D2622-10 (WDXRF)*. These methods are recommended for analyzing sulphur in petroleum and petroleum products (*ASTM Standards* 1998; *ASTM Standards* 2010).

A series of diesel and gasoline fuel standard with known sulphur concentrations were used to set up the calibrations for the determination of sulphur in diesel and gasoline. In this case, sulphur in isooctane and sulphur in diesel fuel from AccuStandard brand were used. These standards were used to set up the calibration lines for quantitative determinations. Precise calibration curves were attained, with correlation of coefficient 0.9999–1.000 (*Figure 1*). The concentration of sulphur in mass % is automatically calculated from the obtained calibration curve (*ASTM Standards* 1998; *ASTM Standards* 2010). Later, it was converted to p.p.m. or µg/g by multiplying 10000.



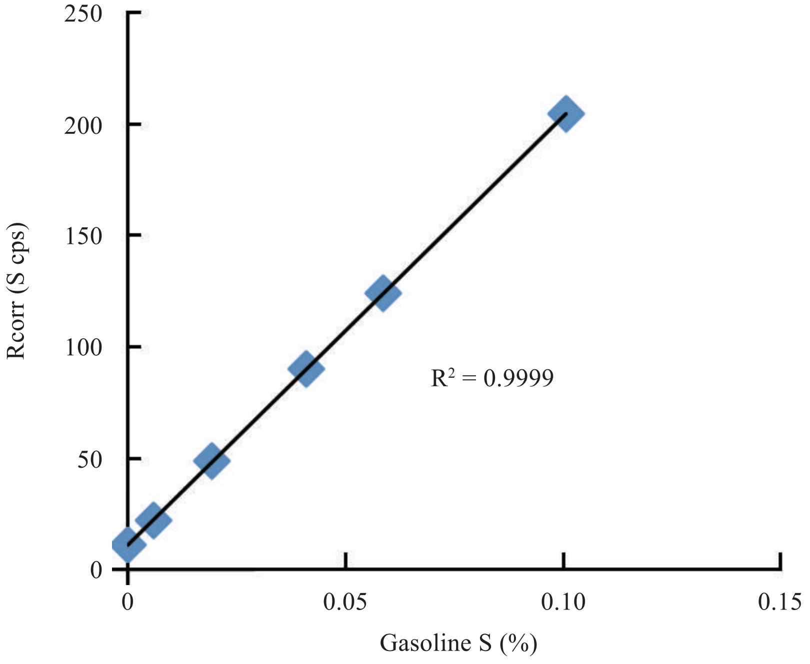


Figure 1. Diesel and gasoline calibration line.

A solution containing 0.003% or 300 µg/g of sulphur in diesel and 0.04% or 400 µg/g of sulphur in gasoline was used as a quality control (QC). The QC sample was analyzed after the measurement of every batch of routine samples.

RESULTS AND DISCUSSION

The concentrations of sulphur analyzed in diesel and gasoline samples across 2006 to 2015 are presented in *Table 1*. In total, 180 diesel samples and 189 gasoline samples were analyzed. Year 2008 was not included in the diesel list as only three samples were sampled for analysis. No values were reported for years 2008, 2007 and 2006 for sulphur in gasoline because there was no legislation requirement to monitor the levels at that time, and as such no samples were collected.

Table 1. Concentration of sulphur in diesel and gasoline samples (2006–2014).

Year		Diesel (p.p.m.)	Gasoline (p.p.m.)
2006	N	32	NA
	Mean	1464	NA
	Median	1170	
	Range	85–2964	NA
2007	N	15	NA
	Mean	1466	NA
	Median	1014	
	Range	283–4310	NA
2009	N	52	62
	Mean	994	95
	Median	434.5	86
	Range	259–3236	28–327
2010	N	22	22
	Mean	457	77
	Median	383	70
	Range	289–698	15–257
2011	N	14	19
	Mean	397	96
	Median	398.5	84
	Range	374–410	22–346
2012	N	15	20
	Mean	382	74
	Median	391	42
	Range	319–436	15–180
2013	N	20	32
	Mean	428	190
	Median	429	207
	Range	196–713	17–322
2014	N	10	34
	Mean	376	125
	Median	340	100
	Range	300–477	10–330

NA: Not avaiable

After *Euro 2 Standards* adoption, the sulphur content in diesel samples across 2010 to 2014 were found to be generally lower than the *Environmental Quality (Control of Petrol and Diesel Properties) Regulations 2007* limit, 500 p.p.m. (*Euro 2 Standards*) with the exception of a few samples. About 18% of the diesel samples in year 2010 and 35% in 2013 exceeded the current sulphur standards limit for diesel (*Figure 2*). On overall, only 4% of samples had levels below 250 p.p.m., 59% between 250 p.p.m.–500 p.p.m. and 37% above 500 p.p.m. (*Figure 2*). Sulphur concentrations tended to be much higher in diesel samples analysed in 2006, 2007 and 2009 (*Table 1*; *Figure 2*), which were consistent with the allowable maximum fuel sulphur content of 3000 p.p.m. at that time. During this period, 60% of diesel samples had sulphur levels above 1000 p.p.m. in 2006, followed by 67% in 2007 and 26% in 2009. Only two samples in 2006, three in 2007 and two in 2009 exceeded the standards limit of 3000 p.p.m.. Although, the concentration of sulphur in all diesel samples in 2011, 2012 and 2014, and in the majority of the 2013 samples were lower than 500 p.p.m., the mean and median concentration for all respective years was close to 400 p.p.m. (*Table 2*). This value was eight times higher than *Euro 4 Standards* Standards that were proposed to be adopted in the future.

Most gasoline sulphur concentrations were substantially lower than the current DOE standard limit of 500 p.p.m. and only 8% samples contained sulphur concentrations in exceedence of 250 p.p.m., while 35% of samples had concentrations above 100 p.p.m. (*Figure 2*). Although the measured concentrations are comparatively low, when compared with the *Euro 4 Standards* requirement (50 p.p.m.), the levels are considered still high. However, the recorded minimum concentrations of sulphur in gasoline samples across the years (2010, 2011, 2012, 2013 and 2014) were < 30 p.p.m. (*Table 1*), which could be considered as an optimistic sign in meeting the *Euro 4 Standards*.

A notable reduction in the annual median sulphur diesel content, close to 62%, was observed over the period 2006–2009 (*Figure 3*). From 2009 to 2013, there seems to be no drastic drop or increase in the annual median sulphur diesel content. The concentrations were rather steady for these years; however, there was a gradual drop in the sulphur content in 2014 (*Figure 3*). In the case of sulphur gasoline, there was no obvious diminution or raise in the annual median concentrations (*Figure 4*). The annual median concentrations were relatively stable, showing small annual variations but no consistent trend in recent years, except in 2013, where the concentration was almost 50% higher than the rest of the years (*Figure 4*).

The obvious sulphur levels in diesel and highly variable sulphur in both diesel and gasoline especially across 2009 to 2014 indicated that although the government had actively adopted stringent vehicle fuel standards, the less aggressiveness in the step towards the use of better quality fuels was a plausible factor impeding the full implementation of fuel emission standards. It was worth noting that regulation reforms were moving ahead to address issues related to fuel quality. More efforts therefore would be required to improve fuel quality by mandating reduced sulphur levels. Upgrading of hydro treatment units at refineries can be a potential solution as it aids the removal of sulphur from the fuel stream (Blumberg *et al*. 2003; Riegel *et al*. 1983; Zhang *et al*. 2009b) and there are refineries that are still using conservative approach and have inadequate advanced refinery equipment.

The results reported here need to be interpreted cautiously as only a number of routine samples were accessible. Moreover, no additional parameters were determined. It would have been informative to assess other parameters stated in the 2007 DOE (Malaysia) standards on fuel regulation in order to determine the quality of diesel and

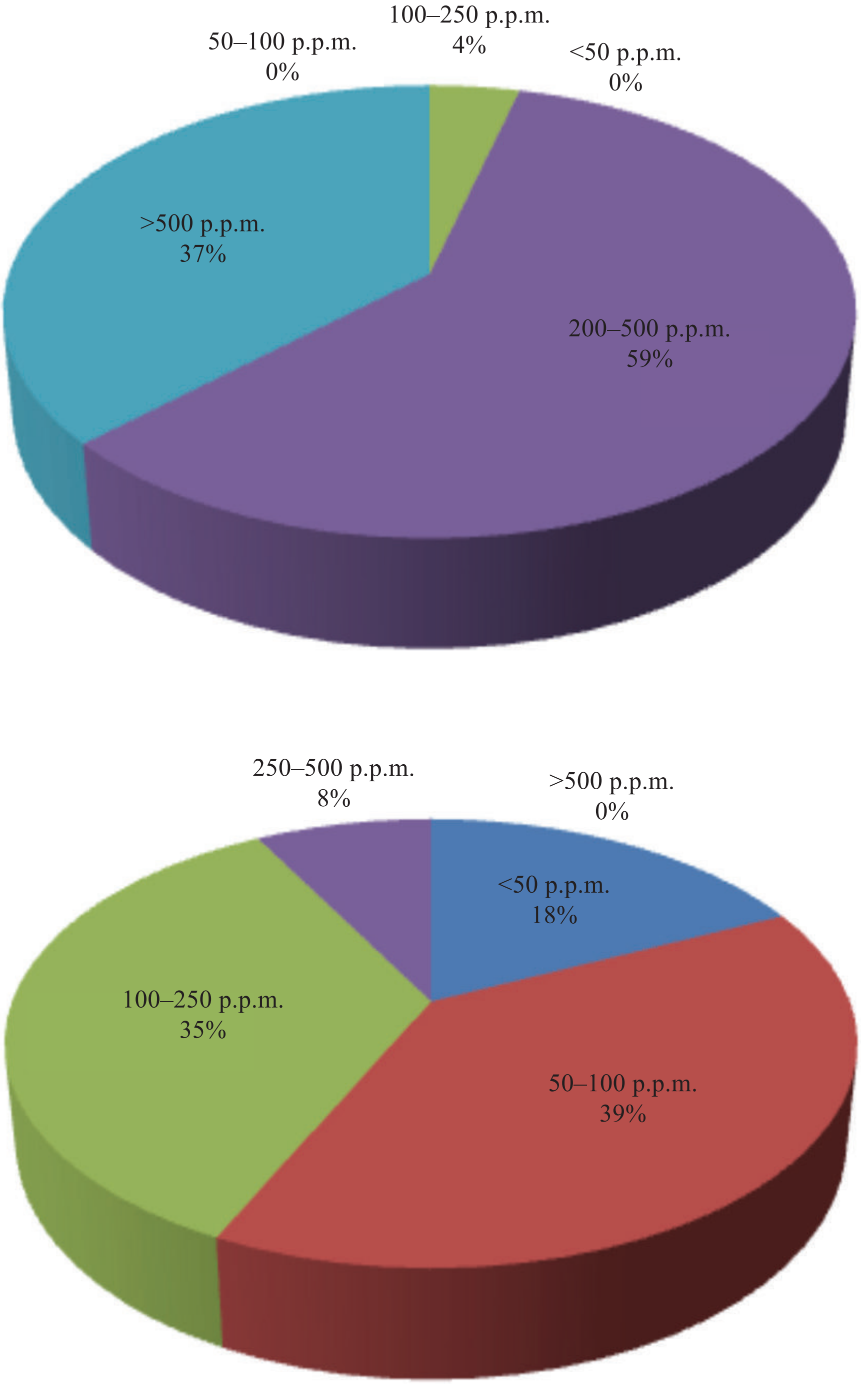


Figure 2. Diesel and gasoline sulphur levels distribution.

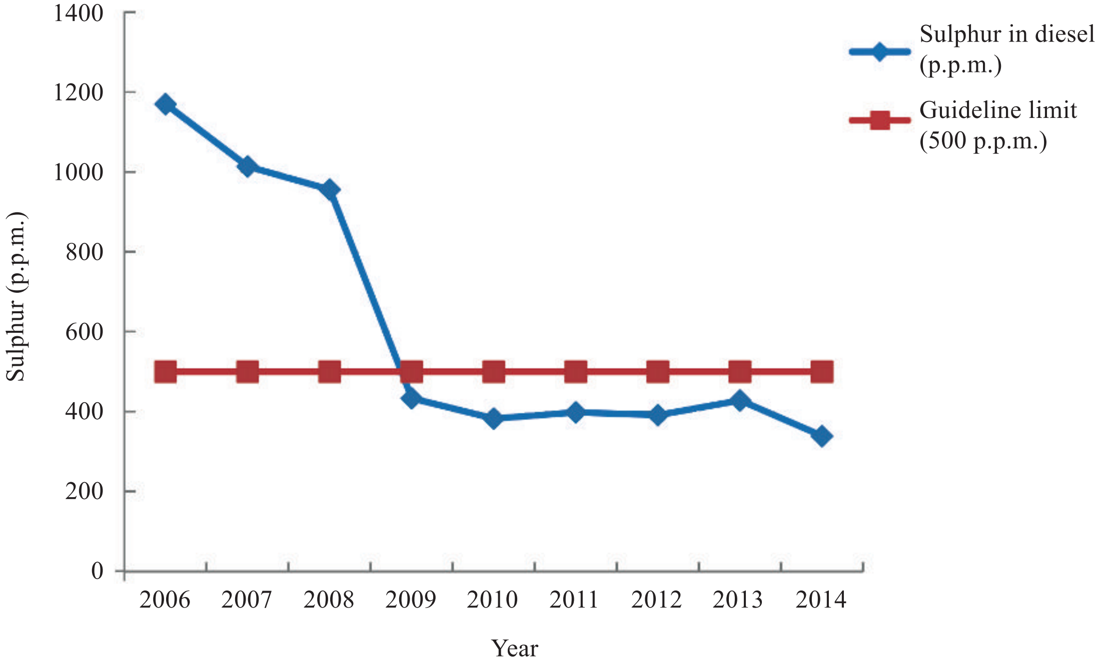


Figure 3. Median diesel sulphur concentration over the period 2006 to 2014.

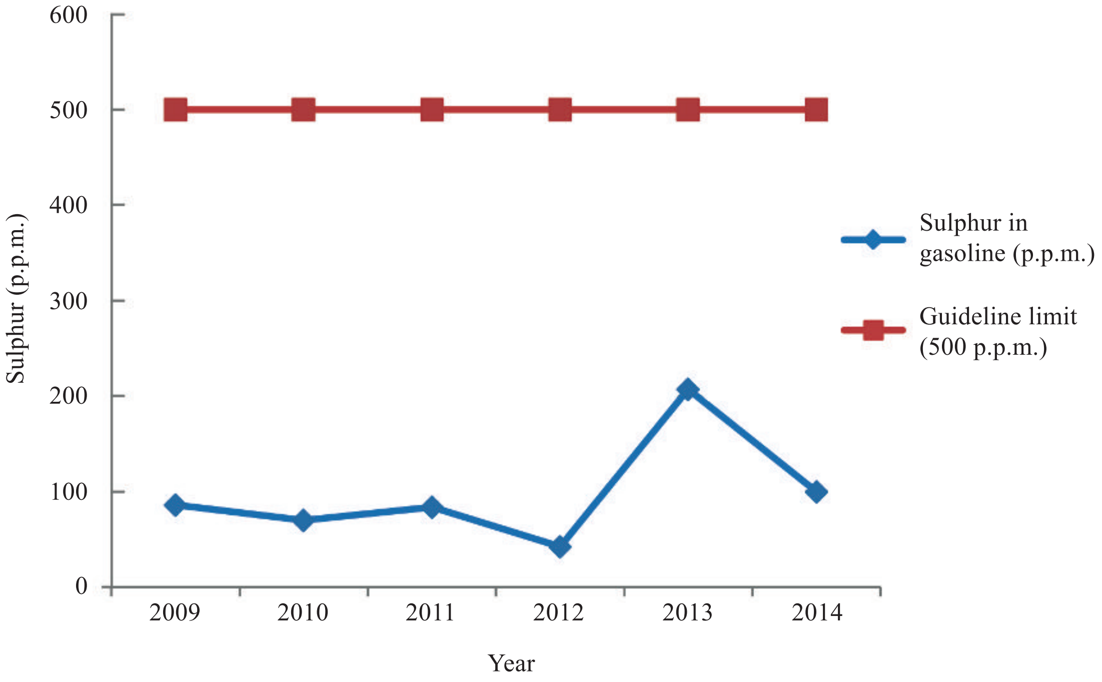


Figure 4. Median gasoline sulphur concentration over the period 2009 to 2014.

gasoline as other fuel specifications also impact emissions (Zhang *et al*. 2009a; Zhang *et al*. 2009b). Although, this was not possible in the current study, it is hoped that those characteristics would be addressed in future.

CONCLUSION

Some information was now available on the current situation of diesel and gasoline sulphur levels in Peninsular Malaysia, with the study which confirmed the presence of sulphur at noteworthy levels for most samples. While the sulphur concentration in fuels was overall slightly below the current DOE (Malaysia) allowable limits, for *Euro 2 Standards*, the concentrations were considered still high if compared with the *Euro 4 Standards*. The results presented here was only based on available samples; additional investigation with the inclusion of more recent samples was, however, recommended to ensure consistency. Finally, with the highly variable sulphur limits in samples, it seemed that there was a need for more rigorous enforcement concerning fuel emissions since there appeared to be inadequate regulation compliance.

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Periphytic Diatoms in the Polluted Linggi (*sensu stricto*) and Kundor Rivers, Negeri Sembilan, Malaysia

I.S.A. NATHER KHAN

The investigation in this paper aimed to describe periphytic diatom assemblage, species composition and distribution in tropical polluted Linggi (*sensu stricto or s.s.*) and Kundor rivers in Negeri Sembilan, Malaysia. Regardless of ecological and environmental conditions, diatoms were the numerically dominant flora among periphytic algae collected monthly over a period of 13 months at nine stations in the Linggi (*s.s.*) and Kundor rivers. The freshwater periphytic diatom samples were collected mainly using artificial substrates and supplemented with natural substrates. The periphytic diatoms thus collected from both natural and artificial substrates comprised 86 taxa (82 pennate and 4 centric forms) belonging to 21 genera. Of the 86 species, 71 species were found colonized in artificial substrates while the remaining 15 species were recorded exclusively on natural substrates. On the whole, the most common diatoms in both rivers combined were *Eunotia vanheurckii, Gomphonema parvulum, Nitzschia palea, Pinnularia braunii, Navicula cryptocephala, Achnanthes saxonica, Achnanthes minutissima* and *Pinnularia microstauron.* The most abundant species were *E. vanheurckii, N. palea, A. saxonica, G. parvulum* and *A. minutissima.*

Key words: Diatom, periphyton, biological assessment, aquatic ecology, lotic ecosystem, tropical river, river ecology, water pollution, water quality, Malaysia

There is a general paucity of scientific research on taxonomy, species composition and distribution of freshwater periphytic diatoms in Malaysia. Prowse (1962) provided the sole detailed taxonomic work on diatoms, although there were many general limnological studies that were carried out in Malaysian rivers, lakes and ponds covered some aspects of diatoms (Hirano 1967; Mizuno & Mori 1970; Prowse & Ratnasabapathy 1970; Arumugam 1972; Furtado & Mori 1982; Nather Khan & Haji Mohamed 1985). Bishop (1973) and Ho (1973) included freshwater algae in their studies on river ecology and attempted to correlate diatoms distribution with water quality and other ecological factors. Nather Khan (1985) conducted an extensive study on biological assessment of water pollution using diatoms community structure, species diversity and productivity in the Linggi River Basin (Nather Khan 1990a, b, c; 1991a, b; 1992a, b; Nather Khan *et al*. 1986a, b; 1987); Nather Khan & Lim 1991; Nather Khan & Firuza 2010, 2012).

Algae which live attached to surfaces of river substrates are called periphyton or Aufwuchs. Periphytons are important primary producers in *lotic* compared to *lentic* ecosystems where phytoplanktons are major primary producers. Normally diatoms are numerically most dominant algal group among periphyton community in lotic system and have been found to be reliable indicators of water pollution. In order to overcome difficulty in collecting

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Ecotone Environmental Management Sdn. Bhd., Suite 912, Block A, Kelana Centre Point, Kelana Jaya, 47301 Petaling Jaya, Selangor, D.E., Malaysia
* Corresponding author (e-mail: ecotonemalaysia@yahoo.com)

quantitative samples from natural substrates, artificial substrates such as pieces of wood, sterilized smooth stones, plastic sheets or glass slides were used for quantitative assessment of periphytic diatoms in *lotic* environment (Sladecek & Sladekova 1964; King & Ball 1967; Bishop 1973; Nather Khan 1985; Nather Khan *et al.* 1987).

As diatoms were numerically abundant in periphyton community in the Linggi (*s.s.*) and Kundor rivers qualitative and quantitative assessment of diatom community was estimated through microscopic counting methods (Nather Khan *et al.* 1987). The investigation aimed to determine and describe the taxonomy, species composition, distribution, diversity, relative abundance and productivity of periphytic diatom community with reference to prevailing pollution and water quality in the rivers (Nather Khan 1990a; Nather Khan 1990b; 1991a; 1991b; 1992a; 1992b; Nather Khan *et al*. 1986a; 1986b; 1987; 1991; 2010; 2012). However, this paper describes mainly the taxonomy and distribution of periphytic diatoms at variatous locations at the Linggi (*s.s.*) and Kundor rivers with reference to water pollution.

MATERIALS AND METHODS

The Linggi (*s.s.*) and Kundor Rivers

The Linggi (*s.s.*) and Kundor were highly polluted sub-basins of Linggi River Basin, and located at 2°24'–2°50' N latitude and 101°53'–102°12' E longitude at south-western part of the state of Negeri Sembilan. The Linggi sub-basin has more than 21 major tributaries, of which seven are located above Seremban town, the state capital. The predominant types of land use in the basin were rubber and oil palm plantations, small areas of rice fields with urban and industrial areas. Water from these rivers were extensively used for domestic, industrial and irrigation purposes. The river sections under investigation at the Linggi (*s.s.*) river received mostly treated and untreated urban and industrial wastes from Seremban municipality area, though the domestic sanitary wastes from Seremban town ranked highest among all pollutants discharged.

The Kundor, a tributary of Pedas sub-basin was highly polluted tributary within the Linggi River Basin which was due to effluent discharged from rubber and oil palm factories from Ulu Kanchong estate. The upstream section of this tributary runs through rubber and oil palm plantations while the downstream section passes through lowland swampy areas including rice paddy fields, where a large volume of water was extracted from several small reservoirs built over the Kundor river for paddy irrigation.

Water Quality and Periphtyic Diatom Sampling Stations

Twelve sampling stations were established at both the rivers to assess water quality on monthly basis over 13 months. Of the twelve sampling stations, eight stations were selected only for quantitative periphytic diatom study. The first four stations (Stations 1 – 4) were located at the Linggi (*s.s.*) river while remaining four stations (Stations 9 – 12) were located at the Kundor river. The locations and general characteristic features of these stations are depicted in *Figure 1*. When selecting sampling stations, steps were taken to minimise habitat heterogeneity and other ecological variation between the stations. The dates, sampling location, sampling methods, effluent type and load discharged were described in detail in several other papers published earlier (Nather Khan 1990a; 1990b; 1990c; 1991a; 1991b; 1992a; 1992b).

Water Quality Measurement

Apart from monitoring river flow and river discharge, water samples were collected on a monthly basis at all the stations for a period of 13 months, from January 1983 to January

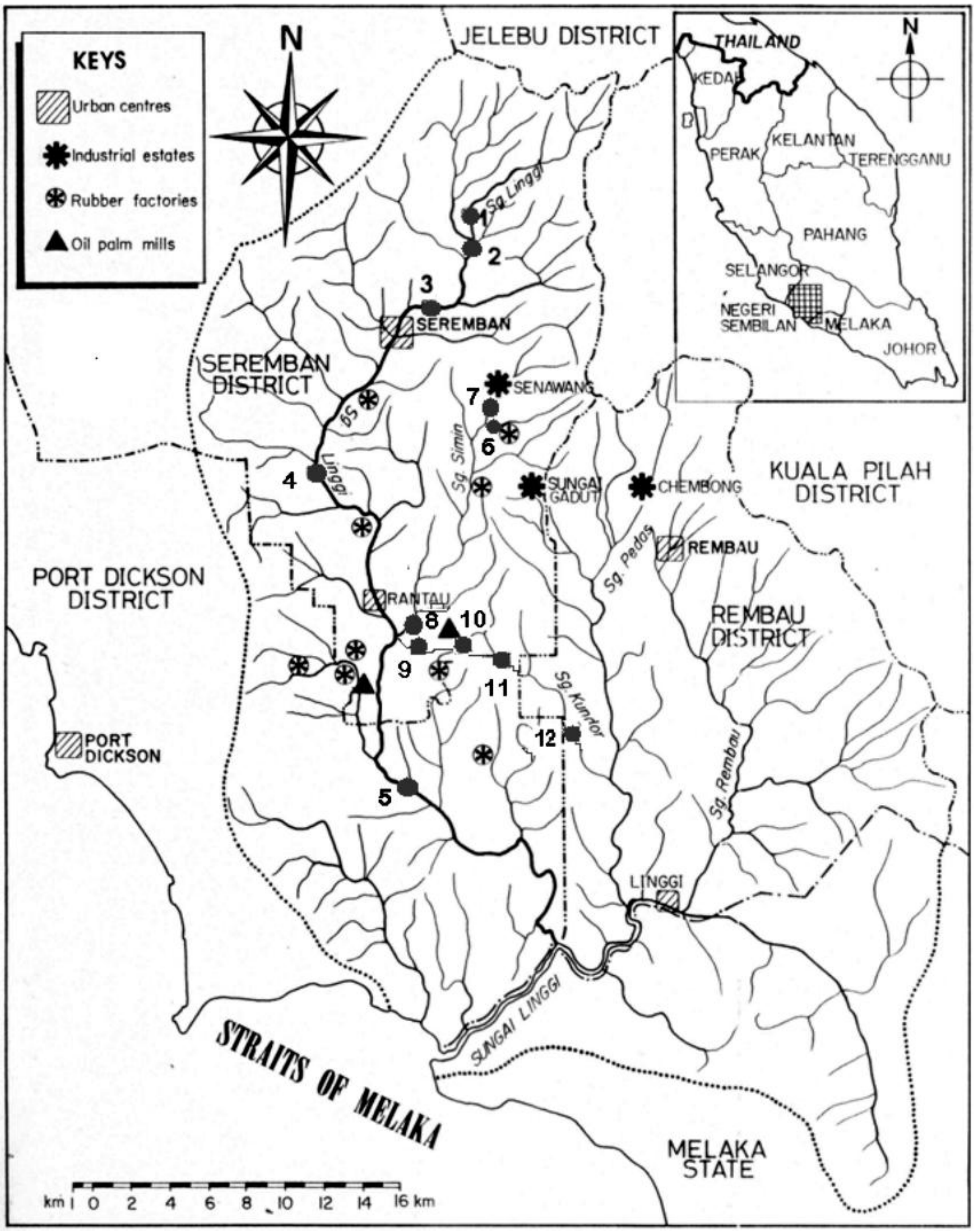


*Figure 1. Sampling stations at Linggi (*s.s.*) and Kundor rivers of Linggi River Basin.*

1984 to analyse 29 physical and chemical parameters. Water samples were collected from middle of the water column using prewashed polyethylene bottles. The temperature, pH, and conductivity were measured in the field and dissolved oxygen was determined immediately upon returning to laboratory. All samples were analysed within 48 hrs after collection except the biochemical oxygen demand (BOD). All determinations were made in duplicate and repeated when precision was needed. The physical and chemical parameters analysed were based on methods outlined in APHA (1975) and Mackereth *et al.* (1978). For details of the methods employed and extensive data collected were discussed in other earlier publications related to the studies (Nather Khan 1992a & 1992b).

Diatoms Sampling Methods

Diatoms were sampled from both natural and artificial substrates. Old leaves, stems and roots of submerged marginal plants were randomly collected within 10 metre river reaches of each station and preserved immediately in 80 ml of 4% formalin in wide mouthed bottles. Additional samples were collected from stones and rocks. Samples thus collected from natural substrates were used for qualitative study only, particularly for species composition and identification purposes (Whitford & Schumacher 1963; Nather Khan 1990a).

For detailed quantitative assessment, diatom samples were collected using glass microscope slides as artificial substrates from January 1983 until January 1984 at Stations 1 to 4 and from March 1983 until January 1984 at Stations 9 to 12. A special diatom collection device was constructed using wooden frames to hold 12 microscope slides (75 × 25 mm). At each station two such devices, one at each side of bank of the river were placed and held vertically parallel to the current and just below the water level by means of iron stakes and wire. Approximately after a month, exposed frames were removed and replaced with new frames for the next colonisation period. Two colonized slides were randomly chosen from frames and preserved immediately in 80 ml of 4% formalin. Additional slides were taken to laboratory in bottles containing river water to examine diatoms in fresh form.

Diatom Identification and Enumeration

In the laboratory, two slides preserved in formalin were scrapped to remove diatom colony and were 'cleaned' by adding nitric acid and potassium dichromate as described by Hohn and Hellerman (1963). The cleaned samples were made up to known quantities and one ml of aliquot of acid-free, homogenised suspension was placed on a 22 ml # 1 cover glass, dried on a hot plate and mounted on a slide with Hyrax mounting medium. In order to determine relative abundance and diversity of diatom species a numerical counting method was used. Each slide was randomly scanned until 500 diatom cells were counted under a magnification of ×600. For identification, diatom frustules were examined under oil immersion (×1000 magnification). The count data for each species were expressed as number of individuals per 500 cells counted. As far as possible, diatoms were identified to species level using taxonomic keys, drawings and descriptions given in such works as Prowse (1962), Sladecek (1963), Mizuno (1964), Patrick & Reimer (1966) and Ho (1973).

RESULTS AND DISCUSSION

Water Quality at Linggi (*s.s.*) and Kundor Rivers

The water quality measurement carried out over a year indicated that Station 1 and 2 were characterised by relatively low ionic content, slightly neutral pH, high silica and oxygen contents, low BOD and permanganate values (*Table 1*). The ammonia-nitrogen, nitrates

Table 1. Water quality at eight sampling stations in the Linggi (*s.s.*) and Kundor rivers.

Stations	Station 1			Station 2			Station 3			Station 4			Station 9			Station 10			Station 11			Station 12		
Parameter	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
pH	6.75	5.26	6.04	6.65	4.64	5.60	6.08	5.02	5.46	6.08	5.02	5.46	6.41	4.45	5.37	7.39	5.60	6.38	6.17	5.75	6.05	6.38	4.65	5.48
Umho / cm	44.00	23.00	29.30	45.00	25.00	32.73	130.00	66.00	86.77	130.00	66.00	86.77	300	42.00	135.20	1500.00	150.00	690.00	410.00	100.00	212.00	110.00	51.00	64.86
SiO_2	22.00	15.80	18.66	22.50	14.90	18.52	18.10	8.20	13.76	18.10	8.20	13.76	10.10	5.60	7.87	-	-	-	12.80	7.70	10.70	9.40	6.70	8.24
HCO^-_3alk	51.00	7.00	25.91	50.00	11.00	30.73	156.00	23.00	81.00	156.00	23.0	81.00	413.00	8.00	128.00	1388.00	53.00	519.60	325.00	116.00	183.50	101.00	13.00	38.43
Cl	7.48	0.90	3.34	7.48	1.00	3.53	19.00	2.30	9.90	19.00	2.30	9.90	23.90	1.50	10.80	160.90	3.30	55.30	53.90	16.90	28.00	22.50	1.80	10.61
PO_4-P	0.033	0.00	0.006	0.033	0.00	0.012	0.591	0.021	0.323	0.591	0.021	0.323	0.269	0.002	0.087	5.208	0.068	2.194	0.644	0.138	0.403	0.199	0.084	0.119
NH_3-N	0.026	0.08	0.13	0.28	0.01	0.12	8.05	1.82	5.17	8.05	1.82	5.17	30.50	0.55	9.70	53.75	3.76	30.17	8.85	0.16	2.95	2.62	0.11	0.76
NO_2-N	0.001	0.00	0.0002	0.001	0.00	0.0004	0.007	0.001	0.004	0.007	0.001	0.004	0.003	0.00	0.001	0.822	0.005	0.083	0.040	0.004	0.024	0.014	0.001	0.003
NO_3-N	0.16	0.03	0.07	0.14	0.03	0.10	0.73	0.1	0.35	0.73	0.1	0.35	0.51	0.05	0.19	2.59	0.13	1.30	6.77	0.45	2.51	0.93	0.40	0.544
O_2	9.20	6.50	8.27	8.80	6.00	7.46	5.80	0.34	2.08	5.80	0.34	2.08	8.01	0.46	3.72	6.67	0.11	3.41	7.93	5.05	6.19	8.10	5.89	6.78
BOD_5	2.03	0.32	1.17	2.82	0.42	1.38	42.23	4.37	11.87	42.23	4.37	11.87	82.40	6.71	21.36	384.00	12.78	117.71	9.50	18.20	12.31	4.12	11.21	6.48
Permanganate value	3.40	0.20	1.42	9.40	0.20	2.46	11.40	2.70	6.08	11.40	2.70	6.08	10.60	1.28	4.30	79.20	4.00	24.29	10.10	7.90	8.62	2.20	11.20	5.76

and phosphates were very low and nitrites were usually present in low or sometimes in undetectable level. Drastic changes in the above physical and chemical parameters were found at Station 3. Very high specific conductivity, ammonia, nitrate and BOD were recorded at this station. This was due to effluent discharged from nearby rubber processing factory. The water quality at Station 4, located south of the Seremban town, revealed increased concentration of substances associated with sewage and its decomposition products. The concentration of dissolved oxygen was reduced to a very low level here. This was due to the large amount of treated and untreated sewage discharged from Seremban town upstream of the Station 4.

Among all the stations, very high physical and chemical values were recorded at Stations 9, 10, 11 and 12 that located at Kundor river (*Table 1*). The conductivity, alkalinity, chloride, ammonical nitrogen, BOD and permanganate values were high at almost all the stations. This was due to oil palm effluent discharged into the river of low dilution capacity. Based on the concentrations of some important water quality parameters, Station 1 and 2 could be considered unpolluted, Station 3 moderately polluted and Stations 4, 9,10, 11 and 12 were severely polluted, though there was a great variation in pollution type and load, water quality river flow and discharge between these stations.

Species Composition of Periphytic Diatoms

The diatoms collected from both natural and artificial substrates comprised 86 taxa (82 pennate and 4 centric forms) belonging to 21 genera (*Table 2*). Of the 86 species, 71 species were found colonized in artificial substrates while the remaining 15 species were recorded exclusively on natural substrates. However, these 15 species were very rare and appeared only at certain stations (Stations 1, 2, 3 and 7) in certain months of the year (Nather Khan 1990b). The number of diatom species observed between stations varied from 22 to 47 species under 8 – 16 genera respectively. Stations 1, 2 and 3 had more than 50% of all species recorded. The maximum numbers of species recorded at Station 1 were 47 and the minimum number of species was recorded at Station 6 were 22. Out of 71 species observed on artificial substrates, 43 species were recorded at Station 1, 42 species at Stations 2 and 3; 27 species at Station 4; 22 species at Station 9; 26 Species at Station 10 and 34 species at Stations 11 and 12 together (*Tables 3* and *4*). On the whole, the annual mean number of species varied from 8 to 22; the minimum (8 species) at Station 9 and the maximum (22 species) at Station 3.

Almost all species of genera Achnanthes, Synedra and several species of genera Cymbella, Gomphonema and Surirella were recorded at Station 1. Several species of Eunotia were recorded at Station 2, several species of Navicula at Station 3, several species of Pinnularia at Station 6 and several species of Nitzschia at Station 7. Among all the genera, Navicula was the most dominant genus with 13 species followed by Cymbella and Eunotia with 9 species each while Achnanthes and Nitzschia were the next with 8 species each.

Spatial Variation of Periphytic Diatoms

The occurrence and abundance of diatoms varied from station to station. Within a few days of exposure at Station 1 thin layer of mucilage with scattered individuals of *Achnanthes minutissima, Cymbella javanica* and *Synedra rumpens* were found, while at Stations 2 and 3, *A. minutissima and Achnanthes saxonica* were the initial colonizers. *A. saxonica* and *A. minutissima* were two most common and abundant species at Station 1 comprising over 75% of cell counts. Both these species showed 100% constancy values which meant, these species were present in all the sampling months. The other common species at Station

Table 2. Periphytic diatoms flora in the Linggi (*s.s.*) and Kundor rivers.

No	Name of the species
1	*Achnanthes minutissima* Kuetz. var. *minutissima*
2	*Achnanthes linearis* (W. Sm.) Grun. var. linearis
3	*Achnanthes saxonica* Krasske
4	*Achnanthes lapidosa* var. *lanceolata* Hustest
5	*Achnanthes stauroneiformis* Prowse
6	*Achnanthes crenulata* Grun. *
7	*Achnanthes brevipes* C.A. Agardh var. intermedia Kuetz.
8	*Achnanthes exigua* Grun.
9	*Amphora normani* Rabenhorst
10	*Cyclotella meneghiniana* Kuetz.
11	*Cyclotella glomerata* Bachmann
12	*Cocconeis thumensis* A. Mayer
13	*Cymbella javanica* Hustedt
14	*Cymbella sumatrensis* Hustedt
15	*Cymbella turgida* Gregory
16	*Cymbella japonica* Reichert *
17	*Cymbella minuta* Hilse. ex. Rabh. var. *minuta* * (Syn. *C. ventricosa*)
18	*Cymbella sumatrensis fo. malayensis nov. fo* (Syn. *C. lanceolata*)
19	*Cymbella kolbei* Hustedt
20	*Cymbella tumida* (Brebisson) van. Heurek. *
21	*Cymbella cuspidata* Kuetz.
22	*Diploneis ovalis* (Hilsse) P. T. Cleve
23	*Desmogonium rabenhorstianum* Grun. *
24	*Eunotia monodon* Ehrenberg var. *constricta* A. Cleve-Euler
25	*Eunotia vanheurckii* Patr. var. *vanheurckii** (Syn.E.faba (Ehr.) Grun.
26	*Eunotia rhomboidea* Hustest *
27	*Eunotia major* (W. Smith) Rabenhorst var. *indica* (Grun.) A. Berg
28	*Eunotia pectinalis* (Kuetz) Rabenhorst *
29	*Eunotia polydentula* (Brun) A. Berg. var. *perminuta* Grun. *lunaris* (Ehr.) Grun. var. *capitata* Grun.
30	*Eunotia lunaris* (Ehr.) Grun. var. *capitata* Grun
31	*Eunotia camelus* Ehr. var. *karveerensis* Gandhi
32	*Eunotia major* (W. Smith) Rabenhorst
33	*Fragilaria vauncheriae* (Kuetz) Boye Petersen
34	*Fragilaria sp.* *
35	*Frustulia rhomboides* (Ehr.) de Toni
36	*Frustulia saxonica* Rabenhorst
37	*Frustulia javanica* Hustedt *
38	*Gomphonema parvulum* (Kuetz) Grun.
39	*Gomphonema gracile* Ehr.
40	*Gomphonema subventricosum* Hustedt
41	*Gomphonema longiceps* Ehr. var. *subclavata* Grun. *F. gracilis* Hustedt
42	*Gomphonema clevei* var. *inaequilongum* H. Kobayasi
43	*Gyrosigma attenuatum* (Kuetz) P.T. Cleve
44	*Melosira granulata* (Ehr.) Ralfs
45	*Melosira italica* (Ehr.) Kuetz.

Table 2 (Cont.). Periphytic diatoms flora in the Linggi (*s.s.*) and Kundor rivers.

No	Name of the species
46	*Navicula senjoensis* H. Kobayasi ?
47	*Navicula liboensis* Schoeman ?
48	*Navicula veneta* Kuetz (Syn. *N. crytocephala* var. *veneta* (Kuetz.) Grun.)
49	*Navicula globosa* Meister
50	*Navicula trituberculata* Prowse
51	*Navicula antiqua* A. Cleve *
52	*Navicula mutica fo. intermedia* Hustedt *
53	*Navicula pupula fo. capitata* Skvortzow u. Mayer
54	*Navicula minima* Grun.
55	*Navicula gastrum* Ehr.
56	*Navicula rhyncocephala* Kuetz.
57	*Navicula cuspidata* Kuetz. var. *ambigua* (Ehr.) P.T. Cleve
58	*Navicula amphibola* P.T. Cleve
59	*Neidium productum* (W. Smith). *P.T. Cleve var. *minor* A. Cleve
60	*Neidium hitchcockii* (Ehr.) P.T. Cleve
61	*Nitzschia palea* (Kuetz) W. Smith
62	*Nitzschia amphioxys* (Ehr.) Grun. *
63	*Nitzschia sigma* (Kuetz.) W. Smith var. *clausii* (Hantz.) Grun.
64	*Nitzschia stagnorum* Rabenhorst
65	*Nitzschia acicularis* (Kuetz.) W. Smith *
66	*Nitzschia sigma* (kuetz.) W. Smith
67	*Nitzschia obtusa* W. Smith var. *scalpelliformis* Grun.
68	*Nitzschia fonticola* Grun.
69	*Pinnularia biceps* Gregory var. *minor* (Boye Petersen). A. Cleve
70	*Pinnularia braunii* var. *amphicephala* (A. Mayer) Hustedt
71	*Pinnularia microstauran* (Ehr.) P.T. Cleve
72	*Pinnularia microstauron* var. *ambigua*
73	*Pinnularia bogotensis* (Grun.) P.T. Cleve var. *continue* A. Cleve
74	*Pinnularia borealis* Ehr.
75	*Pinnularia brevicostata* P.T. Cleve
76	*Pinnularia stauroptera* (Grun.) P.T. Cleve var. *subparallela* Mayer
77	*Rhopalodia gibberula* (Ehr.) O. Muller
78	*Synedra rumpens* var. *fragilarioides* Grun.
79	*Surirella tenuissima* Hustedt
80	*Surirella angusta* Kuetz.
81	*Surirella linearis* W. Smith
82	*Surirella lemmermanii* Hustedt
83	*Surirella robusta Ehr.* var. *splendida* (Ehr.) van. Heurck
84	*Surirella angusticostata* Hustedt *
85	*Stauroneis pusilla* A. Cleve
86	*Stauroneis phoenicenteron* (Nitzsch) Ehr. var. *intermedia* (Dipp.) A. Cleve-Eular

(*) indicates species recorded exclusively from natural substrates.

Table 3. Periphytic diatom occurrence at various stations (in alphabetical order).

No	Diatom species	Stations						
		Linggi (s.s) River				Kundur River		
		St. 1	St. 2	St. 3	St. 4	St. 9	St. 10	St. 11 & 12
1	*Achnanthes minutissima*	√	√	√	√	√	√	√
2	*Achnanthes linearis*	√	√	√	√	√	√	–
3	*Achnanthes saxonica*	√	√	√	√	√	√	–
4	*Achnanthes lapidosa*	√	√	–	–	–	–	–
5	*Achnanthes stauroneiformis*	√	–	√	–	–	–	–
6	*Achnanthes crenulata**	√	–	–	–	–	–	–
7	*Achnanthes brevipes*	√	√	√	–	–	–	–
8	*Achnanthes exigua*	√	√	–	√	–	√	√
9	*Amphora normani*	√	–	√	√	–	–	–
10	*Cyclotella meneghiniana*	–	√	√	√	–	–	√
11	*Cyclotella glomerata*	√	√	–	–	–	–	–
12	*Cocconeis thumensis*	–	√	–	–	–	–	–
13	*Cymbella javanica*	√	√	√	√	√	–	√
14	*Cymbella sumatrensis*	√	√	√	–	–	–	–
15	*Cymbella turgida*	√	√	√	–	–	–	√
16	*Cymbella japonica**	√	–	–	–	–	–	–
17	*Cymbella minuta**	√	√	–	–	–	–	–
18	*Cymbella sumatrensis fo. malayensis*	√	–	–	√	–	–	–
19	*Cymbella kolbei* var. *lanceolata*	√	–	√	–	–	√	–
20	*Cymbella tumida**	√	–	–	–	–	–	–
21	*Cymbella cuspidata*	–	–	–	√	–	–	–
22	*Diploneis ovalis*	√	–	√	–	–	–	–
23	*Desmogonium rabenhorstianum**	–	√	–	–	–	–	–
24	*Eunotia monodon*	√	√	√	√	√	√	√
25	*Eunotia vanheurckii**	–	–	–	–	–	–	√
26	*Eunotia rhomboidea**	–	√	–	–	–	–	–
27	*Eunotia major*	–	√	√	√	√	–	–
28	*Eunotia pectinalis**	–	√	–	–	–	–	–
29	*Eunotia polydentula*	–	–	√	–	–	–	–
30	*Eunotia lunaris*	–	–	–	–	√	–	–
31	*Eunotia camelus*	–	–	–	–	–	√	–
32	*Eunotia major* var. *indica*	–	–	–	–	–	–	√
33	*Fragilaria vaucheriae*	√	–	–	–	–	–	–
34	*Fragilaria sp.**	√	–	–	–	–	–	–
35	*Frustulia rhomboides*	–	–	√	–	–	–	–
36	*Frustulia saxonica*	√	√	√	√	√	√	√
37	*Frustulia javanica**	–	√	–	–	–	–	–
38	*Gomphonema parvulum*	√	√	√	√	√	√	√
39	*Gomphonema gracile*	√	√	√	√	√	√	√
40	*Gomphonema subventricosum*	√	√	√	–	–	–	–
41	*Gomphonema longiceps*	√	√	–	√	√	–	–
42	*Gomphonema Clevei*	√	√	√	–	–	–	–
43	*Gyrosigma attenuatum*	–	√	√	–	–	–	–
44	*Melosira granulata*	–	–	√	–	–	–	–

Table 3 (Cont.). Periphytic diatom occurrence at various stations (in alphabetical order).

No	Diatom species	Stations						
		Linggi (s.s) River				Kundur River		
		St. 1	St. 2	St. 3	St. 4	St. 9	St. 10	St. 11 & 12
45	*Melosira italica*	√	–	√	–	√	–	–
46	*Navicula senjoensis*	√	√	√	–	–	√	–
47	*Navicula liboensis*	√	√	√	–	–	–	–
48	*Navicula veneta*	√	√	√	√	√	√	√
49	*Navicula globosa*	√	√	√	√	–	√	√
50	*Navicula trituberculata*	√	–	–	–	√	–	√
51	*Navicula antigua**	–	–	√	–	–	–	–
52	*Navicula mutica**	–	–	√	–	–	–	–
53	*Navicula pupula*	–	√	√	–	–	√	√
54	*Navicula minima*	–	–	–	–	–	–	√
55	*Navicula gastrum*	√	–	√	–	–	√	√
56	*Navicula rhyncocephala*	–	√	√	√	–	–	√
57	*Navicula cuspidata*	–	–	–	–	–	√	√
58	*Navicula ambhibola*	–	–	–	–	–	–	√
59	*Neidium productum**	–	–	√	–	–	–	–
60	*Neidium hitchockii*	–	–	–	–	–	–	√
61	*Nitzschia palea*	√	√	√	√	√	√	√
62	*Nitzschia amphioxys**	–	–	–	–	–	√	–
63	*Nitzschia sigma* var. *clausii*	√	√	√	√	√	–	–
64	*Nitzschia stagnorum*	–	–	–	√	–	√	√
65	*Nitzschia acicularis**	–	–	–	–	–	√	–
66	*Nitzshia sigma*	–	–	–	–	–	√	√
67	*Nitzschia obtusa*	–	–	–	–	√	–	√
68	*Nitzschia fonticola*	√	–	–	√	–	–	–
69	*Pinnularia biceps*	√	√	√	√	√	√	√
70	*Pinnularia Braunii*	–	√	√	√	√	√	√
71	*Pinnularia microstauron*	√	√	√	√	√	√	√
72	*Pinnularia microstauron* var. *ambigua*	–	√	–	–	–	–	–
73	*Pinnularia bogotensis*	–	√	–	–	√	–	–
74	*Pinnularia borealis*	–	–	–	–	√	√	√
75	*Pinnularia brevicostata*	–	–	√	–	–	–	–
76	*Pinnularia stauroptera*	–	–	√	–	√	–	√
77	*Rhopalodia gibberula*	√	√	–	–	–	–	–
78	*Synedra rumpens*	√	√	√	–	–	–	–
79	*Surirella tenuissima*	√	√	√	√	–	√	√
80	*Surirella angusta*	√	√	–	–	–	√	√
81	*Surirella linearis*	√	–	√	–	–	–	–
82	*Surirella lemmermanii*	√	√	–	–	–	–	–
83	*Surirella robusta*	–	√	√	–	–	–	–
84	*Surirella angusticostata**	–	–	–	–	–	√	—
85	*Stauroneis pusilla*	√	–	√	–	–	–	√
86	*Stauroneis phoenicenteron*	√	–	–	–	–	–	√
	Total	48	45	46	26	23	28	34

Table 4. Periphytic diatom occurrence at various stations based on constancy values.

No	Diatom Species	Stations							
		Linggi River				Kundur River			
		St. 1	St. 2	St. 3	St. 4	St. 9	St. 10	St. 11&12	All
1	*Achnanthes minutissima*	√	√	√	√	√	√	√	7
2	*Eunotia monodon*	√	√	√	√	√	√	√	7
3	*Frustulia saxonica*	√	√	√	√	√	√	√	7
4	*Gophonema parvulum*	√	√	√	√	√	√	√	7
5	*Gophonema gracile*	√	√	√	√	√	√	√	7
6	*Navicula veneta*	√	√	√	√	√	√	√	7
7	*Nitzschia palea*	√	√	√	√	√	√	√	7
8	*Pinnularia microstauron*	√	√	√	√	√	√	√	7
9	*Pinnularia biceps*	√	√	√	√	√	√	√	7
10	*Achnanthes linearis*	√	√	√	√	√	√	–	6
11	*Achnanthes saxonica*	√	√	√	√	√	√	–	6
12	*Cymbella javanica*	√	√	√	√	√	–	√	6
13	*Navicula globosa*	√	√	√	√	–	√	√	6
14	*Pinnularia Braunii*	–	√	√	√	√	√	√	6
15	*Surirella tenuissima*	√	√	√	√	–	√	√	6
16	*Achnanthes exigua*	√	√	–	√	–	√	√	5
17	*Nitzschia sigma* var. *clausii*	√	√	√	√	√	–	–	5
18	*Cyclotella meneghiniana*	–	√	√	√	–	–	√	4
19	*Cymbella turgida*	√	√	√	–	–	–	√	4
20	*Eunotia major*	–	√	√	√	√	–	–	4
21	*Gomphonema longiceps*	√	√	–	√	√	–	–	4
22	*Navicula gastrum*	√	–	√	–	–	√	√	4
23	*Navicula rhyncocephala*	–	√	√	√	–	–	√	4
24	*Navicula senjoensis*	√	√	√	–	–	√	–	4
25	*Navicula pupula*	–	√	√	–	–	√	√	4
26	*Surirella angusta*	√	√	–	–	–	√	√	4
27	*Achnanthes brevipes*	√	√	√	–	–	–	–	3
28	*Amphora normani*	√	–	√	√	–	–	–	3
29	*Cymbella sumatrensis*	√	√	√	–	–	–	–	3
30	*Cymbella kolbei*	√	–	√	–	–	√	–	3
31	*Gomphonema Clevi*	√	√	√	–	–	–	–	3
32	*Gomphonema subventricosum*	√	√	√	–	–	–	–	3
33	*Melosira italica*	√	–	√	–	√	–	–	3
34	*Navicula liboensis*	√	√	√	–	–	–	–	3
35	*Navicula trituberculata*	√	–	–	–	√	–	√	3
36	*Nitzschia stagnorum*	–	–	–	√	–	√	√	3
37	*Pinnularia borealis*	–	–	–	–	√	√	√	3
38	*Pinnularia stauroptera*	–	–	√	–	√	–	√	3
39	*Synedra rumpens*	√	√	√	–	–	–	–	3
40	*Stauroneis pusilla*	√	–	√	–	–	–	√	3
41	*Achnanthes lapidosa*	√	√	–	–	–	–	–	2
42	*Achnanthes stauroneiformis*	√	–	√	–	–	–	–	2
43	*Cyclotella glomerata*	√	√	–	–	–	–	–	2
44	*Cymbella minuta*	√	√	–	–	–	–	–	2

Table 4 (Cont.). Periphytic diatom occurrence at various stations based on constancy values.

No	Diatom Species	Stations							
		Linggi River				Kundur River			
		St. 1	St. 2	St. 3	St. 4	St. 9	St. 10	St. 11&12	All
45	*Cymbella sumatrensis fo. malayensis*	√	–	–	√	–	–	–	2
46	*Diploneis ovalis*	√	–	√	–	–	–	–	2
47	*Gyrosigma attenuatum*	–	√	√	–	–	–	–	2
48	*Navicula cuspidata*	–	–	–	–	–	√	√	2
49	*Nitzschia fonticola*	√	–	–	√	–	–	–	2
50	*Nitzschia obtusa*	–	–	–	–	√	–	√	2
51	*Nitzschia sigma*	–	–	–	–	–	√	√	2
52	*Pinnularia Bogotensis*	–	√	–	–	√	–	–	2
53	*Rhopalodia gibberula*	√	√	–	–	–	–	–	2
54	*Surirella linearis*	√	–	√	–	–	–	–	2
55	*Surirella lemmermanii*	√	√	–	–	–	–	–	2
56	*Surirella robusta*	–	√	√	–	–	–	–	2
57	*Stauroneis phoenicenteron*	√	–	–	–	–	–	√	2
58	*Achnanthes crenulata*	√	–	–	–	–	–	–	1
59	*Cymbella cuspidata*	–	–	–	√	–	–	–	1
60	*Cymbella japonica*	√	–	–	–	–	–	–	1
61	*Cymbella tumida*	√	–	–	–	–	–	–	1
62	*Cocononeis thumensis*	–	√	–	–	–	–	–	1
63	*Desmogonium rabenhorstianum*	–	√	–	–	–	–	–	1
64	*Eunotia camelus*	–	–	–	–	–	√	–	1
65	*Eunotia lunaris*	–	–	–	–	√	–	–	1
66	*Eunotia major van indica*	–	–	–	–	–	–	√	1
67	*Eunotia polydentula*	–	–	√	–	–	–	–	1
68	*Eunotia pectinalis*	–	√	–	–	–	–	–	1
69	*Eunotia rhomboidea*	–	√	–	–	–	–	–	1
70	*Eunotia vanheurckii*	–	–	–	–	–	–	√	1
71	*Fragilaria sp.*	√	–	–	–	–	–	–	1
72	*Fragilaria vaucheriae*	√	–	–	–	–	–	–	1
73	*Frustulia rhomboides*	–	–	√	–	–	–	–	1
74	*Frustulia javanica*	–	√	–	–	–	–	–	1
75	*Melosira granulata*	–	–	√	–	–	–	–	1
76	*Navicula Antigua*	–	–	√	–	–	–	–	1
77	*Navicula mutica*	–	–	√	–	–	–	–	1
78	*Navicula minima*	–	–	–	–	–	–	√	1
79	*Navicula ambhibola*	–	–	–	–	–	–	√	1
80	*Neidium hitchockii*	–	–	–	–	–	–	√	1
81	*Neidium productum*	–	–	√	–	–	–	–	1
82	*Nitzschia acicularis*	–	–	–	–	–	√	–	1
83	*Nitzschia amphioxys*	–	–	–	–	–	√	–	1
84	*Pinnularia microstauron van. ambigua*	–	√	–	–	–	–	–	1
85	*Pinnularia brevicostata*	–	–	√	–	–	–	–	1
86	*Surirella angusticostata*	–	–	–	–	–	√	–	1
	Total	48	45	46	26	23	28	34	

1 were *Achnanthes linearis, C. javanica, Cymbella turgida, Gomphonema parvulum* and *S. rumpens* and also showed 100% constancy values. Most of the above-mentioned diatoms were commonly found in clean and rocky streams.

A. saxonica was the most dominant species *(59%)* at Station 2, followed by *Eunotia vanheurckii* (22%), they together constituted over 81% of total cell count. Except for these two species, all other species at this station showed less than 100% constancy values. *A. minutissima*, the second most dominant species at Station 1, constituted only 25% of cell count with a 75% constancy value here at Station 2. The lower abundance of *A. minutissima,* greater abundance of *E. vanheurckii* and appearance of new species *Gyrosigma attenuatum* differentiated Station 2 from Station1, though both the stations were more or less similar in water quality.

At Station 3, *E. vanheurckii*, *A. saxonica* and *A. minutissima* were the dominant species with percentage abundance of 29%, 20% and 11.4%, respectively. *A. saxonica*, the second most abundant species with constancy value of 92% while the other two species showed 100% constancy values. In addition, four more species namely *C. javanica*, *G. parvulum*, *Navicula cryptocephala* and *Pinnularia braunii*, also showed 100% constancy values and contributed significantly to the total percentage abundance with 4%, 7%, 6.4% and 4%, respectively. Thus, unlike the previous two stations, the Station 3 was not dominated by individuals of one or two species but many species.

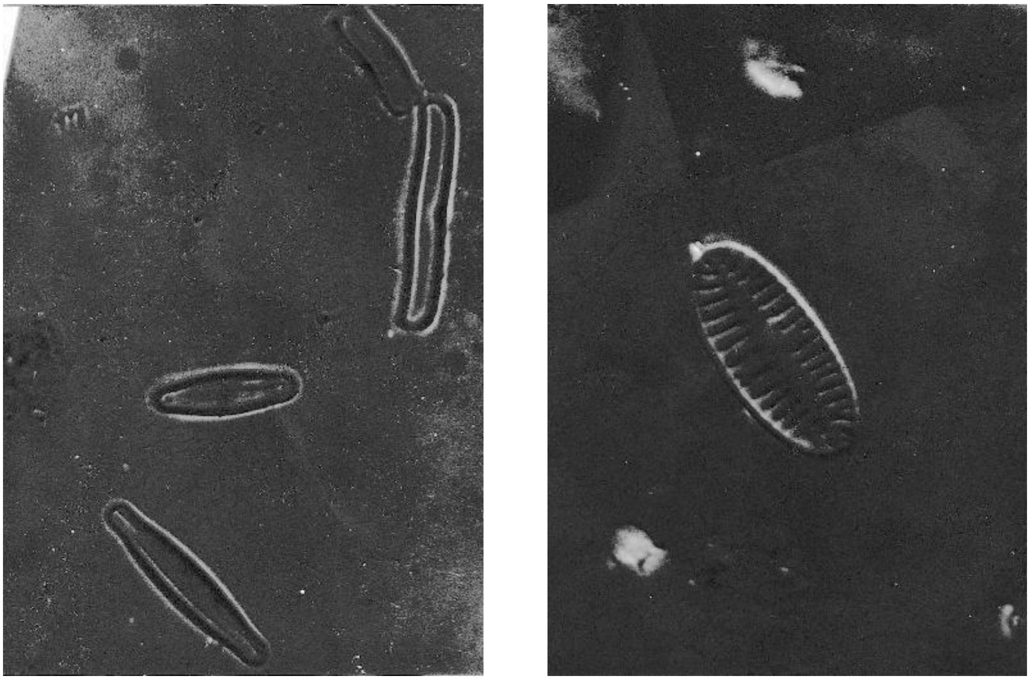
At Station 4, *Nitzschia palea*, *G. parvulum* and *P. braunii* were the most dominant species with percentage abundance of 43%, 37% and 7.2%, respectively. These three species showed 100% constancy values at Station 4. Other common diatoms recorded at this station were *Navicula globosa*, *Navicula gastrum*, *Navicula senjoensis* and *Pinnularia biceps*.

E. vanheurckii was the most dominant species at all three stations of Kundor river (40%, 33.4% and 44.2% at Station 9, 10 and 11, respectively), followed by *N. palea* (28%, 32% and 38% respectively) and *Gomphonema parvulum* (18%, 20% and 2.4%, respectively). Among them only *N. palea* showed 100% constancy values at all the four stations. *P. braunii* which showed 100% constancy values at these stations, but not in abundant in numbers (3.2%, 7.0%, less than 1%). All the species mentioned above were pollution tolerant species (Palmer 1969).

Diversity of Periphytic Diatoms

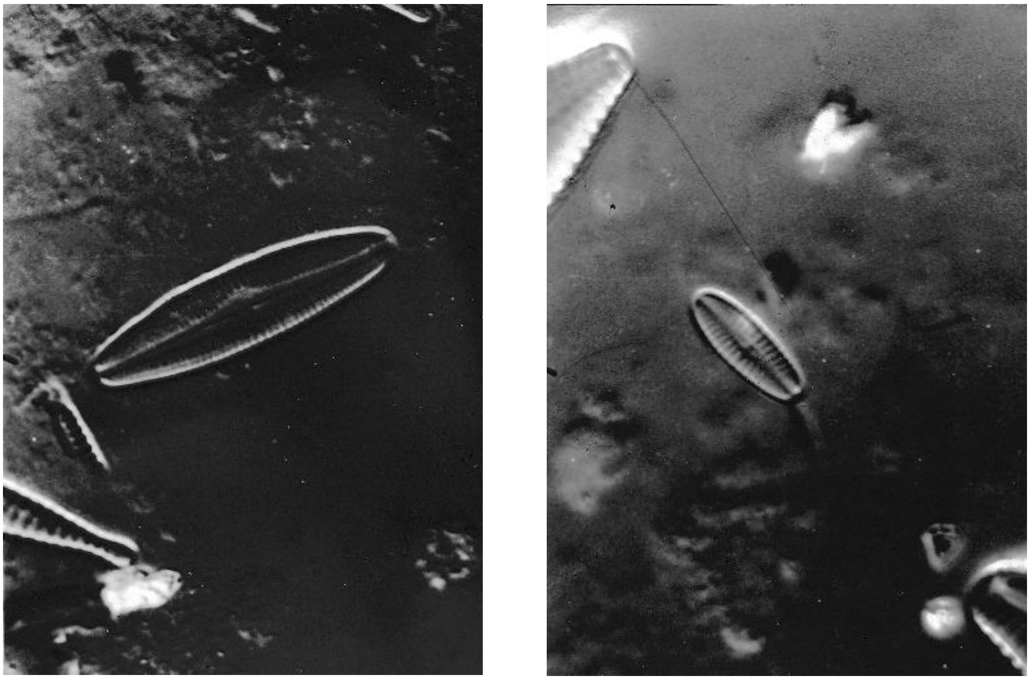
The most common and abundant species were *A. minutissima* and *A. saxonica* at Station 1, *A. saxonica* and *E. vanheurckii* at Station 2, *N. palea*, *G. parvulum* and *P. braunii* at Station 3, *G. parvulum* and *N. palea* at station 4. The lower abundance of *A. minutissima*, greater abundance of *E. vanheurckii*, and the appearance of *Gyrosigma* sp. differentiate Station 2 from Station 1 (*Tables 3* and *4*). Among the four stations in the Linggi (*s.s.*) river, the highest diversity was recorded at Station 3 which was polluted by mainly rubber effluent followed by Station 1 and 2 which were relatively unpolluted. Lowest mean diversity was recorded at Station 4, polluted mainly with urban domestic sewage and industrial wastes.

Among stations located in the Kundor river most common and abundant species of diatoms were *E. vanheurckii*, *Gomphonema parvulum* and *N. palea* at station 9, *E. vanheurckii*, *G. parvulum*, and *Nitzschia stagnorum* at station 10 and *Eunotia vanheurckii* and *N. palea* at station 11 and 12. Among four stations at Kundor river, the highest mean diversity value was recorded at Station 11 followed by Station 12, both located at the downstream pollution recovery zone. The lowest diversity was recorded at Station 9 polluted with rubber effluent (Nather Khan 1985, 1991a; Nather Khan *et al*. 1986a, b).



Achnanthes minutissima var. *minutissima*

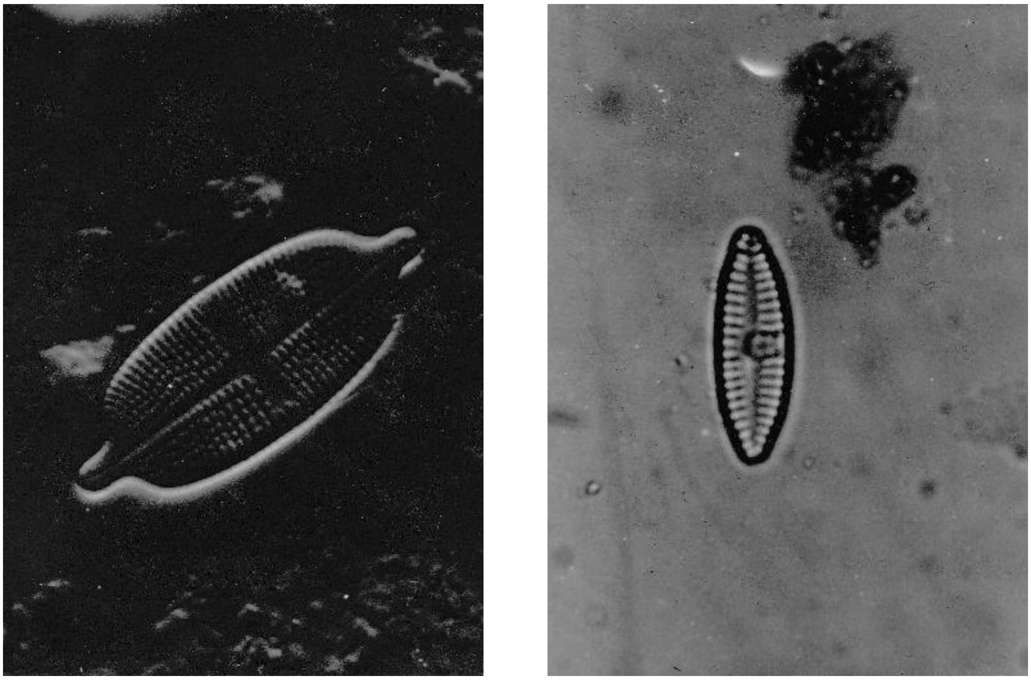
Achnanthes saxonica



Achnanthes lapidosa var. *lanceolate*

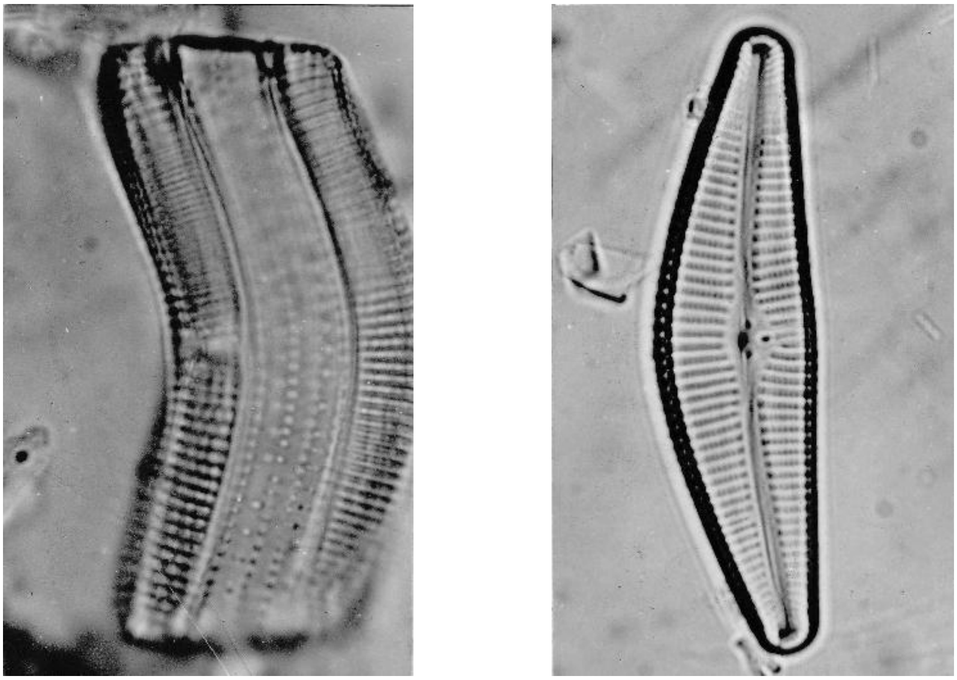
Achnanthes linearis var. *linearis*

Figure 2. Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Achnanthes stauroneiformis

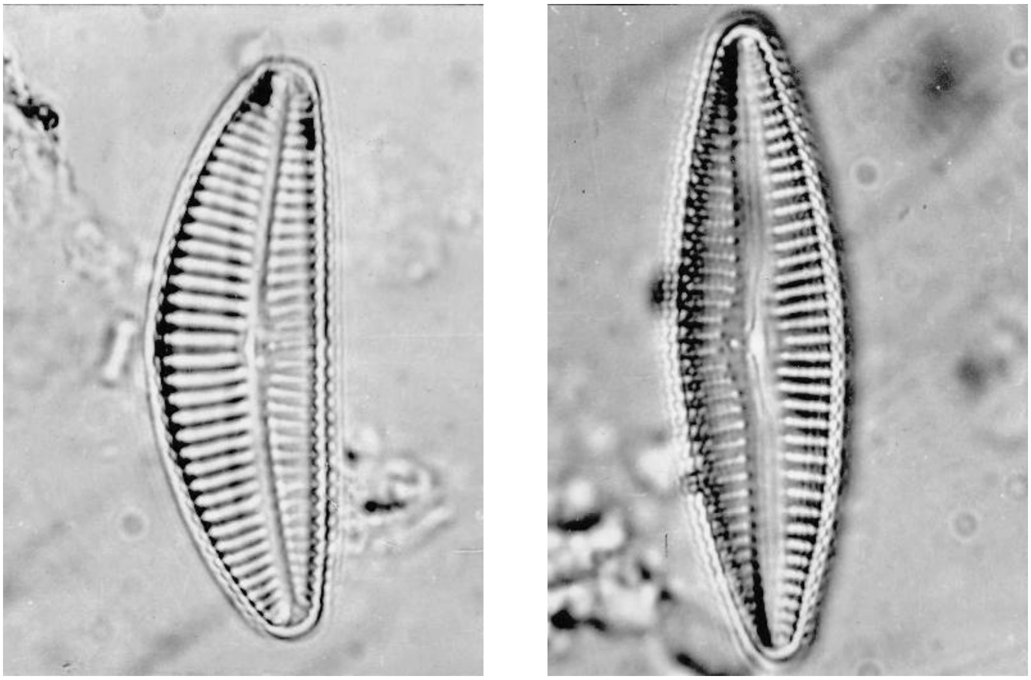
Achnanthes lapidosa var. *lanceolata*



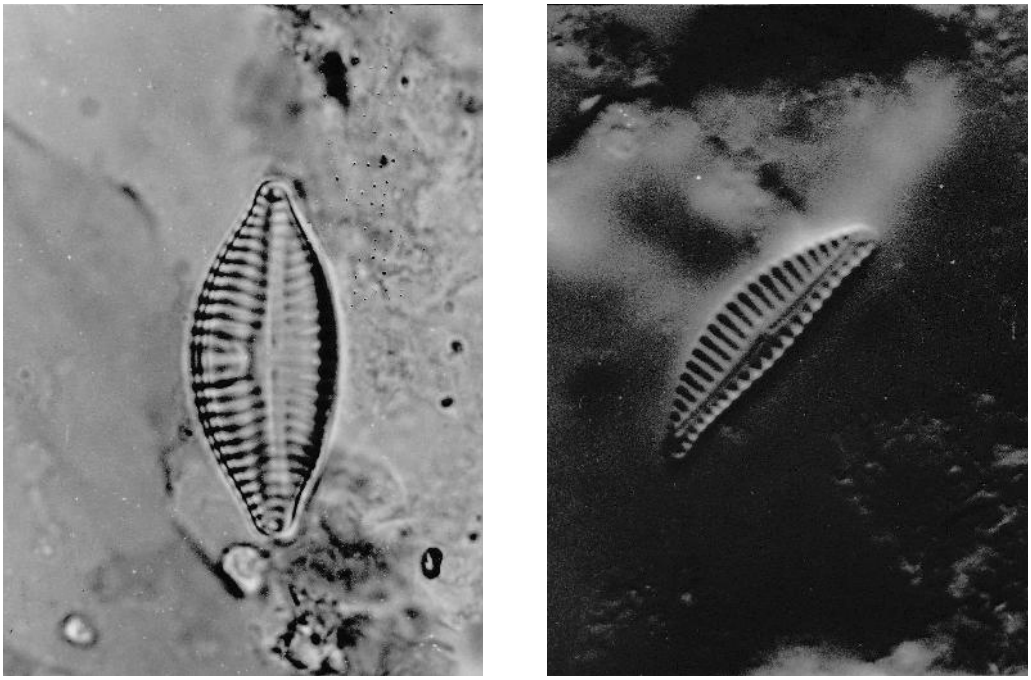
Achnanthes brevipes var. *intermedia*

Cymbella sumatrensis fo. malayensis

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.

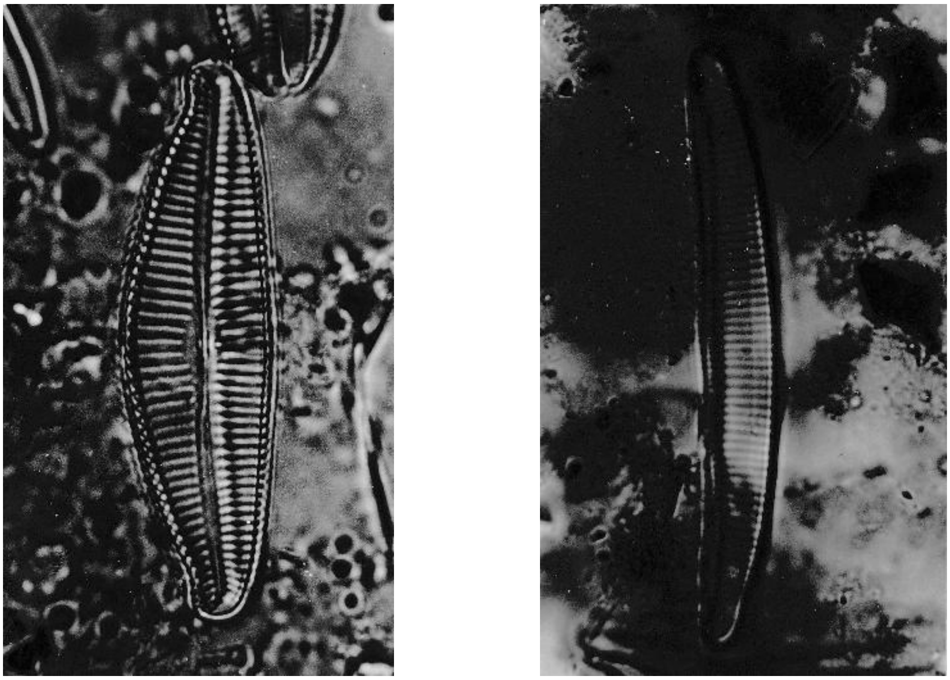


Cymbella sumatrensis *Cymbella japonica*

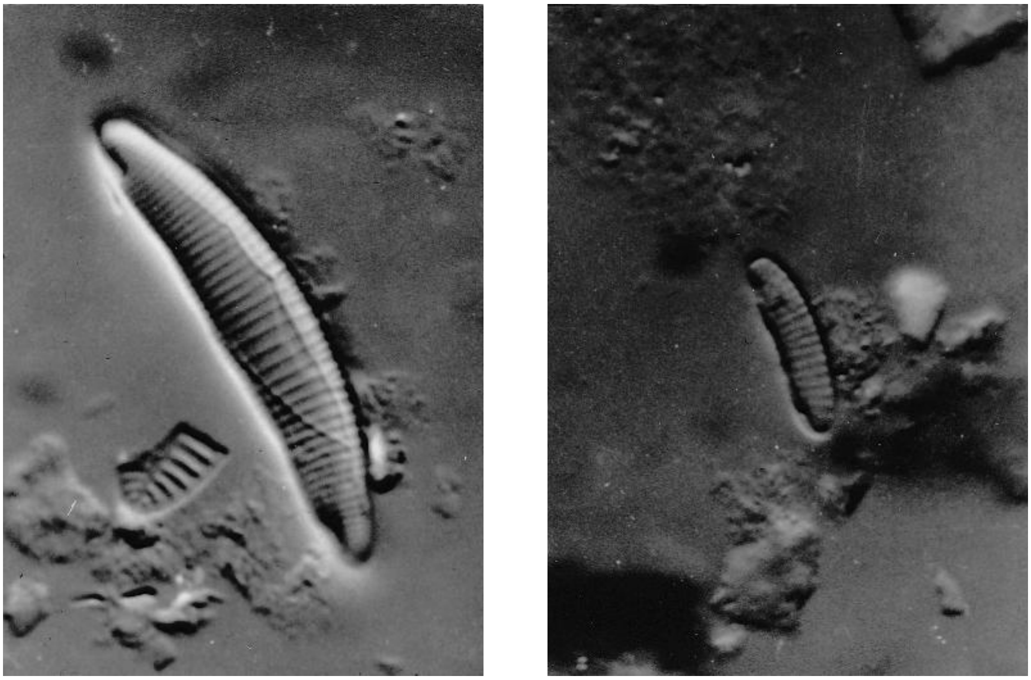


Cymbella cuspidata *Cymbella minuta* var. *minuta*

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.

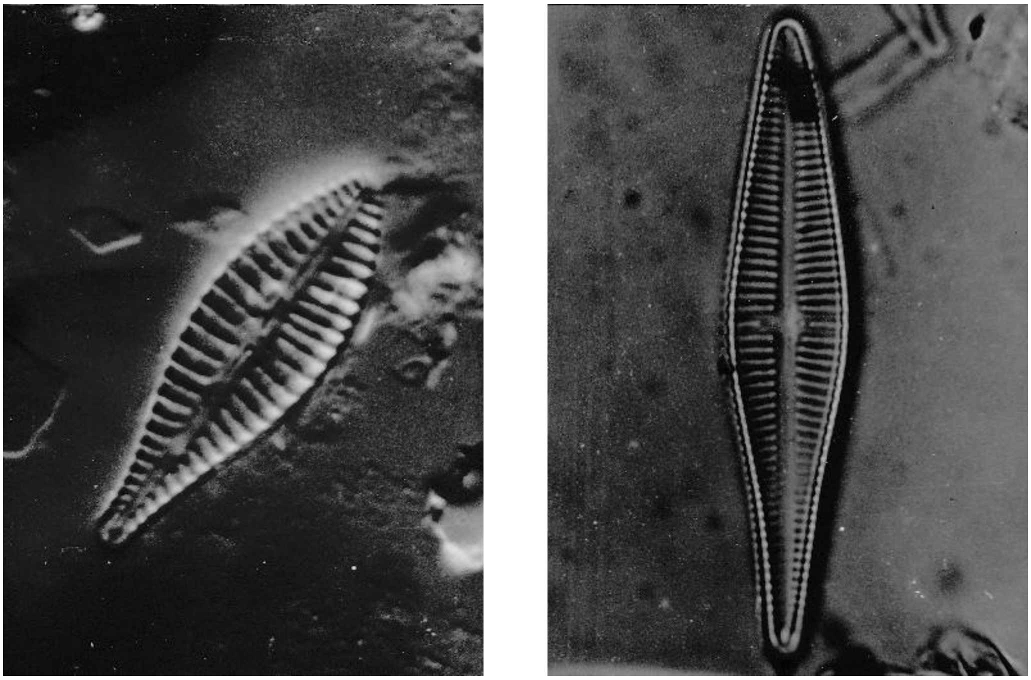


Cymbella turgida *Eunotia pectinalis*



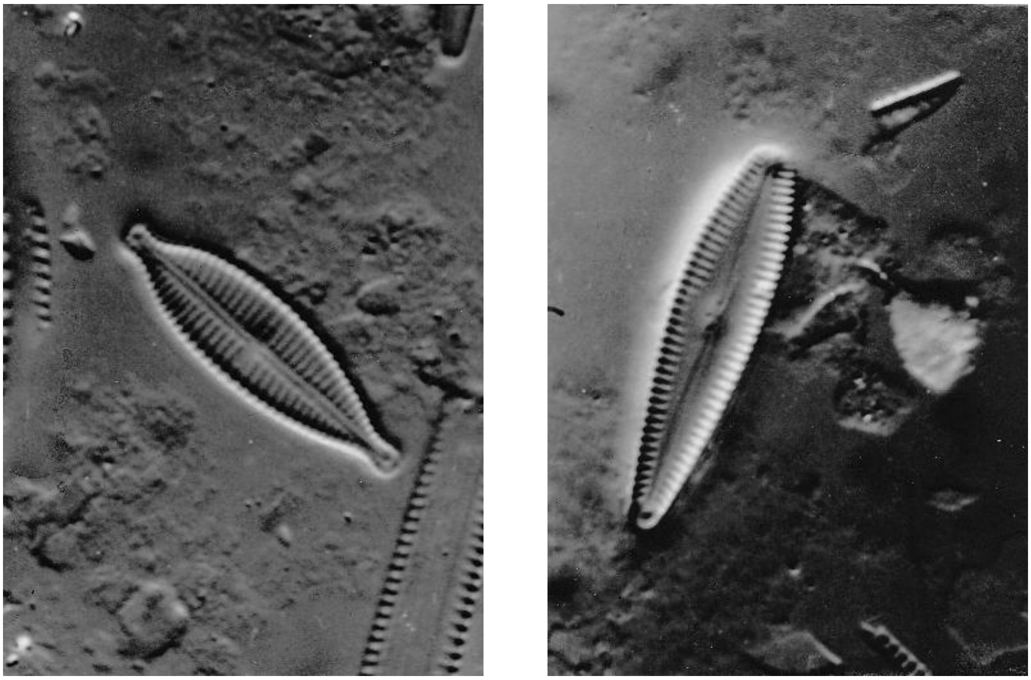
Eunotia vanheurckii *Eunotia rhomboidea*

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Gomphonema augustatum var. *producta*

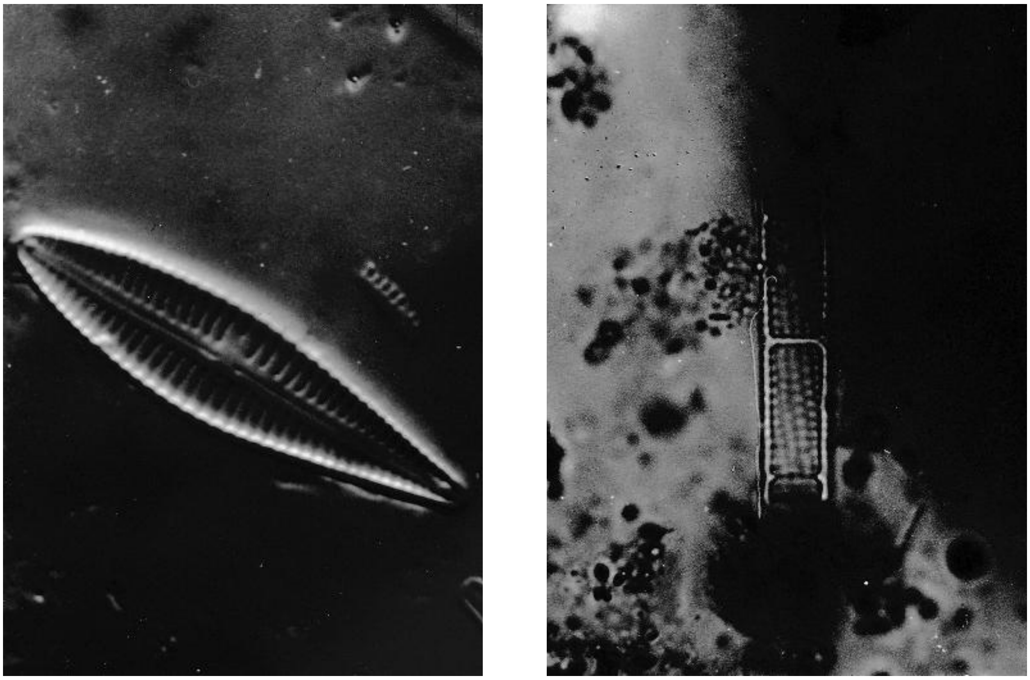
Gomphonema longiceps



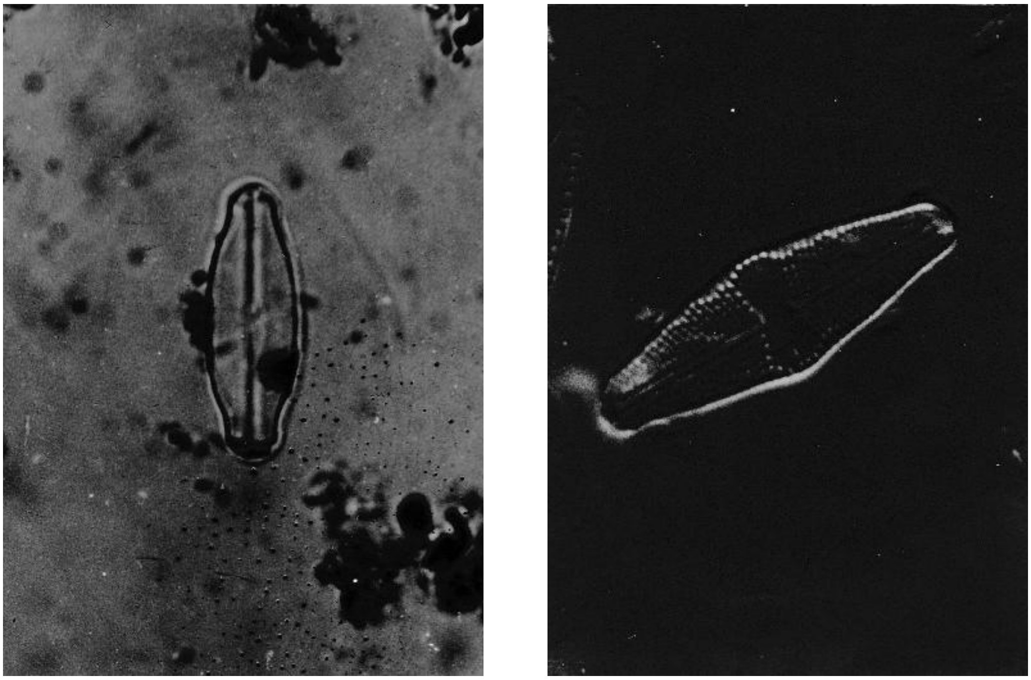
Gomphonema parvulum

Gomphonema clevei var. *inaequilongum*

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Gomphonema gracile *Melosira granulata*

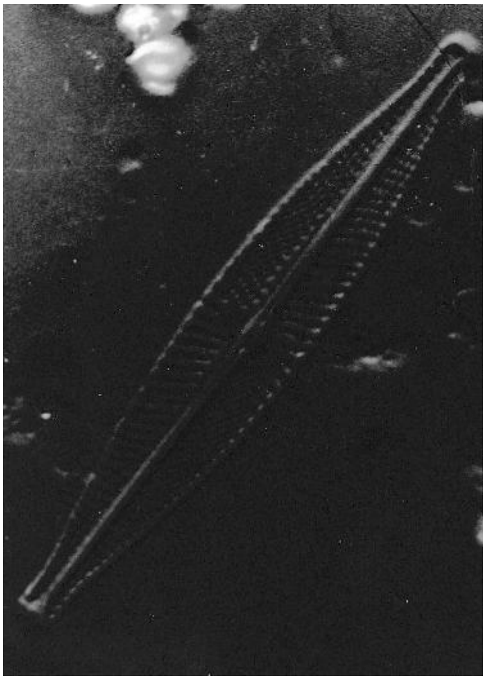


Navicula amphibola *Navicula mutica fo. intermedia*

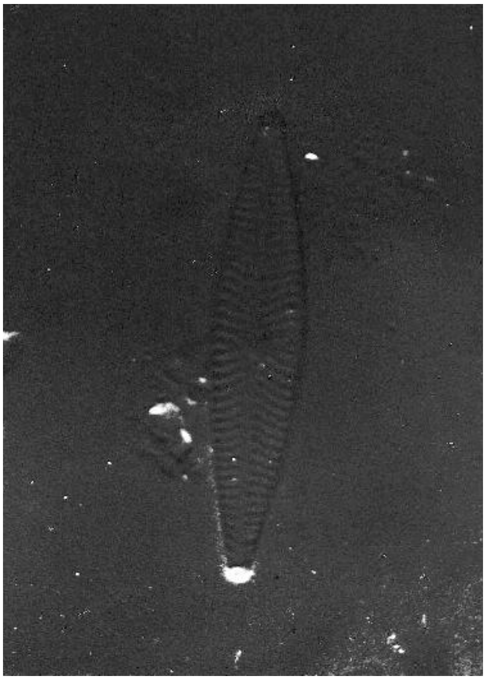
Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.

Navicula pavillardi

Navicula pupula fo. capitata

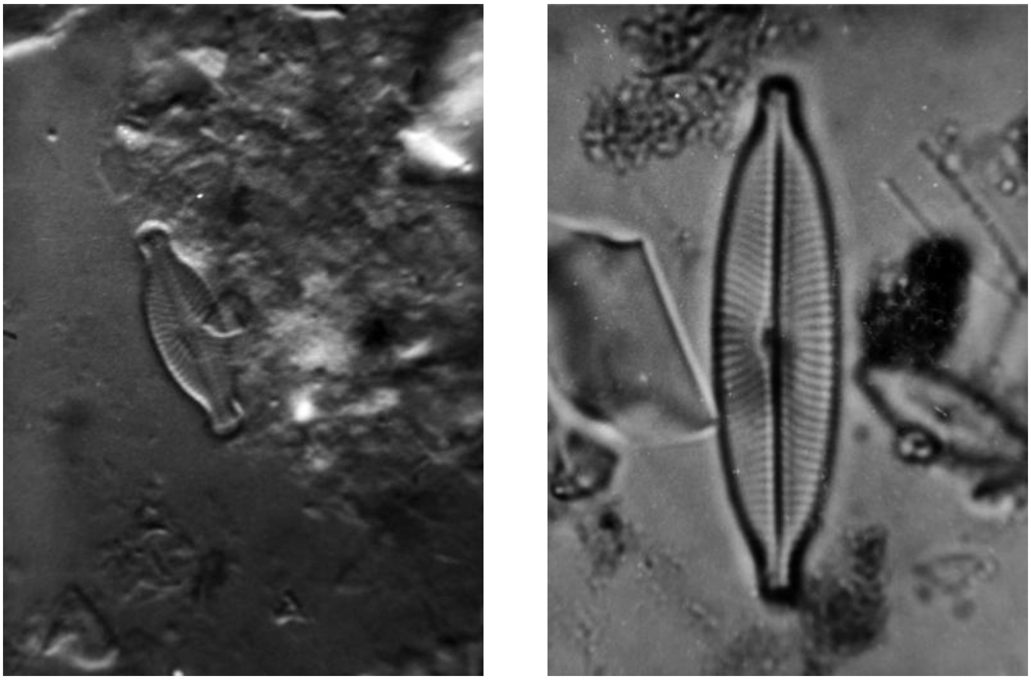


Navicula senjoensis



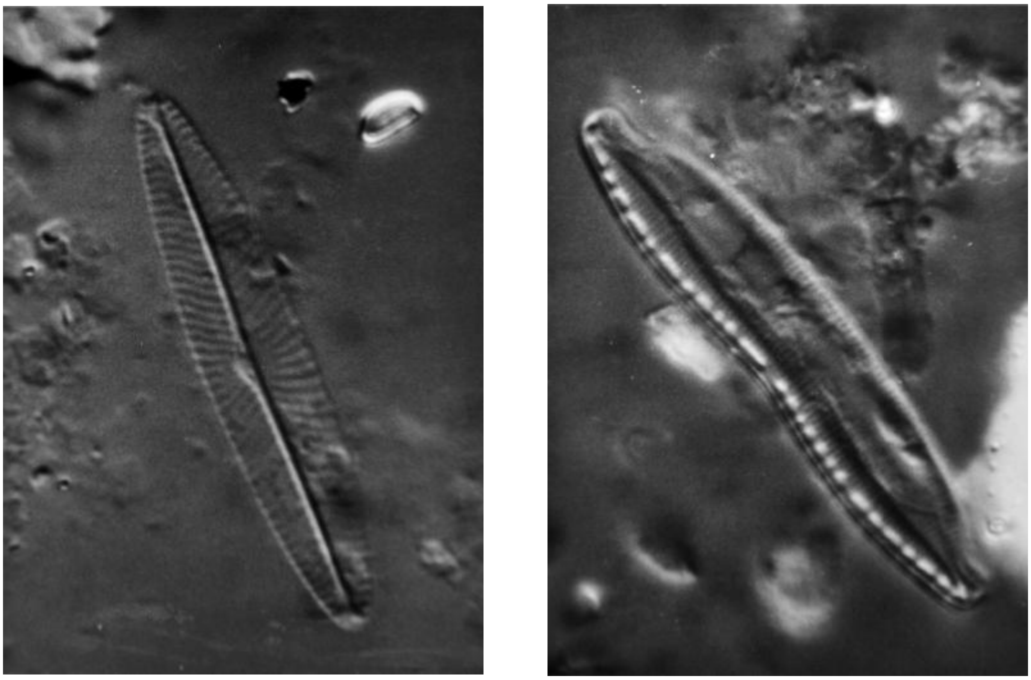
Navicula veneta

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Navicula globosa

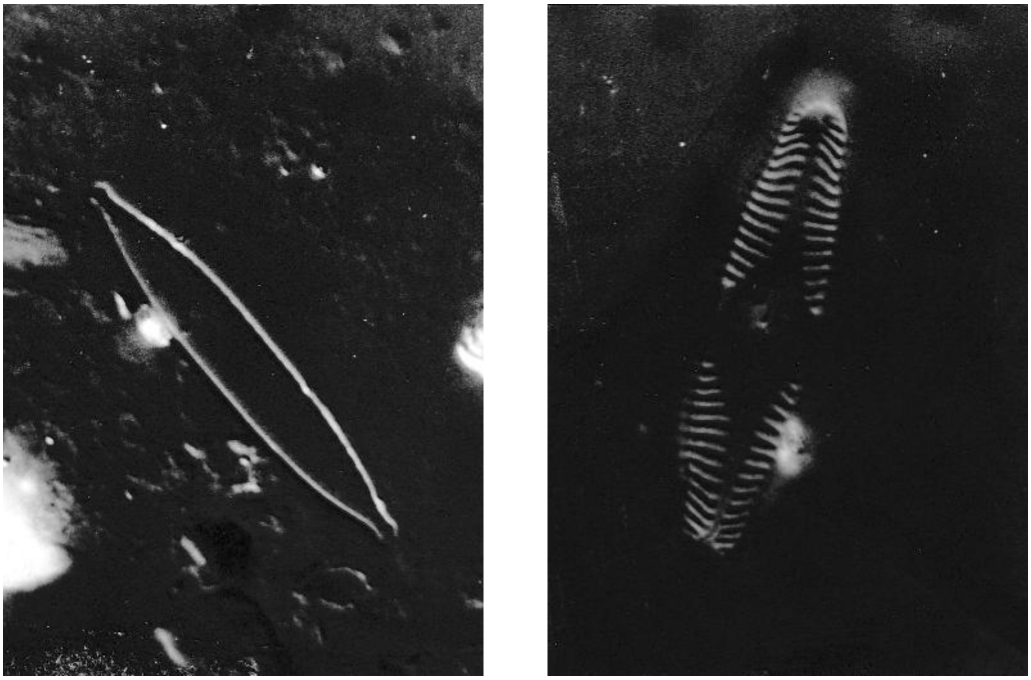
Navicula rhyncocephala



Navicula liboensis

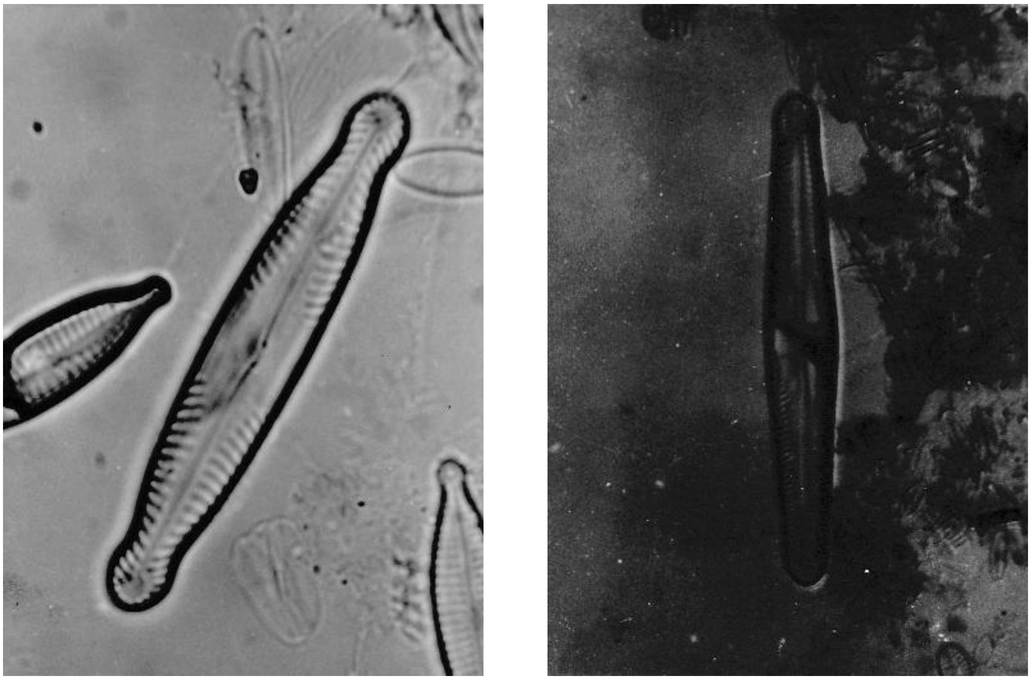
Nitzschia amphioxys

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Nitzschia palea var. *braunii*

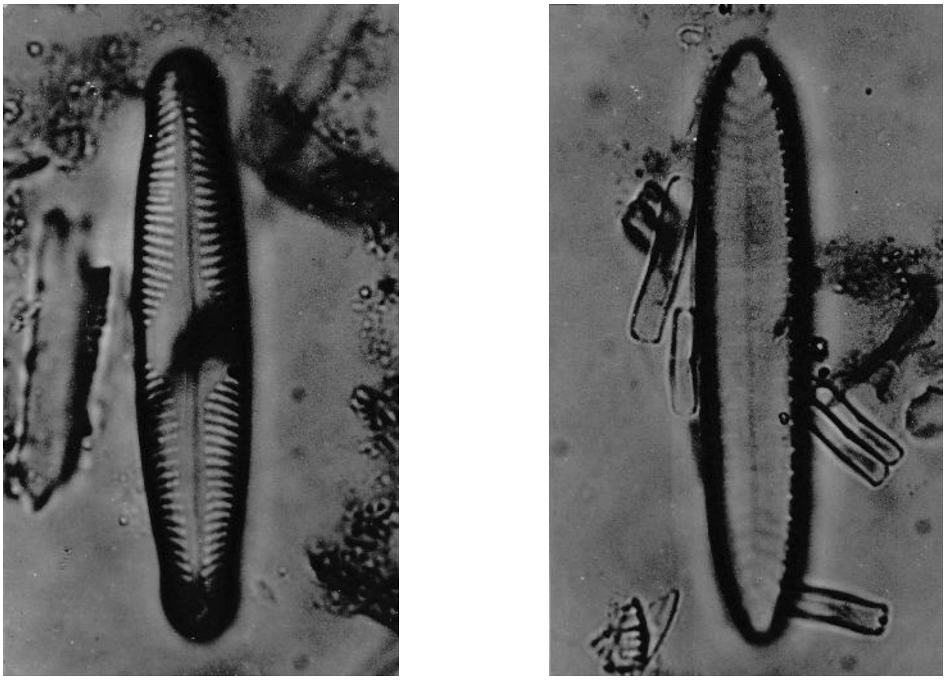
Pinnularia braunii



Pinnularia braunii var. *amphicephala*

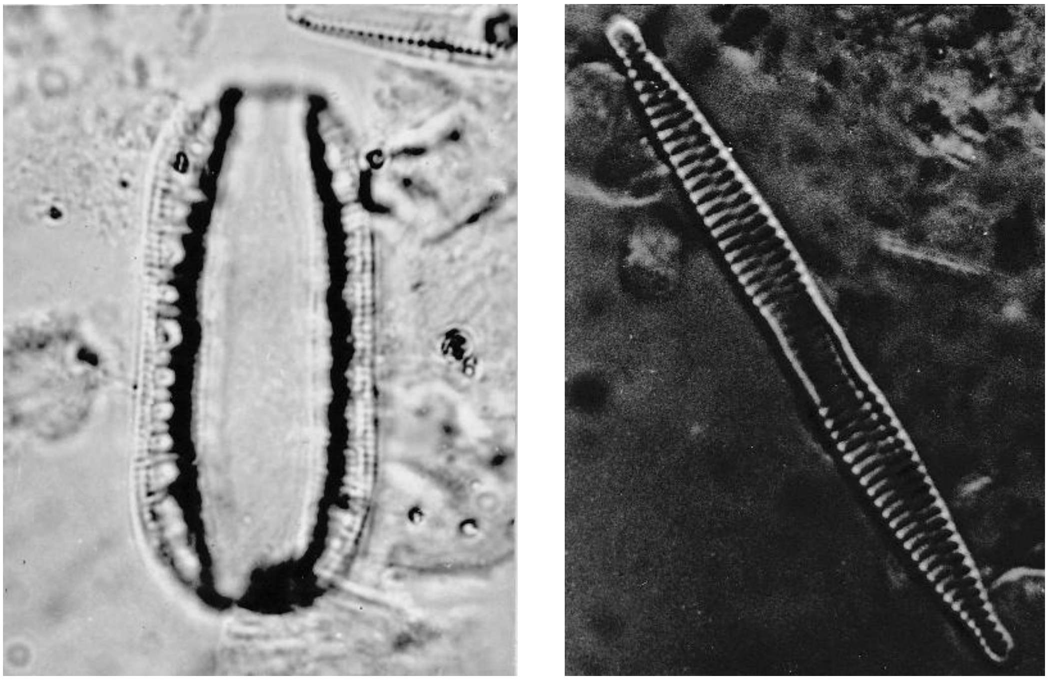
Pinnularia gibba var. *interrupta*

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.



Pinnularia microstauron

Surirella tenuissima



Surirella angusta

Synedra rumpens var. *fragilarioides*

Figure 2. (Cont.) Periphytic diatoms recorded in the Linggi (s.s.) and Kundor rivers.

CONCLUSION

The periphytic diatoms were collected from both natural and artificial substrates comprised of 86 taxa (82 pennate and 4 centric forms) belonging to 21 genera. The number of diatom species observed between stations varied from 22 to 47 species under 8 to 16 genera respectively. On the whole, annual mean number of species varied from 8 to 22; the minimum species at Station 9, heavily polluted with rubber effluent and the maximum species at Station 3 mildly polluted with rubber effluent. The most common diatoms, if both the rivers combined were *E. vanheurckii*, *G. parvulum*, *N. palea*, *P. braunii*, *N. cryptocephala*, *A. saxonica*, *A. minutissima* and *P. microstauron*. The most abundant species were *E. vanheurckii*, *N. palea*, *A. saxonica*, *G. parvulum* and *A. minutissim*.

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