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Synthesis of Bisdimedone Derivatives and Quantum Chemical Studies of 2,2'-[(2,3,4-trimethoxyphenyl) methylene]bis (3-hydroxy-5, 5-dimethylcyclohex-2-enone)

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Abstract

*An easy and efficient method for the synthesis of bisdimedone derivatives using three different green solvents i.e. ethanol, water and an inert solvent p-cymene has been developed to increase the % yield in less reaction time. The quantum chemical calculations on one of the synthesized compound 2,2'-[(2,3,4-trimethoxyphenyl) methylene] bis (3-hydroxy-5,5-dimethylcyclohex-2-enone) (**3a**) were performed using Density Functional Theory (DFT) with Becke3–Lee–Yang–Parr (B3LYP) functional and 6– 31G (d, p) as basis set to study the chemically reactive sites and local reactivity descriptors along with molecular electrostatic potential surfaces and thermodynamic parameters. The intramolecular hydrogen bonding interactions were also calculated with the help of Atom In Molecule (AIM) approach.*

Keywords: *AIM approach, Bisdimedone, Density functional theory, Green solvent*

Introduction

Dimedone (**1**) is the most important active methylene substrate which can exist in tautomeric enol form due to the presence of 1,3-dicarbonyl group having large number of heterocyclic and non-heterocyclic derivatives which are reported in the literature[1-3]. Bisdimedones or tetra-ketones are an important class of oxygen-containing comp-ounds based on dimedone scaffold that have shown various biological properties.[4-6] Therefore, several methods have been reported for the synthetic preparation of these moieties[7-14] although most of the reported methods have some disadvantages such as use of expensive organic solvents, harsh and hazardous reaction conditions, long reaction times, low yields and typical work-up procedure. Therefore, development of simple method for the synthesis of these compounds utilizing mild and effective green condition is needed. An inert hydrocarbon solvent, *p*-cymene as a renewable hydrocarbon is well placed to substitute toluene and other aromatic solvents[15]. The present study describes the Knoevenagel condensation[16] reaction between dimedone (**1**) and various substituted benzldehydes (**2a-2k**) separately in green solvents ethanol, water and *p*-cymene respectively to synthesize the various derivatives of bisdimedone with less reaction time and excellent yields.

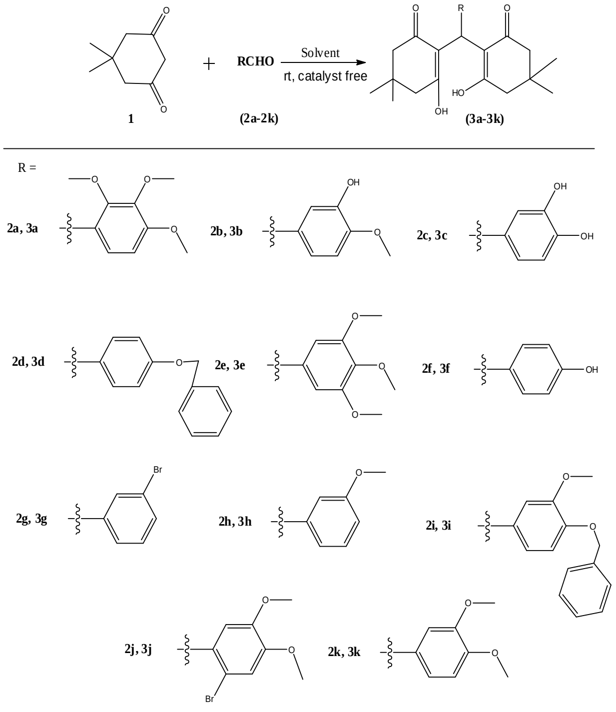
Theoretical investigations on one of the synthesized derivatives, 2,2'-[(2,3,4-trimethoxyphenyl) methylene] bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (**3a**) have been done using density functional theory (DFT) and quantum theory of atoms-in-molecules (QTAIM). DFT is emerging as a useful tool for computational studies to evaluate the various chemical, physical and thermodynamical properties of chemical species[17-20] as well as biomolecules[21]. Our present study is based on the DFT (B3LYP functional and 6-31G (d,p) basis set) to successfully estimate a range of molecular properties (structural and electronic) and topological parameters based on the QTAIM technique. The aim of this work is to provide a description of the experimental and theoretical calculation of bisdimedone, **3a** along with the theoretical investigation of weak intramolecular H—H, C—H and O—H bonding interactions in **3a**. To our knowledge, this is the first report on synthesis and quantum chemical studies calculated from DFT methods and QTAIM analysis.

Materials and Methods

Infrared (IR) spectrum was recorded on Perkin-Elmer FTIR spectrophotometer, ^1H and ^{13}C NMR(Nuclear Magnetic Resonance) spectra were recorded on Bruker 300 MHz instrument using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million (ppm) using Tetramethylsilane (TMS) as internal standard. Ultra violet (UV) spectrum was recorded on UV- visible Double-Beam Spectrophotometer (Systronic-2203) using chloroform as a solvent. Melting point was determined in a melting point apparatus. Thin layer chromatograms (TLC) were visualized in an iodine chamber.

General procedure for the synthesis of bisdimedone derivatives (3a-3k) (Scheme 1)

Dimedone, **1** (1 mole) in solvent was stirred in a round bottom flask for 5 minutes at room temperature. Then, benzaldehydes, (**2a-2k)** (0.5 mole) were added separately. The reaction mixture was stirred for 1 hour at room temperature. The progress of the reaction was monitored by TLC in Ethyl acetate:Hexane (30:70 v/v) as solvent. After completion of the reaction, the reaction mixture was left for a few minutes to allow the solid, to settle down, filtered and washed.



Scheme 1: Synthesis of Bisdismedone derivatives **(3a-3k)**

Computational Details

The DFT calculations were using Gaussian 09 program package and all theoretical analysis were done with the help of Gaussview 5.0 molecular visualization program[22, 23]. The investigation of optimized structural parameters, thermodynamic properties, vibrational assignments, HOMO–LUMO properties etc. were calculated using DFT[24]. A hybrid functional B3LYP which is described as *B3* is Becke's three parameter exchange (local, non local, Hartree–Fock) and *LYP* is Lee–Yang–Parr correlation functional. All the calculations were done using 6-31G (d, p) basis set. All predictions of intramoleular hydrogen bonding were done using *AIMALL* program using *QTAIM* theory.

Results and Discussion

Synthesis

Syntheses of bisdimedone derivatives (**3a-3k)** were done using three different green solvents i.e. (a) ethanol, (b) water, and (c) *p*-cymene without using any catalyst. The % yields and reaction times are given in Table1. It is clear from the table that the polarity of solvent affects the reaction time and % yield. Polar solvents i.e. ethanol and water enhanced the % yield and reduced the reaction time as compared to non polar solvent i.e. *p*-cymene.

Table 1: % yield and reaction time in three different solvents. a=ethanol; b=water; c=*p*-cymene

Compounds	~Yield (%)			Time (hrs)		
	a	b	c	a	b	c
3a	91	86	66	1.5	2.5	4
3b	85	87	68	1	2	3
3c	93	85	71	1	1	3
3d	81	83	65	2	2	3
3e	87	86	68	1.5	2.5	3
3f	79	75	56	1	1	4
3g	88	87	61	2	2	4
3h	94	86	63	2.5	3	3.5
3i	90	93	70	3	3.5	4
3j	85	82	67	3	4	3.5
3k	86	88	61	1	2	3

Molecular Structures

The optimized structure of the theoretically studied compound, **3a** is given in Fig. 1. The values of bond length (Å) and bond angle (degree) are also listed in Table 2.

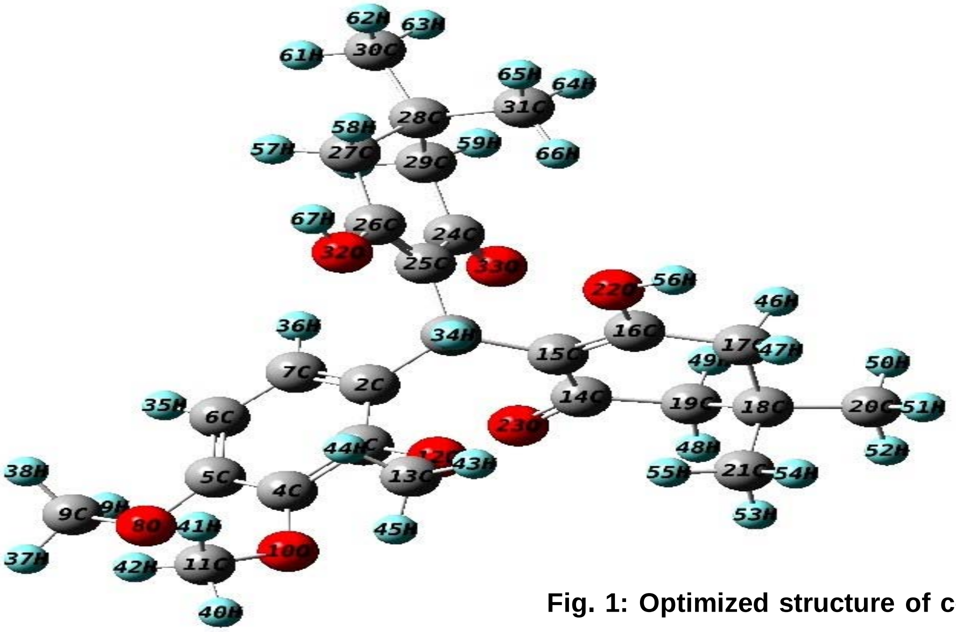


Fig. 1: Optimized structure of compound 3a

Table 2. Geometrical parameters of optimized geometries of 3a at B3LYP/6-31G (d, p) level

Bond -length (Å)		Bond –angle (º)	
R(1,2)	1.5405	A(2,1,15)	109.865
R(1,15)	1.5417	A(2,1,25)	109.078
R(1,25)	1.5441	A(2,1,34)	109.5029
R(1,34)	1.07	A(15,1,25)	109.5846
R(2,3)	1.5396	A(15,1,34)	109.077
R(2,7)	1.3556	A(25,1,34)	109.7213
R(3,4)	1.3532	A(1,2,3)	120.1669
R(3,12)	1.4282	A(1,2,7)	119.894
R(4,5)	1.5395	A(3,2,7)	119.9391
R(4,10)	1.4282	A(2,3,4)	119.9748
R(5,6)	1.3551	A(2,3,12)	120.4347
R(5,8)	1.4298	A(4,3,12)	119.5906
R(6,7)	1.539	A(3,4,5)	120.1272
R(6,35)	1.0711	A(3,4,10)	119.7764
R(7,36)	1.0698	A(5,4,10)	120.0963
R(8,9)	1.4289	A(4,5,6)	119.9212
R(9,37)	1.071	A(4,5,8)	120.0362
R(9,38)	1.0707	A(6,5,8)	120.0426
R(9,39)	1.0707	A(5,6,7)	119.989
R(10,11)	1.4285	A(5,6,35)	120.0188
R(11,40)	1.0708	A(7,6,35)	119.9921
R(11,41)	1.0705	A(2,1,15)	109.865
R(11,42)	1.0699	A(2,1,25)	109.078
R(12,13)	1.4311	A(2,1,34)	109.5029
R(13,43)	1.0698	A(15,1,25)	109.5846
R(13,44)	1.0708	A(15,1,34)	109.077
R(13,45)	1.069	A(25,1,34)	109.7213
R(14,15)	1.5375	A(1,2,3)	120.1669
R(14,19)	1.5347	A(1,2,7)	119.894
R(14,23)	1.2595	A(3,2,7)	119.9391
R(15,16)	1.3593	A(2,3,4)	119.9748
R(16,17)	1.5451	A(2,3,12)	120.4347
R(16,22)	1.4306	A(4,3,12)	119.5906
R(17,18)	1.5424	A(3,4,5)	120.1272
R(17,46)	1.0692	A(3,4,10)	119.7764
R(17,47)	1.0695	A(5,4,10)	120.0963
R(18,19)	1.5386	A(4,5,6)	119.9212
R(18,20)	1.5442	A(4,5,8)	120.0362
R(18,21)	1.5409	A(6,5,8)	120.0426
R(19,48)	1.0703	A(5,6,7)	119.989
R(19,49)	1.0689	A(5,6,35)	120.0188
R(20,50)	1.0709	A(7,6,35)	119.9921
R(20,51)	1.0706	A(2,7,6)	120.0446
R(20,52)	1.0702	A(2,7,36)	119.9563
R(21,53)	1.0699	A(6,7,36)	119.9989

Bond -length (Å)		Bond –angle (º)	
R(21,54)	1.0706	A(5,8,9)	109.1841
R(21,55)	1.0707	A(8,9,37)	109.5264
R(22,56)	0.9603	A(8,9,38)	109.3828
R(24,25)	1.5374	A(8,9,39)	109.4329
R(24,29)	1.5346	A(37,9,38)	109.4903
R(24,33)	1.2586	A(37,9,39)	109.4744
R(25,26)	1.3594	A(38,9,39)	109.5205
R(26,27)	1.5489	A(4,10,11)	109.2409
R(26,32)	1.43	A(10,11,40)	109.5521
R(27,28)	1.542	A(10,11,41)	109.4068
R(27,57)	1.07	A(10,11,42)	109.4003
R(27,58)	1.0695	A(40,11,41)	109.4398
R(28,29)	1.5358	A(40,11,42)	109.4818
R(28,30)	1.5418	A(41,11,42)	109.5464
R(28,31)	1.5395	A(3,12,13)	108.801
R(29,59)	1.0695	A(12,13,43)	109.5047
R(29,60)	1.0699	A(12,13,44)	109.5084
R(30,61)	1.0707	A(12,13,45)	109.356
R(30,62)	1.0705	A(43,13,44)	109.5499
R(30,63)	1.0701	A(43,13,45)	109.4412
R(31,64)	1.0699	A(44,13,45)	109.4669
R(31,65)	1.0704	A(15,14,19)	119.0541
R(31,66)	1.0706	A(15,14,23)	120.4717
R(32,67)	0.9586	A(19,14,23)	120.4743
R(18,19)	1.5386	A(2,7,6)	120.0446
R(18,20)	1.5442	A(2,7,36)	119.9563
R(18,21)	1.5409	A(6,7,36)	119.9989
R(19,48)	1.0703	A(5,8,9)	109.1841
R(19,49)	1.0689	A(8,9,37)	109.5264
R(20,50)	1.0709	A(8,9,38)	109.3828
R(20,51)	1.0706	A(8,9,39)	109.4329
R(20,52)	1.0702	A(37,9,38)	109.4903
R(21,53)	1.0699	A(37,9,39)	109.4744
R(21,54)	1.0706	A(38,9,39)	109.5205
R(21,55)	1.0707	A(4,10,11)	109.2409
R(22,56)	0.9603	A(10,11,40)	109.5521
R(24,25)	1.5374	A(10,11,41)	109.4068
R(24,29)	1.5346	A(10,11,42)	109.4003
R(24,33)	1.2586	A(40,11,41)	109.4398
R(25,26)	1.3594	A(40,11,42)	109.4818
R(26,27)	1.5489	A(41,11,42)	109.5464
R(26,32)	1.43	A(3,12,13)	108.801
R(27,28)	1.542	A(12,13,43)	109.5047
R(27,57)	1.07	A(12,13,44)	109.5084
R(27,58)	1.0695	A(12,13,45)	109.356

Bond -length (Å)		Bond –angle (°)	
R(28,29)	1.5358	A(43,13,44)	109.5499
R(28,30)	1.5418	A(43,13,45)	109.4412
R(28,31)	1.5395	A(44,13,45)	109.4669
R(29,59)	1.0695	A(15,14,19)	119.0541
R(29,60)	1.0699	A(15,14,23)	120.4717
R(30,61)	1.0707	A(19,14,23)	120.4743
R(30,62)	1.0705	A(1,15,14)	120.5252
R(30,63)	1.0701	A(1,15,16)	119.3803
R(31,64)	1.0699	A(14,15,16)	120.0945
R(31,65)	1.0704	A(15,16,17)	121.0181
R(31,66)	1.0706	A(15,16,22)	119.3745
R(32,67)	0.9586	A(17,16,22)	119.6073
		A(16,17,18)	110.1781
		A(16,17,46)	109.2777
		A(16,17,47)	109.2214
		A(18,17,46)	109.351
		A(18,17,47)	109.2255
		A(46,17,47)	109.5734
		A(17,18,19)	109.5024
		A(17,18,20)	109.5106
		A(17,18,21)	109.316
		A(19,18,20)	109.3895
		A(19,18,21)	109.5284
		A(20,18,21)	109.5806
		A(14,19,18)	108.9747
		A(14,19,48)	109.7283
		A(14,19,49)	109.4671
		A(18,19,48)	109.6054
		A(18,19,49)	109.6187
		A(48,19,49)	109.4328
		A(18,20,50)	109.4663

Hydrogen Bonding Interactions

The *QTAIM* calculations have been done with the help of *AIMALL* Program. These calculations describe the theoretical investigation of intramolocular hydrogen bonding within the molecule. The hydrogen bonding is defined by the bond critical point (*BCP*) between a proton donor and proton acceptor atom. The second order derivative of the electron density, the Laplacian ($\nabla^2_{\rho BCP}$), charge density (*ρBCP*), potential energy density (V_{BCP}), kinetic energy density (G_{BCP}), total energy density(*H*) and energy of interaction (*E*) are the parameters of *BCP* which are used to describe the chemical behavior of a compound[25]. For the charge concentrated in covalent (shared) interaction $\nabla^2_{\rho BCP} > 0$ and $H > 0$; the hydrogen bonds are weak and electrostatic in nature. In our study, it was found that in **3a** compound there are five types of bonding interactions i.e. O8....H42, O10....H45, O22....-H66, O23....C2 and O33....C15. The bonding interactions in molecule **3a** are displayed in Fig. 2. The theoretical values of parameters of *BCP* are shown in Table 3 for **3a** compound.

According to the Espinosa correlation[26] the *QTAIM* theory is also able to investigate the interaction energy of H-Bond as

$$E\ (\text{au}) = 1/2\ \boldsymbol{V_{BCP}}\ (\text{au}) = 313{:}754\ \boldsymbol{V_{BCP}}\ (\text{kcal mol}^{-1}) = E\ (\text{kcal mol}^{-1}) \qquad (1)$$

where $\boldsymbol{V_{BCP}}$ is the potential energy density and *E* is the energy of interatomic interaction (a.u. or kcal mol^{-1}), which can be easily calculated using the *AIMAll* package. Table 3 showed that interaction energy, *E,* at their critical points, O10—H45 (E = 191.70 kcal mol^{-1}), O22....H66 (E = 198.29 kcal mol^{-1}) and O33....C15 (E = 170.04 kcal mol^{-1}) are the strongest interactions for compound **3a**. These studies show that **3a** molecule is considered as the reactive molecule for further study.

UV-Visible Absorption Spectroscopy

The theoretically UV-visible calculations of compound **3a** were done by DFT calculation and UV-visible spectrum was obtained by taking chloroform solvent. Table 4 depicts the value of percentage contribution of probable transitions, oscillator strengths (f), calculated and experimental $\lambda_{max,}$ and molecular orbital electronic transitions. The correlation graph between theoretical and experimental values of absorbance and wavelength is shown in Fig. 3. The energy transitions between the HOMO-LUMO molecular orbitals are shown in Fig. 4. The calculated λ_{max} of **3a** is 231.57 nm with oscillator strength f = 0.3085 for p-p* transition which correlates well with the experimental value i.e. 244 nm. The electronic transition from HOMO to LUMO+4 showed maximum percentage contribution that is 27.32 due to p-p* transition.

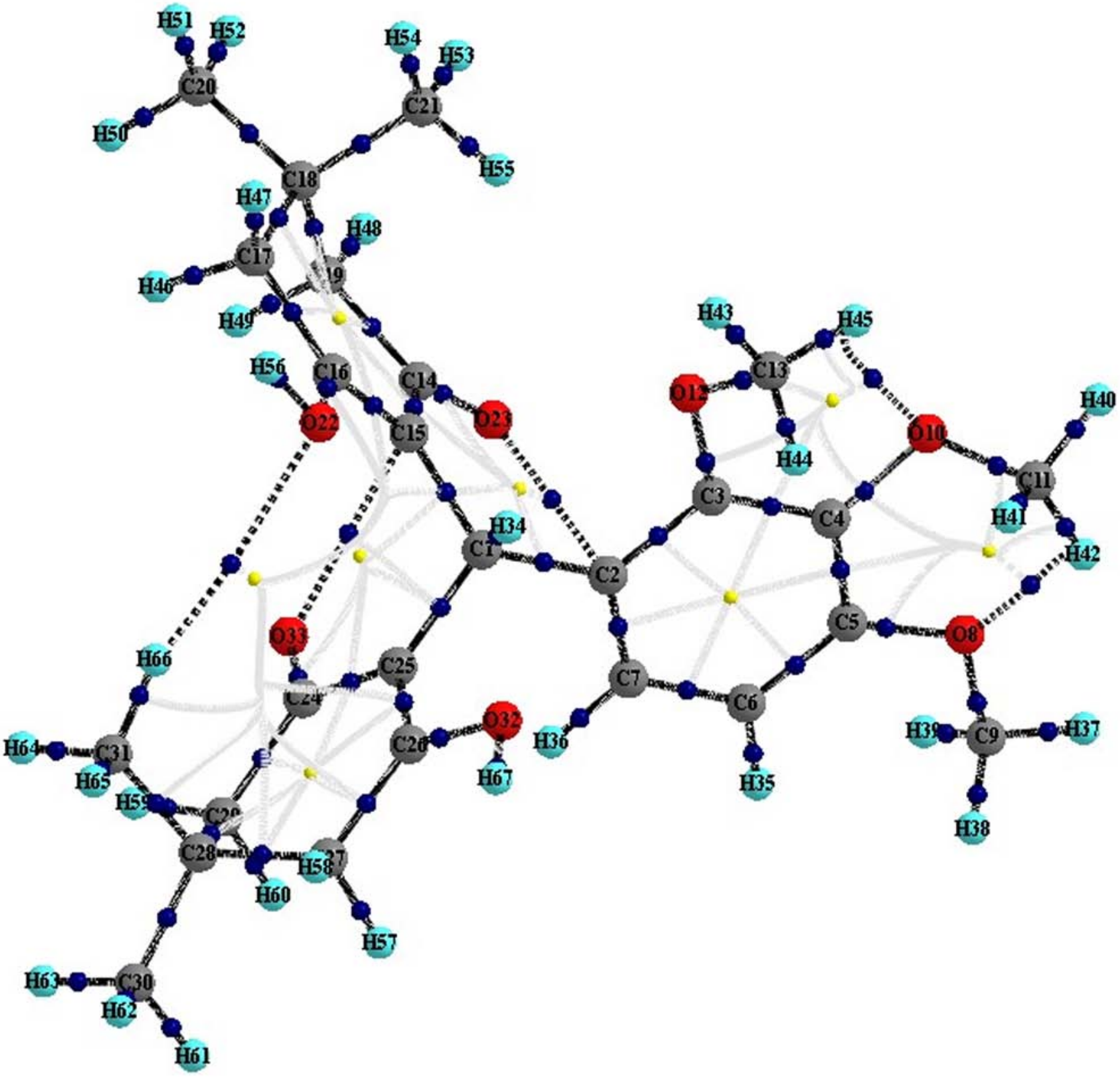


Fig. 2: Molecular graphs showing intramolecular interactions (blue dotted lines) in 3a. The yellow points correspond to ring critical points, the blue points represent BCPs and light blue lines correspond to RCPs to BCPs path. Red spheres correspond to oxygen atoms, dark grey spheres correspond to carbon atoms, and green spheres correspond to hydrogen atoms.

Table 3: Topological values of 3a at B3LYP/6-31G (d, p) level

Interactions	O8---H42	O10---H45	O22---H66	O23---C2	O33---C15
ρBCP	+0.014300	+0.0133	+0.0028	+0.0129	+0.0123
$\nabla^2_{\rho BCP}$	+0.051132	+0.0521	+0.0110	+0.0426	+0.0425
G_{BCP}	+0.0117	+0.0114	+0.0020	+0.0098	+0.0096
V_{BCP}	-0.0107	-0.0098	-0.0013	-0.0089	-0.0087
H_{BCP}	-0.0014	-0.0014	-0.0000	-0.0012	-0.0013
E_{HB}	44.553	191.70	198.29	49.512	170.04
$\|V(r)\|/G(r)$	0.9140	0.8600	0.6529	0.9109	0.9016
BPL	+4.4762	+4.8495	+5.8439	+5.4027	+5.4795

UV-Visible Absorption Spectroscopy

The theoretical UV-visible calculations of compound **3a** were done by DFT calculation and UV-visible spectrum was obtained using chloroform solvent. Table 4 depicts the value of percentage contribution of probable transitions, oscillator strengths (f), calculated and experimental $\lambda_{max,}$ and molecular orbital electronic transitions. The correlation graph between theoretical and experimental values of absorbance and wavelength is shown in Fig. 3. The energy transitions between the HOMO-LUMO molecular orbitals are shown in Fig. 4. The calculated λ_{max} of **3a** is 231.57 nm with oscillator strength f = 0.3085 for Π-Π* transition which correlates well with the experimental value i.e. 244 nm. The electronic transition from HOMO to LUMO+4 showed maximum percentage contribution that is 27.32 due to Π-Π* transition.

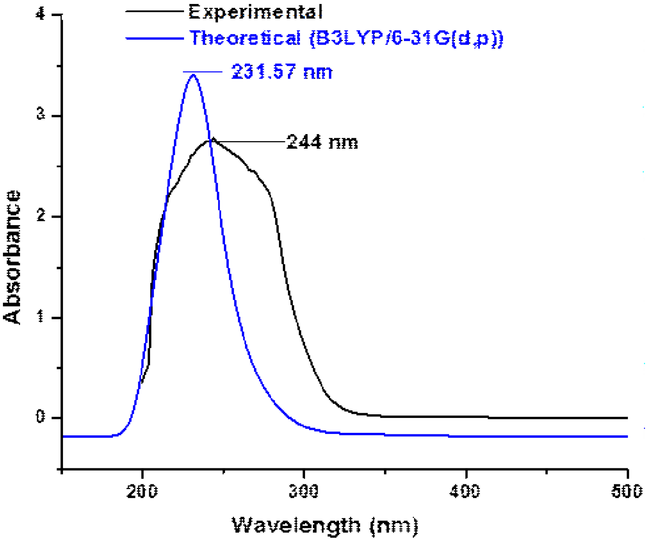


Fig. 3: Experimental and Theoretical UV- visible spectrum of compound 3a

Table 4: Experimental and theoretical absorption wavelengths (nm) and excitation energies E (eV) of title compound 3a using B3LYP functional and 6–31-G / (d, p) basis set.

S. No.	Electronic transitions (molecular orbitals involved)	Excitation energies (eV)	Calculated λ_{max} (in nm)	Oscillatory strength (f)	Percent contribution of probable transition	Observed λ_{max} (in nm)
1.	H-5 → L+4 H-1 → L+3 H → L+4	6.954 6.213 5.924	231.57	0.3085	12.36 3.28 27.32	244

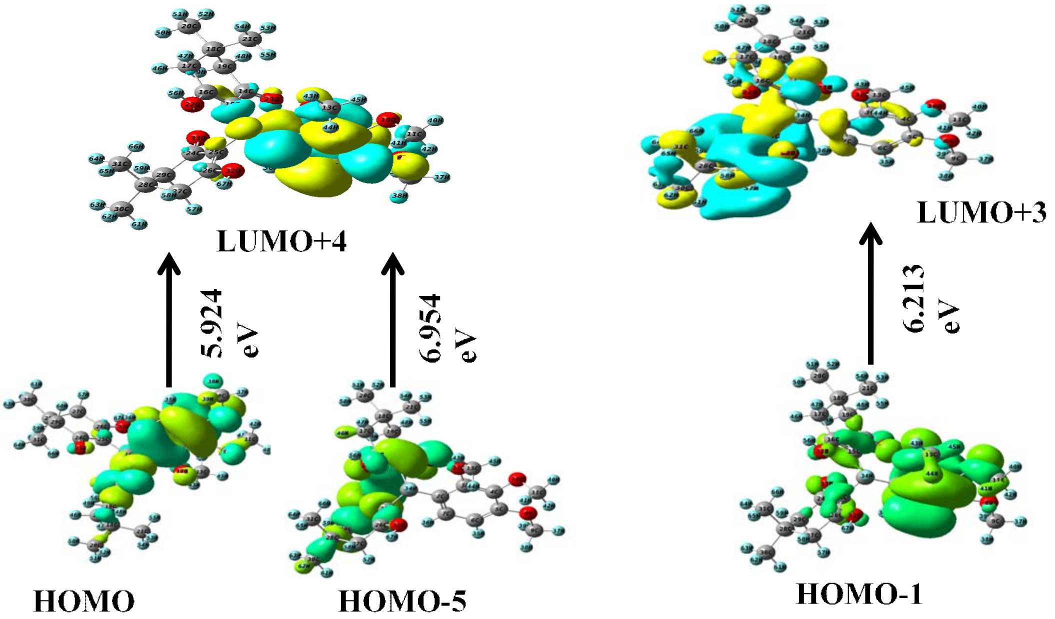
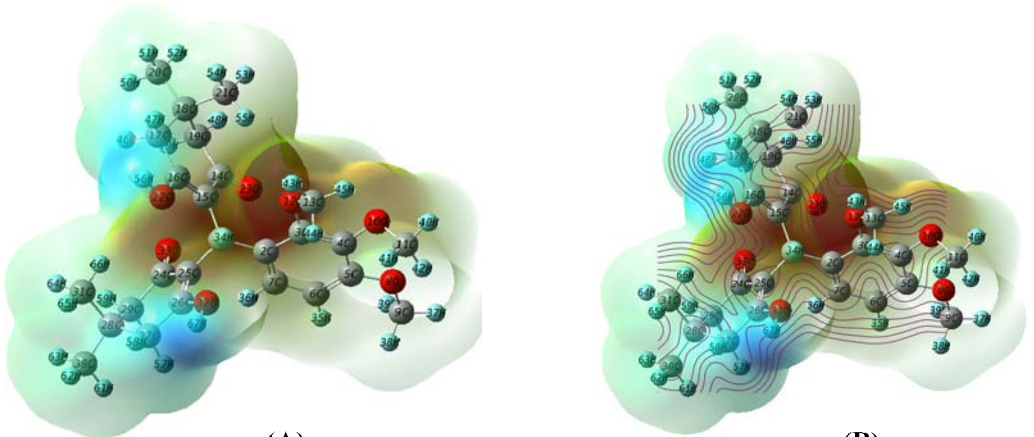


Fig. 4: Calculated HOMO-LUMO energy gaps of compound 3a

The Molecular Electrostatic Potential Surface

Molecular electrostatic potential (MESP) is a function of DFT for the recognition of the reactive sites (nucleophilic and electrophilic attack) using color based pattern[27]. The MESP surface for compound **3a** was calculated at B3LYP/ 6-31G (d, p) basis set. Figures 5A and 5B showed the chemically reactive site and total electron density using charge surface contour around the atoms of compound **3a** respectively. The MESP surface indicated that electrophilic reactivity is represented by the negative (red) regions, nucleophilic reactivity is shown by the positive (blue) regions and green represents region of zero potential of MEP. Potential decreases in the order red < orange < yellow < green < blue. In the compound **3a** the positive region is appearing at atoms 32O and 27O of hydroxyl groups. The positive region is appearing at 24C and 14C atoms.



(A) (B)

Fig. 5: (A) MESP surface diagram (B) Charge surface contour of compound 3a

Electronic Parameters

DFT studies have also been used to calculate the electronic parameters such as HOMO-LUMO energy gap (εLUMO - εHOMO), ionization potential (***IP***), electron affinity (***EA***), electronegativity (χ), global hardness (η), global electrophilicity index (ω), chemical potential (***μ***), global softness (***S***) and additional electronic charge ***(ΔNmax)*** by B3LYP/6-31G (d, p) level for compound **3a**. The chemical reactivity of molecules is related to the *global reactivity descriptors* which could be described with the help of these electronic parameters. These descriptors have been used to elucidate the electronic structure of compounds. The mechanism involved in the covalent bond formation between the electron rich and electron poor centers has also been described by these parameters. These electronic parameters of studied compound **3a** are listed in Table 5. These calculations have been done using following equations[28-32].

$$IP = -\varepsilon_{HOMO} \quad (2)$$

$$EA = -\varepsilon_{LUMO} \quad (3)$$

$$\chi = -\frac{1}{2}(\varepsilon_{LUMO} + \varepsilon_{HOMO}) \quad (4)$$

$$\eta = \frac{1}{2}(\varepsilon_{LUMO} - \varepsilon_{HOMO}) \quad (5)$$

$$\mu = -\chi = 1/2(\varepsilon_{LUMO} + \varepsilon_{HOMO}) \quad (6)$$

$$\omega = \mu^2 / 2\eta \quad (7)$$

$$S = \frac{1}{2}\eta \quad (8)$$

$$\Delta N_{\max} = -\mu / \eta \quad (9)$$

Table 5: Calculated electronic parameters in eV for Compound 3a using B3LYP/6-31 Ga(d,p)

ε_H	εL	εH -ε L	IP	EA	χ	η	μ	ω	S	ΔN_{max}
-5.27	-0.516	-4.754	5.27	0.516	2.893	2.377	-2.893	1.7605067	0.2103492	1.21708035

The values of ***IP*** and ***EA*** are equals to 5.27 eV and 0.516 eV respectively which indicate that the compound **3a** is a stable molecule. A good nucleophile is characterized by lower value of **ω** and conversely a good electrophile is characterized by higher value of **ω**. Compound **3a** acts as neither good electrophile nor good electrophile as the compound showed moderate value for global electrophilicity index (**ω**) equal to 1.76 eV.

Thermodynamic Parameters

Thermodynamic parameters of compound **3a** *viz.* heat capacity (C_V), entropy (S), zero point vibrational energy, rotational constants and total energy E_{total} etc. at various temperatures (50-298 K) for compound **3a** using B3LYP/6-31G (d, p) level are listed in Table 6 and 7 respectively. It is observed that the thermodynamic functions increase with temperature (50 to 298 K), due to the fact that the molecular vibrational intensities increase with temperature[33]. All thermodynamic calculations (theoretical) were done in the gas phase and they could not be used in solution. The correlation graph of total energy, entropy and heat capacity versus temperature is shown in Fig. 6.

Table 6: Thermodynamic functions at different temperatures at the B3LYP /6-31-G (d,p) level

Temperature (K)	**Heat capacity (C_V) cal/mol K**	**Entropy (S) (cal/mol K)**	**Total energy E_{total} (kcal/mol)**	**Translational**	**Rotational**	**Vibrational**
50	30.375	87.527	353.830	0.149	0.149	353.532
100	54.151	117.357	355.951	0.298	0.298	355.355
150	72.453	136.789	358.761	0.385	0.385	3357.991
200	94.831	169.238	363.470	0.596	0.596	362.278
250	113.381	192.839	368.676	0.745	0.745	367.186
298	131.178	214.682	374.564	0.889	0.889	372.787

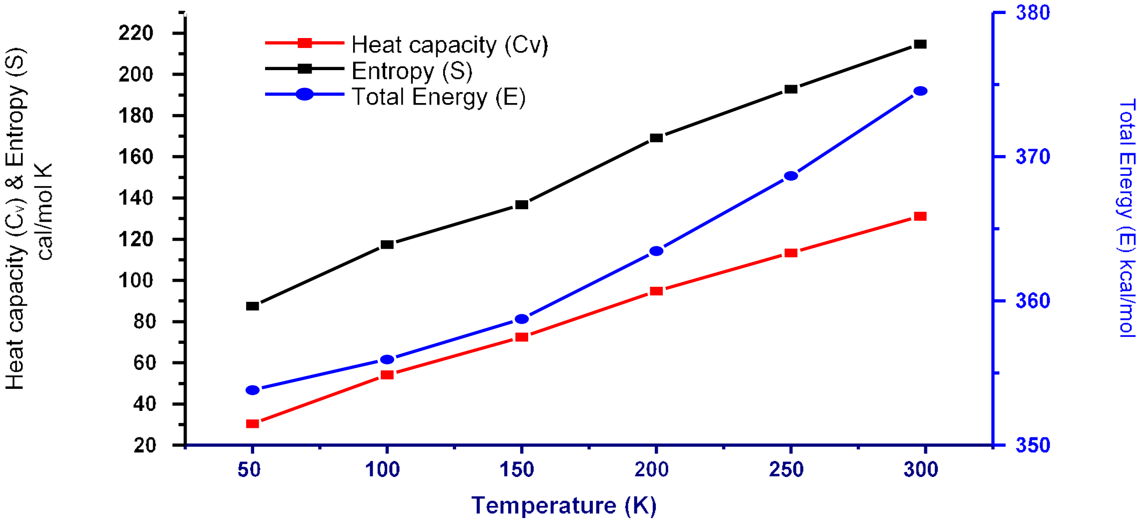


Fig. 6: Correlation graph of total energy, entropy and heat capacity versus temperature

Table 7: Calculated Thermodynamic parameters at 298 K

Parameters	**B3LYP 6-31G (d,p)**
Zero point vibrational Energy (kcal/mol)	352.94821
Rotational temperature (K)	
X	0.00719
Y	0.00517
Z	0.00375
Rotational constant (GHZ)	
X	0.14989
Y	0.10772
Z	0.07808

Non Linear Optical Analysis

The relationship between molecular structure and first hyperpolarizability (β_{total} **(esu)**) and related properties such as polarizability ***(α)*,** and total dipole moment (***μ)*** have been calculated using B3LYP/6-31G (d, p) based on the finite-field approach. First hyperpolarizability is a third rank tensor and can be described by a 3 x 3 x 3 matrix. The 27 components of the 3 Dimensional matrixes can be reduced to 10 components using Kleinman symmetry[34]. All NLO parameters were calculated according to the equations given below.

$$\mu_{tot} = (\mu_x{}^2 + \mu_y{}^2 + \mu_z{}^2)^{1/2} \qquad (11)$$

$$\alpha_{tot} = 1/3(\alpha_{xx} + \alpha_{yy} + \alpha_{zz}) \qquad (12)$$

$$<\beta> = [(\beta_{xxx} + \beta_{xyy} + \beta_{xzz})^2 + [(\beta_{yyy} + \beta_{yzz} + \beta_{yxx})^2 + [(\beta_{zzz} + \beta_{zxx} + \beta_{zyy})^2]^{1/2} \qquad (13)$$

The investigated compound **3a** showed large values of the polarizability and hyperpolarizability indicting that there is delocalization of charge in this compound. The values of the dipole moment (μ), polarizability (α), first hyperpolarizability (β) are given in Table 8. It is clear from Table 8 that **3a** compound showed the values of dipole moment (μ) equal to 12.48; polarizability **(α)** equal to 58.61 esu and Hyperpolarizability (β) equal to 5.158 esu in comparison to Gaussian 09 output as reported in atomic unit (a.u.). These values are converted into electrostatic unit (esu) using converting factors viz. for **(α)**: 1 a.u. = 0.1482 x 10^{-24} esu; for **(β_{total})**: 1 a.u = 0.008639 x 10^{-30} esu).

Table 8: Dipole Moment μ, Polarizability α_{tot} (10^{-24} esu) and first order static hyperpolarizability β_{tot} (10^{-30}) data for compound 3a calculated at DFT/B3LYP/6-31G(d,p) level of theory

Dipole moment		**Hyperpolarizability**	
μ_x	-6.6988		
μ_y	-4.9159	β_{xxx}	-503.369
μ_z	9.3158	β_{xxy}	99.3556
μ	12.4829	β_{xyy}	200.634
Polarizability		β_{yyy}	-215.080
α_{xx}	425.027	β_{xxz}	-83.8008
α_{xy}	-13.9054	β_{xyz}	-78.0588
α_{yy}	378.539	β_{yyz}	134.991
α_{xz}	-21.7170	β_{xzz}	-216.044
α_{yz}	21.5640	β_{yzz}	14.0665
α_{zz}	383.061	β_{zzz}	-328.897
(α)	58.61	β_{total}(esu)	5.158

Local Reactivity Descriptors

Fukui function (**FF**), local softness (***S$_k$***) and local electrophilicity index (ω_k) are the tools mostly used in Computational Chemistry to identify the chemical reactivity and site selectivity of the molecule. Using Hirshfield population analysis of neutral, cation and anion state of molecule, Fukui Functions are calculated using following equations:

$$f_k^{+} = [q(N+1) - q(N)] \text{ for nucleophilic attack} \qquad (14)$$

$$f_k^{-} = [q(N) - q(N-1)] \text{ for electrophilic attack} \qquad (15)$$

$$f_k^{0} = 1/2[q(N+1) - q(N-1)] \text{ for radical attack} \qquad (16)$$

where N, N-1, N+1 are total electrons present in neutral, anion and cation state of molecule respectively. Local softness (***S$_k$***) functions are calculated according to the following equations:

$$S_k^{+} = Sf_k^{+}, S_k^{-} = Sf_k^{-}, S_k^{0} = Sf_k^{0} \qquad (17)$$

$$S_k^{+} = Sf_k^{+}, S_k^{-} = Sf_k^{-}, S_k^{0} = Sf_k^{0} \qquad (18)$$

Selected Fukui functions (***f_k*+, *f_k*-**), local softness (**sk$^+$, sk$^-$**) and local electrophilicity indices (**ωk$^+$, ωk$^-$**), Huckel charges for compound **3a** are listed in Table 9. It is clear from Table 9 that for **3a** maximum values of

f_k^+, s_k^+, ω_k^+ are 0.128884, 0.031359 and 0.415033 respectively for atomic site C24, hence it is the most vulnerable site for nucleophilic attack as compared to the other sites listed in Table 5. Maximum values of f_k^-, s_k^-, ω_k^- are 0.135906, 0.033067 and 0.191237 respectively for atomic site O15 hence these sites are more prone for the electrophilic attack in **3a** compound. Fig. 7 represents the charge distribution on different atoms in singlet state for neutral and doublet state for anions and cations respectively.

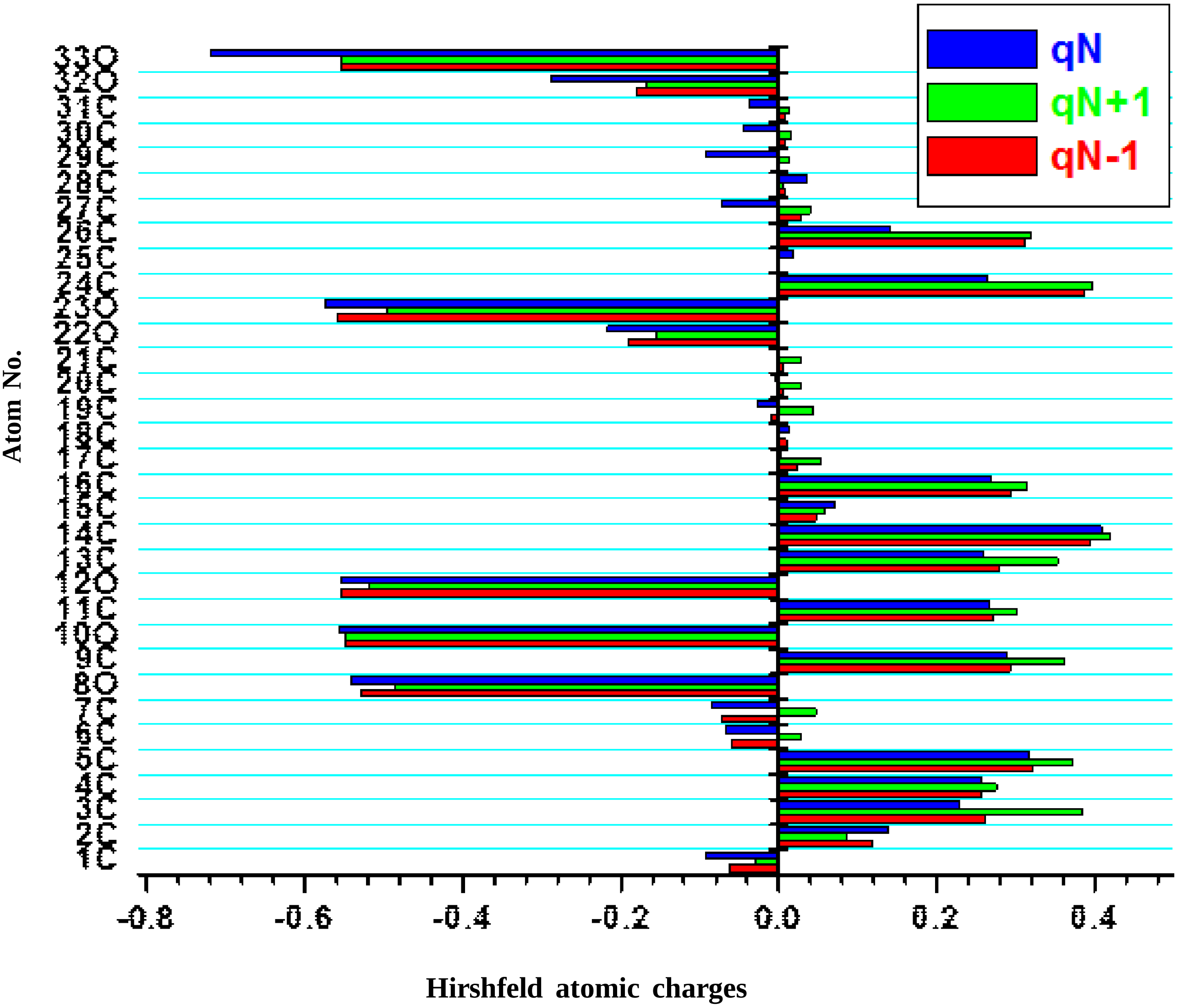


Fig. 7: Graphical representation of charge distribution on different atoms of 3a

Table 9: Fukui functions (fk^+, fk^-), Local softnesses (sk^+, sk^-) in eV, local electrophilicity indices (wk^+, wk^-) in eV for specific atomic sites of compound 3a

Atom no.	Hirshfeld atomic charges			Fukui functions		Local softnesses		Local electrophilicity indices	
	q_N	q_N+1	q_N-1	f_k+	f_k-	sk^+	sk^-	ωk^+	ωk^-
1C	-0.063	-0.030	-0.0927	0.032707	0.0294	0.0068	0.006201	0.057564	0.051881
2C	0.118	0.085	0.13938	-0.03369	-0.0204	-0.0081	-0.00429	-0.0593	-0.0359
3C	0.262	0.384	0.22958	0.121121	0.0333	0.02946	0.007017	0.213173	0.058715
4C	0.257	0.275	0.25675	0.017759	0.0005	0.00432	0.000123	0.031256	0.001026
5C	0.322	0.371	0.31667	0.049358	0.005705	0.01200	0.0012	0.08687	0.010041
6C	-0.06	0.028	-0.0679	0.090744	0.005642	0.02207	0.001187	0.159709	0.00993
7C	-0.072	0.047	-0.0849	0.120229	0.012398	0.0292	0.002608	0.211603	0.02182
8O	-0.53	-0.487	-0.5417	0.05181	0.00245	0.01260	0.000515	0.091186	0.004312
9C	0.293	0.361	0.28769	0.068751	0.005349	0.01672	0.001125	0.121002	0.009414
10O	-0.55	-0.548	-0.5562	0.006024	0.001955	0.00146	0.000411	0.010602	0.003441
11C	0.27149	0.302	0.26658	0.030891	0.004908	0.00751	0.001032	0.054368	0.008638
12O	-0.554	-0.52	-0.5536	0.031519	-0.00052	0.00766	-0.00011	0.055473	-0.00091
13C	0.279	0.353	0.2587	0.074149	0.020656	0.01804	0.004345	0.130502	0.036355
14C	0.395	0.420	0.40849	0.024858	-0.01309	0.00604	-0.00275	0.04375	-0.02303
15C	0.047	0.059	0.06933	0.011817	-0.02186	0.00287	-0.0046	0.020798	-0.03847
16C	0.294	0.315	0.2678	0.021284	0.026618	0.00517	0.005599	0.03746	0.046848
17C	0.022	0.054	0.00306	0.031907	0.019167	0.00776	0.004032	0.056156	0.033734
18C	0.010	-0.001	0.01298	-0.01268	-0.0022	-0.0030	-0.00046	-0.02231	-0.00386
19C	-0.01	0.044	-0.0278	0.055228	0.016756	0.01343	0.003525	0.097201	0.029491
20C	0.005	0.027	-0.0038	0.022553	0.008951	0.00548	0.001883	0.039693	0.015754
21C	0.006	0.0275	-0.0015	0.020817	0.008228	0.00506	0.001731	0.036638	0.014481
22O	-0.190	-0.154	-0.2168	0.035918	0.026314	0.00873	0.005535	0.063216	0.046313
23O	-0.560	-0.497	-0.5752	0.062463	0.014864	0.01519	0.003127	0.109935	0.026161
24C	0.388	0.397	0.26432	0.009203	0.124008	0.00223	0.026085	0.016197	0.218254
25C	-3E-05	-0.001	0.01781	-0.00192	-0.01784	-0.0004	-0.00375	-0.00337	-0.03139
26C	0.311	0.320	0.14048	0.00864	0.171448	0.00210	0.036064	0.015206	0.301748
27C	0.028	0.040	-0.0714	0.012704	0.099644	0.00309	0.02096	0.022359	0.175373
28C	0.009	0.005	0.0366	-0.00382	-0.02687	-0.0009	-0.00565	-0.00673	-0.0473
29C	0.001	0.014	-0.0917	0.013765	0.09287	0.00334	0.019535	0.024226	0.163451
30C	0.008	0.015	-0.0444	0.007003	0.052547	0.00170	0.011053	0.012325	0.092483
31C	0.008	0.013	-0.0373	0.005645	0.045427	0.00137	0.009556	0.009935	0.079952
32O	-0.180	-0.167	-0.2887	0.013449	0.108193	0.00327	0.022758	0.02367	0.19042
33O	-0.555	-0.555	-0.72055	-0.00021	0.16524	-0.00139	0.034758	-0.00037	0.290822

Analytical analysis of bisdimedone derivatives, 3a-3k

2,2'-((2,3,4-trimethoxyphenyl)methylene)bis(3-hydroxy-5,5- dimethylcyclohex-2-enone) (3a)

The pure compound **3a** was obtained as a white crystalline solid, TLC solvent: Ethyl acetate: Hexane (40:60), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3200, 3330, 2900, 2960, 1660, 1095, 1160, 1130, 950. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 7.268 (1H, s, CH), 7.00 (1H, d, Ar-H), 6.54 (1H, d, J = 8.4 Hz, Ar-H), 3.82 (9H, s, OCH$_3$), 2.18 (4H, dd, CH$_2$), 2.437(4H, s, CH$_2$), 1.05(12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm):196.85, 162.74, 153.57, 141.84, 128.98, 125.70, 114.89, 106.44, 60.71, 55.94, 51.13, 41.20, 32.34, and 28.55. ESI-MS *m/z* 459 [M+Na]$^+$; Anal. Calcd. for $C_{26}H_{34}O_7$: C 68.10, H 7.47, O 24.42.

2,2'-((4-hydroxy-3-methoxyphenyl)methylene)bis (3-hydroxy-5,5-dimethylcyclohex-2-enone) (3b)

The pure compound **3b** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (35:65), Rf value: 0.54; mp 163-165°C; IR (KBr; υ_{max}, cm^{-1}) 3200, 3330, 2900, 2960, 1660, 1095, 1160, 1130, 950. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.986 (2H, s, OH), 6.57-7.27(3H, m, Ar-H), 5.49 (1H, s, CH), 3.77 (9H, s, OCH$_3$), 2.35-2.42 (8H, m, CH$_2$), 1.17(12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 190.61, 189.56, 146.46, 143.73, 129.92, 119.68, 115.97, 114.23, 109.93, 55.84, 47.26, 46.57, 32.50, 31.44, 30.08, and 27.27; ESI-MS *m/z* 415 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{30}O_6$: C 69.54, H 7.30, O 23.16.

2,2'-((3,4-dihydroxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3c)

The pure compound **3c** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (40:60), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3250, 3130, 2950, 2900, 1650, 1095, 1160, 1130. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.80 (2H, s, OH), 6.82-6.95(3H,m, Ar-H), 6.04 (1H, s, CH), 5.35(1H, s, OH), 5.32(1H, s, OH), 1.88-2.14 (8H, m, CH$_2$), 0.99 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 198.91, 180.81, 145.56, 144.43, 136.29, 116.18, 115.27, 108.83, 109.93, 51.54, 46.76, 46.78, 31.50, 31.01, 27.56; ESI-MS *m/z* 401 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{28}O_6$: C 68.98, H 7.05, O 23.97.

2,2'-((4-(benzyloxy)phenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3d)

The pure compound **3d** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (40:60), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3350, 2970, 2915, 1700, 1690, 1525, 1490, 1205, 1170, 1150(C-O). ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.80 (2H, s, OH), 7.48 (2H, m, Ar-H), 7.38(3H, m, Ar-H), 7.12(2H, m, Ar-H), 6.87 (2H, m, Ar-H), 6.04(1H, s, CH), 5.36(2H, s, CH$_2$), 1.42-1.11 (8H, m, CH$_2$), 0.97 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 200.11, 197.56, 156.90, 136.70, 130.00, 128.67, 128.29, 127.60, 127.13, 114.27, 70.80, 59.88, 52.54, 50.30, 33.70, 25.52; ESI-MS *m/z* 475 [M+H]$^+$; Anal. Calcd. for $C_{30}H_{34}O_5$: C 75.92, H 7.22, O 16.86.

2,2'-((3,4,5-trimethoxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3e)

The pure compound **3e** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (30:70), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3250, 3230, 2800, 2800, 1700, 111, 1160, 1050, 930. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.50 (2H, s, OH), 6.45 (1H, s, Ar-H), 6.30 (1H, s, Ar-H), 5.90 (1H, s, CH), 3.99 (9H, s, CH3), 1.97-2.30 (8H, m, CH$_2$), 1.20 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 196.81, 178.82, 152.86, 136.50, 135.20, 108.80, 106.48, 60.80, 56.14, 51.56, 48.78, 31.50, 30.01, and 25.36, ESI-MS *m/z* 459 [M+H]$^+$; Anal. Calcd. for $C_{26}H_{34}O_7$: C 68.10, H 7.47, O 24.42.

2,2'-((4-hydroxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3f)

The pure compound **3f** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (30:70), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3350, 3230, 2940, 2820, 1690, 1065, 1060, 930. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.80 (2H, s, OH), 7.02-6.65(4H,m, Ar-H), 5.75(1H, s, OH), 6.10 (1H, s, CH), 1.68-1.94 (8H, m, CH$_2$), 1.49 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 188.91, 170.61, 155.56, 137.43, 136.29, 130.18, 115.27, 50.44, 45.56, 34.50, 30.51, 29.56. ESI-MS *m/z* 385 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{28}O_5$: C 71.85, H 7.34, O 20.81.

2,2'-((3-bromophenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3g)

The pure compound **3g** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (30:70), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3400, 2900, 2850, 1640, 1465, 1450, 630 (C-Br). ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 7.17-7.41(4H, m, Ar-H), 6.05(1H, s, CH), 1.88-1.94 (8H, m, CH$_2$), 0.98 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 185.91, 175.61, 140.56, 133.43, 129.29, 128.18, 126.27, 123.56, 51.54, 46.70, 34.50, 30.90, 26.30; ESI-MS *m/z* 448 [M+H]$^+$; Anal. Calcd. for $C_{23}H_{27}BrO_4$: C 61.75, H 6.08, Br 17.86, O 14.31.

2,2'-((4-methoxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3h)

The pure compound **3h** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (25:75), Rf value: 0.54; **mp 163-165R°C;** IR (KBr; υ_{max}, cm^{-1}) 3300, 2950, 2800, 1650,1645, 1455, 1150(C-O). ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 10.90 (2H, s, OH), 7.17 (2H, d, Ar-H), 6.87 (2H, d, Ar-H), 6.05(1H, s, CH), 3.33(3H, s, CH$_3$), 1.88-1.94 (8H, m, CH$_2$), 0.98 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 185.91, 175.61, 140.56, 133.43, 129.29, 128.18, 126.27, 123.56, 51.54, 46.70, 34.50, 30.90, 26.30. ESI-MS *m/z* 399 [M+H]$^+$; Anal. Calcd. for $C_{24}H_{30}O_5$: C 72.34, H 7.59, O 20.07.

2,2'-((4-(benzyloxy)-3-methoxyphenyl)methylene)bis(3-hydroxy-5,5 dimethylcyclohex-2-enone) (3i)

The pure compound **3i** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (40:60), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3400, 2900, 2950, 2810, 1680, 1650, 1355, 1100, 1050(C-O). ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.00 (2H, s, OH), 7.38 (3H, m, Ar-H), 7.47 (2H, m, Ar-H), 6.76 (3H, m, Ar-H), 6.04(1H, s, CH), 5.16(1H, s, CH), 3.83(3H, s, CH$_3$), 1.60-1.84 (8H, m, CH$_2$), 0.88 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 198.91, 180.61, 149.56, 146.78, 135.43, 136.76, 128.29, 127.18, 122.47, 112.56, 75.90, 57.78, 52.54, 47.70, 31.50, 27.50. ESI-MS *m/z* 505 [M+H]$^+$; Anal. Calcd. for $C_{31}H_{36}O_6$: C 73.79, H 7.19, O 19.02.

2,2'-((2-bromo-4,5-dimethoxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3j)

The pure compound **3j** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (25:75), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3300, 3100, 3010, 2800, 1650, 1670, 1435, 1230, 1105, 660. ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 11.05 (2H, s, OH), 7.01 (1H, s, Ar-H), 6.79 (1H, s, Ar-H), 6.14(1H, s, CH), 3.99(3H, s, CH$_3$), 3.85(3H, s, CH$_3$), 1.70-1.99 (8H, m, CH$_2$), 1.02 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 198.91, 180.61, 148.57, 147.70, 134.43, 120.25, 117.28, 116.41, 56.68, 46.80, 35.70, 30.33; ESI-MS *m/z* 508 [M+H]$^+$; Anal. Calcd. for $C_{25}H_{31}BrO_6$: C 59.8, H 6.16, Br 15.75, O 18.92.

2,2'-((3,4-dimethoxyphenyl)methylene)bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (3k)

The pure compound **3k** was obtained as a white crystalline solid. TLC solvent: Ethyl acetate: Hexane (35:65), Rf value: 0.54; mp 163-165R°C; IR (KBr; υ_{max}, cm^{-1}) 3300, 2915, 2710, 1680,1675, 1535, 1050, 1010(C-O). ^{1}H (CDCl$_3$, 300 MHz; δ, ppm): 12.05(2H, s, OH), 6.95 (1H, s , Ar-H), 6.68-6.76 (2H, m , Ar-H), 6.90 (1H, s, CH), 4.01 (6H, s, CH3), 1.45-2.10 (8H, m, CH$_2$), 1.05 (12H, s, CH$_3$). ^{13}C (CDCl$_3$, 75 MHz; δ, ppm): 190.95, 185.51, 145.65, 132.12, 130.79, 122.28, 121.22, 121.53, 55.45, 48.90, 31.90, 32.40, 21.10; ESI-MS *m/z* 429 [M+H]$^+$; Anal. Calcd. for $C_{25}H_{32}O_6$: C 70.07, H 7.53, O 22.40.

Conclusions

In conclusion, using green solvents ethanol, water and p-cymene, bisdimedone derivatives were synthesized easily in short reaction time with excellent yield (~90%). Also, the quantum chemical calculations, using DFT and QTAIM analyses, have been performed on one of the derivatives, 2,2'-[(2,3,4-trimethoxyphenyl)methylene] bis(3-hydroxy-5,5-dimethylcyclohex-2-enone) (**3a**) to investigate their bonding interactions and electronic parameters in an attempt to elucidate their chemical reactivity. The calculated results showed that reactivity descriptors based on DFT calculations consistently predict the higher chemical reactivity of compound **3a**.

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References

1. Mark S., Bagrat S., Pnina K., Igor U., Nina C. and Larisa O., 2011, *J. Mol. Struct.*, **1006**, 234.
2. Atar A.B. and Jeong Y.T., 2013, *Tetrahedron Lett.*, **54,** 1302.
3. Mosaddegh E. and Hassankhani A., 2012, *Arabian J. Chem.*, **5**, 315–318
4. Kassaee M.Z. and Bekhradnia A. R., 2003, *J. Biosci. Bioeng.*, **95**, 526.
5. Azizi S.N., Chaichi M.J., Shakeri P. and Bekhradnia A., 2013, *J. Fluoresc.*, **23**, 227.
6. Shafiee A., Kassaee M.Z. and Bekhradnia A.R., 2007, *J. Heterocycl. Chem.*, **44**, 471.
7. Khan K.M., Maharvi G.M., Khan M.T.H., Shaikh A.J., Perveen S., Begum S. and Choudhary, M.I., 2006, *Bioorg. Med. Chem.*, **14**, 344.
8. Ilangovan A., Malayappasamy S., Muralidharan S. and Maruthamuthu S., 2011, *Chem. Cent. J.*, **5**, 81.
9. Nemati F., Heravi M.M. and Saeedi-Rad R., 2012, *Chin. J. Catal.*, **33,** 1825.
10. Jung D.H., Lee Y.R., Kim S.H. and Lyoo W.S., 2009, Bull. *Korean Chem. Soc.*, **30, 50,** 1989.
11. Rao V.K., Kumar M.M. and Kumar A., 2011, *Indian J. Chem. Sec. B: Org. Incl. Med.*, 1128.
12. Khurana J. and Vij K., 2012, *J. Chem. Sci.*, **124,** 907.
13. Tajbakhsh M., Heidary M. and Hossseinzadeh R., 2016, *Res. Chem. Intermed.*, **42**, 1425.
14. Rastroshan M., Sayyahi S., Zare-Shahabadi V. and Badri R., 2012, *J. Iranian Chem. Res.*, 5, 265.
15. Paggiola G., Hunt A.J., McElroy C.R., Sherwood J. and Clark J.H., 2014, *Green Chem.*, **16**, 2107–2110.
16. Gilanizadeh M. and Zeynizadeh B., 2018, *New J. Chem.*, DOI: 10.1039/C8NJ00788H
17. Sharma S., Brahmachari G., Banerjee B., Nurjamal K., Kumar A., Srivastava A.K., Misra N., Pandey S.K., Rajnikant V.K., Gupta, 2016, *J. Mol. Struct.*, **1118,** 344. doi:10.1016/*J.MOLSTRUC*.2016.04.038
18. Srivastava A.K., Pandey S.K. and Misra N., 2016, *Chem. Phys. Lett.*, **71**, 655–656. doi:10.1016/ J.CPLETT.2016.05.039
19. Kageyama, T., Uneme, S., Takase, M., Nomura, K.

and Nishinaga, T., 2014, *Aust. J. Chem.*, **67**, 722. doi:10.1071/CH13522

20. Geetha K., Rekha T.N., Umadevi M., Rajkumar B.J.M., Sathe G.V., Vanelle P., Terme T. and Khoumeri O., 2015, *Aust. J. Chem.*, **69,** 76. doi:10.1071/CH15161

21. Vovusha H., Sanyal B., 2015, *RSC Adv.*, **5**, 67427. doi:10.1039/ C5RA14664J

22. Computer program Gauss View 3.09, Ver. 2, Gaussian Inc., Pittsburgh, PA.

23. Frisch E., Hratchian H.P., Dennington R.D.II, Keith T.A., Millam J., Nielsen B., Holder A.J., Hiscocks. J. and Gaussian, Inc. GaussView Version 5.0.8, 2009.

24. Shahab S., Kumar R., Darroudi M., Borzehandani M.Y., 2015, *J. Mol. Struct.*, **1083**, 198-203.

25. Cremer D., Kraka E., 1984, *Croat. Chem. Acta,* **57,** 1259

26. Rozas I., Alkorta I. and Elguero J., 2000, *J. Am. Chem. Soc,* **122,** 11154. doi:10.1021/JA0017864

27. Politzer P. and Murray J.S., Theoretical biochemistry and molecular biophysics: a comprehensive survey in: D. L. Beveridge, R. Lavery Eds., 1991

28. Guo L. J., Wei C. X., Jia J. H., Zhao L. M. and Quan Z. S., 2009, *Eur. J. Med. Chem*., **44** 954–958

29. Bawa S., Kumar S., Drabu S. and Kumar R., 2010, *Pharm. Bioallied Sci.*, **2**, 64–71.

30. Zhao S., Zhou W. and liu J., 2009**,** *Bioorg Med Chem.*, **17**, 7915–7923

31. Green G.R., Evans J.M., Vong A.K., Katritzky A.R., Rees C.W. and Scriven E.F.V., **5**, 469, Pergamon Press, Oxford, UK, 1995

32. Abdelrazek F.M., Metz P., Kataeva O., Jager A. and El-Mahrouky S.F., 2007, *Arch. Pharm.***, 340,** 543–548

33. Sajan D., Josepha L., Vijayan N. and Karabacak M., 2011, *Spectrochim. Acta, Part A.*, **81,** 85-98

34. R.P. Singh, Kant, R., Singh, K., Sharma, S. and Sethi, A., **2015**, *J. Mol. Structure,* **1095,** 125-134.

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Possibilities of Accumulations of Atomic Fuel without the Use of Physical Methods such as Ultracentrifugation and Diffusion

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Abstract

The Tokyo Electric Power Company's, Fukushima Dai-ichi nuclear power plant in Fukushima-Ken (Fukushima Prefecture), Japan, was destroyed in March 2011 by a massive magnitude 9 earthquake (centred offshore to the northeast of Honshu Island) and by the subsequent historic tsunami of March 11, 2011. Because of the nuclear meltdown, hydrogen-explosion damage to the buildings that housed the reactors, and the contamination of the cooling water from the reactor cores, large quantities of radioisotopes were emitted into the atmosphere and adjacent seawater. Fishing is currently restricted off the coast of Fukushima-Ken because intermittent surveys have found part of the fishery products still contain high levels of radioisotopes. The Tokyo University of Marine Science and Technology has measured radioisotope levels in fishery species off Iwaki-Shi, Fukushima-Ken and this data could be used to understand the relationship between the accumulation of specific nuclides (radioisotopes) and certain species of fish. Some fish species have the ability to accumulate a specific nuclide (radioisotope). To date, ultra-centrifugation and diffusion methods have been used to accumulate specific nuclides for atomic fuel. However, if we could use the ability of some fish species to accumulate specific nuclides, we would have additional methods to concentrate nuclides.

Introduction

The Tokyo Electric Power Company's Fukushima Dai-ichi nuclear power plant, located in Futaba-Gun, Fukushima-Ken, Japan, was destroyed in March 2011 by a massive magnitude-9 earthquake (centred offshore to the northeast of Honshu Island) and by the subsequent historic tsunami on March 11, 2011. Because of the resulting nuclear meltdown, the hydrogen explosion damage to the buildings that housed the reactors, and the contamination of the cooling water from the reactor cores, large quantities of radioisotopes were emitted into the atmosphere and the adjacent seawater. Fishing was restricted off the coast of Fukushima-Ken because intermittent surveys had found that some fishery products still contained radioisotope levels that exceeded the Japanese Standard Value. The Japanese government had measured radioisotope levels in fishery species offshore not only near the destroyed reactors but also throughout the Japanese Exclusive Economic Zone (EEZ). The Japanese government had regularly released a subset of these measurements to the public via the internet and other media[1,2,3,4]. Despite the ample funds provided by Japanese taxpayers, the reports did not contain detailed measurements of individual fishes, such as their weight, sex, length, and collection site, or measured radioisotope levels in fishery species. Therefore, from November 22-23, 2012, the Tokyo University of Marine Science and Technology independently sampled radioisotope levels in fishery species off Iwaki-Shi, Fukushima-Ken, just south of the former nuclear power plant. These data included detailed measurements of individual fish, such as weight, sex, length, and collection site; these data can be used to better understand the accumulation of radioisotopes in fishes. "Feminam *Okamejei kenojei* (English name: Common skate; Japanese name: Komon-Kasube-No-Mesu) had a negative linear relationship between fish weight and $^{137}_{Cs}/^{134}_{Cs}$ ratio, indicating the ability to accumulate a specific nuclide"; this finding was reported based on the sampling conducted offshore of Fukushima-Ken, Japan in November 2012[5]. Despite the large amount of data collected and the research funds provided by the Ministry of Environment Japan, there have been no additional reports on other characteristics or results of the accumulation of specific nuclides by specific fish species. This report provides new insights into the accumulation of specific nuclides by specific fish species, based on the same sampling as indicated in Katsura's report[5].

Materials and Methods

To sample local fish, trawl (dragnet) fishing was conducted on November 22, 2012, off Yotsukura and Ena, Iwaki-Shi, Fukushima-Ken, Japan, in a total fishing area of 9,450 m^2 and 9,775 m^2, respectively[6]. Gill-net fishing was conducted from November 22-23, 2012, in the same location, with a total fishing area of 28,900 m^2 and 50,141 m^2 off Yotsukura and Ena, respectively[7]. The edible portion of the sampled fish was minced and placed into 100 mL plastic containers (U-8 containers). The concentrations of $^{134}_{Cs}$ and $^{137}_{Cs}$ radioisotopes in the fish biomass were measured by IDEA Consultants, Inc., Tokyo, Japan, using a germanium semiconductor detector (Seiko EG&G Co. Model GEM20-70)[8,9].

Results and Discussion

1) The total fish collection area for November 22-23, 2012, as shown in Table 1, was calculated as follows[10]:
   9,450+28,900+9,775+50,141= 98,266 m^2

2) Table 2 shows the following:
i) [Percentage of Total Fish Weight]=[%TFW] is the percentage of the weight of each species of fish $^{134}_{Cs}$with respect to the total fish weight sampled.
ii) ([$^{134}_{Cs}$ per Fish Species]/[Total $^{134}_{Cs}$ for All Species]) x 100 = ([$^{134}_{Cs}$] / [T$^{134}_{Cs}$]) x 100 = [$^{134}_{Cs}$%] is the percentage of $^{134}_{Cs}$ becquerel from each fish species of the total $^{134}_{Cs}$ becquerel for all sampled fish species. Bq is the symbol for "becquerel", an SI-derived unit of radioactivity. One Bq is defined as the activity of a quantity of radioactive material in which one nucleus decays per second[11].
iii) ([$^{137}_{Cs}$ per Fish Species]/[Total $^{137}_{Cs}$ All Species]) x100 = ([$^{137}_{Cs}$] / [T$^{137}_{Cs}$]) x 100 = [$^{137}_{Cs}$%] is the percentage of $^{137}_{Cs}$ becquerel from each fish species of the total $^{137}_{Cs}$ becquerel for all sampled fish species.
iv) The total fish weight % of *Okamejei kenojei* (Common skate; Komon Kasube) and *Sebastes cheni* (Japanese rockfish, Japanese sea perch; Shiro-Mebaru) in this study were 26.6824% and 13.700005%, respectively. The % of $^{134}_{Cs}$ becquerels from *Okamejei kenojei* and *Sebastes cheni* in this

study were 49.322578% and 33.037159%, respectively, and the % of $^{137}_{Cs}$ becquerels from *Okamejei kenojei* and *Sebastes cheni* in this sampling in offshore Fukushima-ken, Japan in November 2012 were 50.479187% and 31.779293%, respectively (Figs. 1 and 2).

The accumulation ratio of the percentage of 134_{Cs} becquerels to the weight percentage in *Okamejei kenojei* was: $[^{134}_{Cs}\text{okame}] = [^{134}_{Cs}\%] / [\%TFW] = ((49.322578\%\ Bq) / (26.6824\ W\%\ g)) \times 100 = 1.8485$

The accumulation ratio of the percentage of $^{137}_{Cs}$ becquerels to the weight percentage in *Okamejei kenojei* was $[^{137}_{Cs}\text{okame}] = [^{137}_{Cs}\%] / [\%TFW] = ((50.479187\%\ Bq) / (26.6824\ W\%\ g)) \times 100 = 1.92168$

The accumulation ratio of the percentage of $^{134}_{Cs}$ becquerels to the weight percentage in *Sebastes cheni* was:
$[^{134}_{Cs}\text{Seba}] = [^{134}_{Cs}\%] / [\%TFW] = ((33.037159\%\ Bq)/(13.700005)$

The accumulation ratio of the percentage of $^{137}_{Cs}$ becquerels to the weight percentage in *Sebastes cheni* was:
$[^{137}_{Cs}\text{Seba}] = [^{137}_{Cs}\%] / [\%TFW] = ((31.779293\%\ Bq)/(13.700005$

Therefore, *Okamejei kenojei* can accumulate 1.8485 times the percentage of their weight in the total fish sample of $^{134}_{Cs}$ and 1.92168 times the percentage of their weight of $^{137}_{Cs}$. *Sebastes cheni* can accumulate 2.411 times the percentage of their weight in the total fish sample of $^{134}_{Cs}$ and 2.3195 times the percentage of their weight of $^{137}_{Cs}$.

If the seawater concentrations of $^{134}_{Cs}$ and $^{137}_{Cs}$ were equal and constant, *Okamejei kenojei* sp. and *Sebastes cheni* would accumulate these radioisotopes to the concentrations calculated above.

3) Tables 3 and 4 show the proportion of $^{134}_{Cs}$ and $^{137}_{Cs}$ accumulated by each fish species based on their percentage of the total fish weight in descending order, as follows:

$[^{137}_{Cs}\%] / [\%TFW] \times 100$
$[^{137}_{Cs}\%] / [\%TFW] \times 100$

If there was a proportional relationship between the amount of $^{134}_{Cs}$ or $^{137}_{Cs}$ Bq (i.e., the quantity of $^{134}_{Cs}$ or $^{137}_{Cs}$ radioactive material) in the sampled fish body weight regardless of species, the percentages of $^{134}_{Cs}$ or $^{137}_{Cs}$ Bq per percentage of total fish weight would always be 100%. However, these percentages vary depending on the fish species, from 0 to 242.855% for $^{134}_{Cs}$ and from 0 to 231.9655554% for $^{137}_{Cs}$.

The values of $[^{137}_{Cs}\%] / [\%TFW] \times 100$ and $[^{137}_{Cs}\%]/[\%TFW] \times 100$ for the following species are 0%: *Acanthopagrus schlegelii* (Japanese black seabream; Kurodai), *Clupea pallasii* Valenciennes (Pacific herring; NISHIN), *Engraulis japonica* (Japanese anchovy; Katakuchi Iwashi), *Lepidotrigla microptera* Gunther (Gurnard, Sea-robin; Kanagashira), *Liparis tanakae* (English name not available=N/A; Kusauo), *Mustelus manazo* (Starspotted smooth hound; Hoshi-Zame), *Oncorhynchus keta* (Chum salmon, Salmon; Sake), *Oplegnathus punctatus* (Spotted knifejaw; Ishigakidai), *Pagrus major* (Red seabream; Madai (Seigyo)), *Pagrus major (fry)* (Red seabream (fry); Madai (Chigyo)), *Paralichthys olivaceus (fry)* (Bastard halibut (fry); Hirame (Chigyo)), *Platycephalus sp.* (Flathead; Magochi), *Platycephalus sp. (fry)* (Flathead (fry); Magochi (Chigyo)), *Takifugu poecilonotus* (Pufferfish; Name: Komon Fugu), *Takifugu snyderi* (Globefish, Blowfish, Pufferfish; Shousai Fugu), *Takifugu stictonotus* (Globefish, Blowfish, Puffer; Goma Fugu), *Trachurus japonicus* (Japanese jack mackerel. Japanese horse mackerel, Japanese scad; Maaji) and *Zeus faber* Linnaeus (John Dory; Matou Dai). These findings indicate that these fish species do not have the ability to accumulate $^{134}_{Cs}$ and $^{137}_{Cs}$ radioisotopes. In other words, these species eliminate $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ radioisotopes from their bodies.

4) Tables 3 and 4 show the differences in the $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ accumulation ratio by fish species; some species accumulate more $^{134}{}_{Cs}$, whereas other species accumulate more $^{137}{}_{Cs}$.

Thus, the order of the values of ([$^{134}{}_{Cs}$%] / [%TFW]) x 100% and ([$^{137}{}_{Cs}$%] / [%TFW]) x 100% can be reversed. Specifically, the species and values have the following features.

i) The value of ([$^{137}{}_{Cs}$%] / [%TFW]) x 100% for *Sebastes cheni* (Japanese rockfish, Japanese sea perch; Shiro Mebaru) is higher than that for *Kareius bicoloratus* (Stone flounder; Ishigarei). However, the value of ([$^{134}{}_{Cs}$%] / [%TFW]) x 100% for *Kareius bicoloratus* is higher than that for *Sebastes cheni*. Therefore, it may be possible to use *Sebastes cheni* and *Kareius bicoloratus* for the separation or accumulation of $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ nuclides.

Sebastes cheni shows higher weight% accumulation of $^{137}{}_{Cs}$ than *Kareius bicoloratus*. However, *Kareius bicoloratus* shows higher weight % accumulation of $^{134}{}_{Cs}$ than *Sebastes cheni*.

Therefore, it may be possible to use *Sebastes cheni* and *Kareius bicoloratus* for the accumulation or separation of the specific nuclides $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$.

ii) The value of ([$^{137}{}_{Cs}$%] / [%TFW]) x 100% for *Ditrema temminckii* (Surfperch; Japanese Name: Umitanago) is higher than that for *Cynoglossus joyneri* (Red tongue sole; Akashita Birame) and the value of ([$^{134}{}_{Cs}$%] / [%TFW]) x 100% for *Cynoglossus joyneri* is higher than that for *Ditrema temminckii*. Therefore, it may be possible to use *Ditrema temminckii* and *Cynoglossus joyneri* for the separation or accumulation of $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ nuclides. *Ditrema temminckii* shows higher weight % accumulation of $^{137}{}_{Cs}$ than *Cynoglo-ssus joyneri*. However, *Cynoglossus joyneri* shows higher weight% higher accumulation of $^{134}{}_{Cs}$ than *Ditrema temminckii*. Therefore, it may be possible to use *Ditrema temminckii* and *Cynoglossus joyneri* for the accumulation or separation of the specific nuclides $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$.

5) Tables 3 and 4 show the differences in accumulation ratio between adult fish and fry of the same species.

i) *Pagrus major* (Adult Fish) (Red seabream (Adult Fish); Madai (Seigyo)), *Pagrus major* (Fry) (Red seabream (Fry); Madai (Chigyo)), *Platycephalus* sp. (Adult Fish) (Flathead (Adult Fish); Magochi (Seigyo)) and *Platycephalus* sp. (fry) (Flathead (Fry); Magochi (Chigyo)) do not have the ability to accumulate either $^{134}{}_{Cs}$ or $^{137}{}_{Cs}$. Additionally, these do not appear to be any differences between these adult fish and fry in the accumulation of $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$.

ii) *Paralichthys olivaceus* (Adult Fish) (Bastard halibut (Adult Fish); Hirame (Seigyo)) has the ability to accumulate both $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$; however, *Paralichthys olivaceus* (Fry) (Bastard halibut (Fry); Hirame (Chigyo)) does not have the ability to accumulate either $^{134}{}_{Cs}$ or $^{137}{}_{Cs}$. There are differences between adult fish and fry of *Paralichthys olivaceus* in terms of the accumulation of $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$.

6) Specific fish species are known to have the ability to accumulate specific elements, ions and molecules[12]. However, the accumulation of specific radioisotopes had not been reported so far[5].

7) *Okamejei kenojei* (Common skate; Komon Kasube) can detect weak electrical signals, which enables fish of this species to receive electrical information on the position of their prey, the drift of ocean currents and their magnetic compass heading. The radioisotopes $^{137}{}_{Cs}$ and $^{137}{}_{Cs}$ are β-emitting nuclides and emit β- and γ-rays. A β-ray is an electron beam or electric current, and a γ-ray is a radio or electro-

magnetic wave of small wavelength [13,14,15,16,17]. This may be related to the ability of this species to accumulate specific radioisotopes, although to date, there is no theoretical explanation for nuclide accumulation in *Okamejei kenojei*. However, it is not possible to explain why specific radioisotopes (nuclides) accumulate in these species of fish. The disaster at Fukushima on March 11, 2011 provides the opportunity to gain new insight into the accumulation of specific radioisotopes by fishes. We must discover the theoretical reasons for these phenomena in order to use fishes as a new method for atomic fuel production and for the clean-up of specific radioisotope contamination.

Conclusions

1) The total fish weight % of *Okamejei kenojei* (Common skate; Komon Kasube) and *Sebastes cheni* (Japanese rockfish, Japanese sea perch; Shiro Mebaru) in this sampling in offshore Fukushima-ken, Japan were 26.6824% and 13.700005%, respectively; additionally, *Okamejei kenojei* and *Sebastes cheni* generated 49.322578% and 33.037159% of $^{134}{}_{Cs}$ becquerels, respectively, and generated 50.479187% and 31.779293% of $^{137}{}_{Cs}$ becquerels, respectively. Therefore, *Okamejei kenojei* has the ability to accumulate 1.8485 times its weight % of $^{134}{}_{Cs}$ and 1.92168 times its weight % of $^{137}{}_{Cs}$. However, *Sebastes cheni* has the ability to accumulate 2.411 times its weight % of $^{134}{}_{Cs}$ and 2.3195 times its weight % of $^{137}{}_{Cs}$. Several fish species are able to eliminate $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ radioisotopes.

2) It is possible to accumulate or separate specific radioisotopes ($^{134}{}_{Cs}$ or $^{137}{}_{Cs}$) by combining the following fish species: *Sebastes cheni* (Japanese rockfish, Japanese sea perch; Shiro-Mebaru) with *Kareius bicoloratus* (Stone flounder; Ishigarei) and *Ditrema temminckii* (Surfperch; Umitanago) with *Cynoglossus joyneri* (Red tongue sole; Akashita Birame).

3) Neither *Pagrus major* (Red seabream; Madai) nor *Platycephalus* sp. (Flathead; Magochi) adult fish and fry accumulated $^{134}{}_{Cs}$ or $^{137}{}_{Cs}$. There were differences in the accumulation of $^{134}{}_{Cs}$ and $^{137}{}_{Cs}$ between adult fish and fry of *Paralichthys olivaceus* (Bastard halibut; Hirame).

4) Physical methods such as ultracentrifugation and diffusion have been used to obtain high concentrations of nuclides (e.g., 235 U). This study suggests that physical methods are not required to accumulate high concentrations of specific radioisotopes.

Table 1: Total fish collection area on 22-23 November, 2012

		YOTSUKURA	YOTSUKURA	ENA	ENA (Land Side)	
		Trawl (Net) [Dragneta Trawlnet] (HIKIAMI)	Gill Net (SASHIAMI)	Trawl (Net) [Dragneta Trawlnet] (HIKIAMI) (2 Times)	Gill Net (SASHIAMI)	
	Sampling date for weight of fishes	22-Nov-2012	22 to 23 Nov. 2012	22-Nov-2012	22 to 23 NOV-2012	Total Fish Collecting Area in 22 to 23 NOV-2012 [m2] = [TFCA]
Area [m^2]		9450	28900	9775	50141	98266

Table 2 The Amount of Fishery Resources and the Total Amount of Cesium Radioisotopes in Fish Bodies in Offshore Fukushima-Ken, Japan in NOV2012

Scientific Name	Japanese Name	Weight [g] at YOTSUKURA by Trawl (Net)	Weight % at YOTSUKURA by Trawl (Net)	Weight [g] at YOTSUKURA by Gill Net	Weight % at YOTSUKURA by Gill Net	Weight [g] at ENA by Trawl (Net) (2 Times)	Weight % at ENA by Trawl (Net) (2 Times)	Weight [g] at ENA (Land Side) by Gill Net	Weight % at ENA (Land Side) by Gill Net	Weight [g] at ENA (Offshore Side) by Gill Net	Weight % at ENA (Offshore Side) by Gill Net	Each Species of Total Fish Weight [g]	The percentage that each species of fish has with respect to the total fish sampled = [%TFW]	(134Cs per Fish Species] [becquerel]/[kg] = [134Cs]	([134Cs per Fish Species]/[Total 134Cs for All Species])x100 [%] = [134Cs%]	(137Cs per Fish Species) [becquerel]/[kg] = [137Cs]	([137Cs per Fish Species]/[Total 134Cs for All Species])x100 [%] =[137Cs%]
Acanthopagrus schlegeli	KURODAI	2000	1.456261195	0	0	0	0	0	0	0	0	2000	0.743555235	0	0	0	0
Chelidonichthys spinosus	HOUBOU	0	0	1170	3.549757282	0	0	0	0	0	0	1170	0.434979812	7.015	0.057003374	10.085	0.047709706
Clupea pallasii Vakenciennes	NISHIN	23	0.016747004	0	0	0	0	0	0	0	0	23	0.008550885	0	0	0	0
Cynoglossus joyneri	AKASHITA BIRAME	600	0.436878359	0	0	1810	4.815110402	300	2.284843869	0	0	2710	1.007517343	14.1	0.265384423	22.25	0.243805938
Ditrema temminkii	UMI TANAGO	0	0	0	0	0	0	210	1.599390708	2960	6.171809842	3170	1.178535047	12.445	0.273994086	22.3	0.285830849
Engraulis japonica	KATAKUCHI IWASHI	88	0.064075493	0	0	0	0	0	0	0	0	88	0.03271643	0	0	0	0
Hexagrammos otakii	AINAME	0	0	2130	6.462378641	0	0	1060	8.073115004	1810	3.773978315	5000	1.858888088	52.19230769	1.812439544	88.01538462	1.779399929
Hexagrammos stelleri	EZO ISO AINAME	0	0	0	0	0	0	330	2.513328256	940	1.959966639	1270	0.472157574	21.9	0.193167857	37.36666667	0.191881477
Kareius bicoloratus	ISHI GAREI	0	0	750	2.275485437	920	2.447459431	0	0	0	0	1670	0.620868621	130	1.507810796	213	1.438272074
Lateolabrax japonicus	SUZUKI	0	0	0	0	1300	3.458366587	0	0	0	0	1300	0.483310903	53.9	0.486652706	98.6	0.518280944
Lepidotrigla microptena Gunther	KANA GASHIRA	280	0.203876567	0	0	0	0	0	0	0	0	280	0.104097733	0	0	0	0
Liparis tanakai	KUSAUO	310	0.225720485	0	0	0	0	0	0	0	0	310	0.115251061	0	0	0	0
Microstomus achn	BABA GAREI	0	0	2600	7.888349515	0	0	0	0	850	1.772310259	3450	1.28263278	107.8333333	2.583801526	187.3333333	2.61323899
Mustelus manazo	HOSHI ZAME	50500	36.77059517	0	0	11100	29.52913009	0	0	0	0	61600	22.90150124	0	0	0	0
Nibea mitsukurii	NIBE	0	0	0	0		0	1140	8.682406702	1040	2.168473728	2180	0.810475206	16.66	0.252242647	29.9	0.263555847
Okamejei kenojei	KOMON KASUBE (TSUMARI KASUBE9	56900	41.430631	1390	4.21723301	13480	35.86060122	0	0	0	0	71770	26.68247961	98.95	49.32257817	173.95	50.47918713
Oncorhynchus keta	SAKE	0	0	0	0	0	0	0	0	500	1.042535446	500	0.185888809	0	0	0	0
Oplegnathus punctatus	ISHIGAKI DAI	0	0	640	1.941747573	0	0	0	0	0	0	640	0.237937675	0	0	0	0
Pagrus major	MADAI	0	0	0	0	0	0	0	0	290	0.604670559	290	0.107815509	0	0	0	0
Pagrus major (Fry)	MADAI (CHIGYO)	322	0.234458052	0	0	0	0	0	0	0	0	322	0.119712393	0	0	0	0
Paralichthys olivaceus	HIRAME	1600	1.165008956	6280	19.05339806	5340	14.20590583	0	0	2500	5.212677231	15720	5.844344147	14.98	1.635503132	25.74727273	1.636548003
Paralichthys olivaceus (Fry)	HIRAME (CHIGYO)	34	0.02475644	0	0	0	0	0	0	0	0	34	0.012640439	0	0	0	0
Platycephalus sp.2	MAGOCHI	580	0.422315747	0	0	450	1.197126895	0	0	0	0	1030	0.382930946	0	0	0	0
Platycephalus sp.2 (fry)	MAGOCHI (CHIGYO)	10	0.007281306	0	0	0	0	0	0	0	0	10	0.003717776	0	0	0	0

Scylicrhinus torazame	TORA ZAME	5200	3.786279107	250	0.758495146	3190	8.486299548	0	0	0	0	8640	3.212158615	27.00583333	1.620534759	47.58	1.662199524
Sebastes cheni	SHIRO MEBARU	0	0	200	0.606796117	0	0	8890	67.70753998	27760	57.88156797	36850	13.7000052	129.0857143	33.03715945	213.2857143	31.77929316
Sebastes inermis	AKA MEBARU	0	0	0	0	0	0	0	0	1020	2.12677231	1020	0.37921317	96.175	0.681317261	160.15	0.660498884
Sebastes pachycephalus pachycephalus	MURASOI	0	0	0	0	0	0	330	2.513328256	1760	3.669724771	2090	0.777015221	40.175	0.583161973	70.65	0.597039996
Sebastes vulpes	KITSUNE MEBARU	0	0	1260	3.822815534	0	0	590	4.493526276	6530	13.61551293	8380	3.115496435	32.4	1.885714992	59.068	2.001433931
Sebastiscus marmoratus	KASAGO	0	0	0	0	0	0	280	2.132520944	0	0	280	0.104097733	38.3	0.074480714	67.1	0.075967096
Seriola quinqueradiata	BURI	0	0	1670	5.066747573	0	0	0	0	0	0	1670	0.620868621	4.03	0.046742135	5.09	0.034369976
Takifugu poecilonotus	KOMON FUGU	70	0.050969142	0	0	0	0	0	0	0	0	70	0.026024433	0	0	0	0
Takifugu rubipes	TORA FUGU	1610	1.172290262	0	0	0	0	0	0	0	0	1610	0.598561964	83.9	0.938155571	135	0.878829483
Takifugu snyderi	SHOUSAI FUGU	16200	11.79571568	0	0	0	0	0	0	0	0	16200	6.022797404	0	0	0	0
Takifugu stictonotus	GOMA FUGU	120	0.087375672	0	0	0	0	0	0	0	0	120	0.044613314	0	0	0	0
Trachurus japonicus	MA AJI	887	0.64585184	0	0	0	0	0	0	0	0	887	0.329766747	0	0	0	0
Triakis scyllium	DOCHI ZAME	0	0	14620	44.35679612	0	0	0	0	0	0	14620	5.435388768	27.00583333	2.742154881	47.58	2.812657065
Zeus faber Linnaeus	MATOU DAI	4	0.002912522	0	0	0	0	0	0	0	0	4	0.00148711	0	0	0	0
	Total Fish Weight	137338	0	32960	0	37590	0	13130	0	47960	0	268978					

Table 3: The order value of ([$^{134}{}_{Cs}$%] / [%TFW]) x 100%

Number (Order value of the right is less)	([134$_{Cs}$%] / [%TFW%]) X 100 [%]	Scientific Name	Japanese Name of Species
1	0	*Acanthopagrus schlegeli*	KURODAI
2	0	*Clupea pallasii Vakenciennes*	NISHIN
3	0	*Engraulis japonica*	KATAKUCHI IWASHI
4	0	*Lepidotrigla microptena Gunther*	KANA GASHIRA
5	0	*Liparis tanakai*	KUSAUO
6	0	*Mustelus manazo*	HOSHI ZAME
7	0	*Oncorhynchus keta*	SAKE
8	0	*Oplegnathus punctatus*	ISHIGAKI DAI
9	0	*Pagrus major*	MADAI
10	0	*Pagrus major (Fry)*	MADAI (CHIGYO)
11	0	*Paralichthys olivaceus (Fry)*	HIRAME (CHIGYO)
12	0	*Platycephalus sp.2*	MAGOCHI
13	0	*Platycephalus sp.2 (fry)*	MAGOCHI (CHIGYO)
14	0	*Takifugu poecilonotus*	KOMON FUGU
15	0	*Takifugu snyderi*	SHOUSAI FUGU
16	0	*Takifugu stictonotus*	GOMA FUGU
17	0	*Trachurus japonicus*	MA AJI
18	0	*Zeus faber Linnaeus*	MATOU DAI
19	7.5285065	*Seriola quinqueradiata*	BURI
20	13.104832	*Chelidonichthys spinosus*	HOUBOU
21	23.248701	*Ditrema temminkii*	UMI TANAGO
22	26.340432	*Cynoglossus joyneri*	AKASHITA BIRAME
23	27.984374	*Paralichthys olivaceus*	HIRAME
24	31.122809	*Nibea mitsukurii*	NIBE
25	40.911735	*Hexagrammos stelleri*	EZO ISO AINAME
26	50.450023	*Scyliorhinus torazame*	TORA ZAME
27	50.450023	*Triakis scyllium*	DOCHI ZAME
28	60.526951	*Sebastes vulpes*	KITSUNE MEBARU
29	71.548834	*Sebastiscus marmoratus*	KASAGO

Number (Order value of the right is less)	([134$_{Cs}$%] / [%TFW%]) X 100 [%]	Scientific Name	Japanese Name of Species
30	75.051551	*Sebastes pachycephalus pachycephalus*	MURASOI
31	97.501273	*Hexagrammos otakii*	AINAME
32	100.69144	*Lateolabrax japonicus*	SUZUKI
33	156.73491	*Takifugu rubipes*	TORA FUGU
34	179.66603	*Sebastes inermis*	AKA MEBARU
35	184.85005	*Okamejei kenojei*	KOMON KASUBE
36	201.44515	*MIicrostomus achn*	BABA GAREI
37	241.14706	*Sebastes cheni*	SHIRO MEBARU
38	242.85505	*Kareius bicoloratus*	ISHI GAREI

Table 4: The order value of ([$^{137}{}_{Cs}$%]/[%TFW]) x 100%

Number (Order value of the right is less)	([137$_{Cs}$%] / [%TFW%]) X 100 [%]	Scientific Name	Japanese Name of Species
1	0	*Acanthopagrus schlegeli*	KURODAI
2	0	*Clupea pallasii Vakenciennes*	NISHIN
3	0	*Engraulis japonica*	KATAKUCHI IWASHI
4	0	*Lepidotrigla microptena Gunther*	KANA GASHIRA
5	0	*Liparis tanakai*	KUSAUO
6	0	*Mustelus manazo*	HOSHI ZAME
7	0	*Oncorhynchus keta*	SAKE
8	0	*Oplegnathus punctatus*	ISHIGAKI DAI
9	0	*Pagrus major*	MADAI
10	0	*Pagrus major (Fry)*	MADAI (CHIGYO)
11	0	*Paralichthys olivaceus (Fry)*	HIRAME (CHIGYO)
12	0	*Platycephalus sp.2*	MAGOCHI
13	0	*Platycephalus sp.2 (fry)*	MAKOCHI (CHIGYO)
14	0	*Takifugu poecilonotus*	KOMON FUGU
15	0	*Takifugu snyderi*	SHOUSAI FUGU
16	0	*Takifugu stictonotus*	GOMA FUGU
17	0	*Trachurus japonicus*	MA AJI
18	0	*Zeus faber Linnaeus*	MATOU DAI

Number (Order value of the right is less)	([^{137}Cs%] / [%TFW%]) X 100 [%]	Scientific Name	Japanese Name of Species
19	5.53578884	*Seriola quinqueradiata*	BURI
20	10.96825746	*Chelidonichthys spinosus*	HOUBOU
21	24.19868403	*Cynoglossus joyneri*	AKASHITA BIRAME
22	24.25306309	*Ditrema temminkii*	UMI TANAGO
23	28.00225246	*Paralichthys olivaceus*	HIRAME
24	32.51868101	*Nibea mitsukurii*	NIBE
25	40.63928808	*Hexagrammos stelleri*	EZO ISO AINAME
26	51.74711847	*Triakis scyllium*	DOCHI ZAME
27	51.74711847	*Scyliorhinus torazame*	TORA ZAME
28	64.2412525	*Sebastes vulpes*	KITSUNE MEBARU
29	72.97670554	*Sebastiscus marmoratus*	KASAGO
30	76.83761917	*Sebastes pachycephalus*	MURASOI
31	95.72388683	*Hexagrammos otakii*	AINAME
32	107.2355166	*Lateolabrax japonicus*	SUZUKI
33	146.8234761	*Takifugu rubipes*	TORA FUGU
34	174.1761459	*Sebastes inermis*	AKA MEBARU
35	189.1847679	*Okamejei kenojei*	KOMON KASUBE
36	203.7402311	*MIicrostomus achn*	BABA GAREI
37	231.6548179	*Kareius bicoloratus*	ISHI GAREI
38	231.9655554	*Sebastes cheni*	SHIRO MEBARU

Table 5: Total Grand Fish Weight % = [%TFW] of fish species in the sampling in offshore Fukushima-ken, Japan in November 2012

Number (Order value of the right is less)	Total Fish Weight % = [%TFW]	Scientific Name	Japanese Name of Species
1	0.0014871	*Zeus faber Linnaeus*	MATOU DAI
2	0.0037178	*Platycephalus sp.2 (fry)*	MAKOCHI (CHIGYO)
3	0.0085509	*Clupea pallasii Vakenciennes*	NISHIN
4	0.0126404	*Paralichthys olivaceus (Fry)*	HIRAME (CHIGYO)
5	0.0260244	*Takifugu poecilonotus*	KOMON FUGU

Number (Order value of	Total Fish Weight % = [%TFW]	Scientific Name	Japanese Name of Species
6	0.0327164	*Engraulis japonica*	KATAKUCHI IWASHI
7	0.0446133	*Takifugu stictonotus*	GOMA FUGU
8	0.1040977	*Lepidotrigla microptena Gunther*	KANA GASHIRA
9	0.1040977	*Sebastiscus marmoratus*	KASAGO
10	0.1078155	*Pagrus major*	MADAI
11	0.1152511	*Liparis tanakai*	KUSAUO
12	0.1197124	*Pagrus major (Fry)*	MADAI (CHIGYO)
13	0.1858888	*Oncorhynchus keta*	SAKE
14	0.2379377	*Oplegnathus punctatus*	ISHIGAKI DAI
15	0.3297667	*Trachurus japonicus*	MA AJI
16	0.3792132	*Sebastes inermis*	AKA MEBARU
17	0.3829309	*Platycephalus sp.2*	MAGOCHI
18	0.4349798	*Chelidonichthys spinosus*	HOUBOU
19	0.4721576	*Hexagrammos stelleri*	EZO ISO AINAME
20	0.4833109	*Lateolabrax japonicus*	SUZUKI
21	0.598562	*Takifugu rubipes*	TORA FUGU
22	0.6208686	*Kareius bicoloratus*	ISHI GAREI
23	0.6208686	*Seriola quinqueradiata*	BURI
24	0.7435552	*Acanthopagrus schlegeli*	KURODAI
25	0.7770152	*Sebastes pachycephalus*	MURASOI
26	0.8104752	*Nibea mitsukurii*	NIBE
27	1.0075173	*Cynoglossus joyneri*	AKASHITA BIRAME
28	1.178535	*Ditrema temminkii*	UMI TANAGO
29	1.2826328	*MIicrostomus achn*	BABA GAREI
30	1.8588881	*Hexagrammos otakii*	AINAME
31	3.1154964	*Sebastes vulpes*	KITSUNE MEBARU
32	3.2121586	*Scyliorhinus torazame*	TORA ZAME
33	5.4353888	*Triakis scyllium*	DOCHI ZAME
34	5.8443441	*Paralichthys olivaceus*	HIRAME
35	6.0227974	*Takifugu snyderi*	SHOUSAI FUGU
36	13.700005	*Sebastes cheni*	SHIRO MEBARU
37	22.901501	*Mustelus manazo*	HOSHI ZAME
38	26.68248	*Okamejei kenojei*	KOMON KASUBE

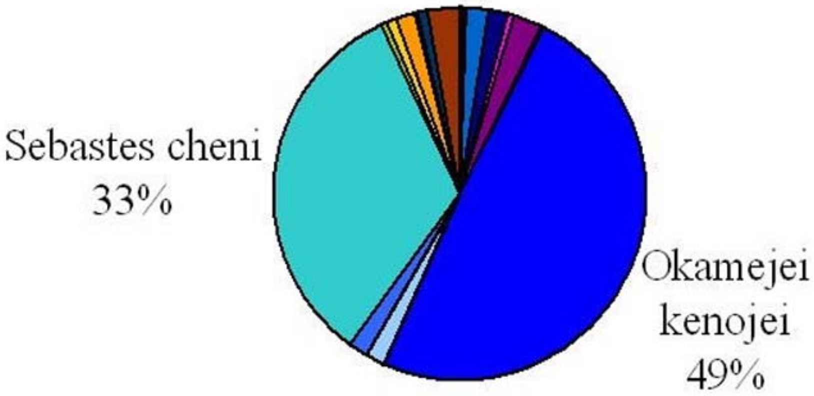

Fig. 1 **([$^{134}_{Cs}$ per Fish Species] / [Total $^{134}_{Cs}$ for All Species]) x 100 = ([$^{134}_{Cs}$] / [T$^{134}_{Cs}$]) x 100 = [$^{134}_{Cs}$%]**

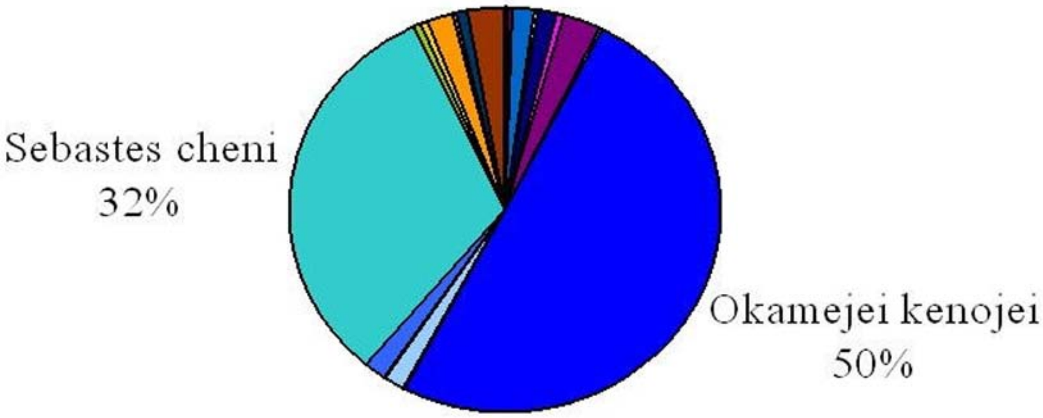

Fig. 2 **([$^{137}_{Cs}$ per Fish Species] / [Total $^{137}_{Cs}$ for All Species]) x 100 = ([$^{137}_{Cs}$] / [T$^{137}_{Cs}$]) x 100 = [$^{137}_{Cs}$%]**

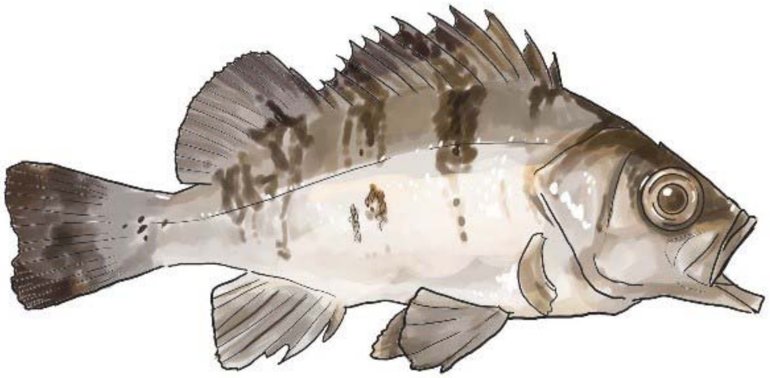

Fig. 3.1 *Sebastes cheni* (English Name: Japanese rockfish or Japanese sea perch; Japanese Name: Shiro Mebaru)

Fig. 3.2 ***Okamejei-kenojei*** **(English Name:Common skete;Japanese-Name: Komon Kasube) spp.**

Fig. 4 It may be possible to use *Sebastes cheni* (English Name: Japanese rock fish or Japanese sea perch; Japanese Name: Shiro-Mebaru) and *Kareius bicoloratus* (English Name: Stone flounder; Japanese Name: ISHIGAREI) for the separation or accumulation of nuclide $^{134}{}_{CS}$ and $^{137}{}_{Cs}$.

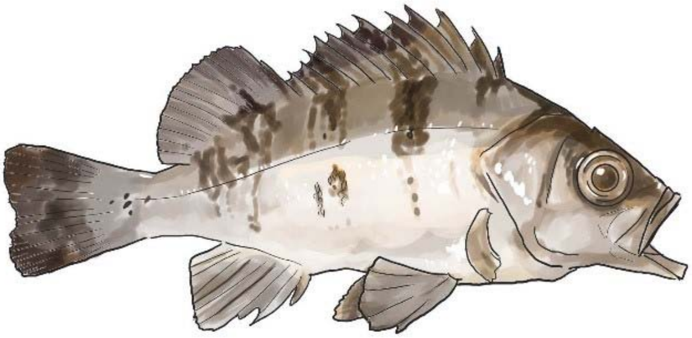

Fig. 4.1 $^{137}{}_{Cs} > {}^{134}{}_{Cs}$
Sebastes cheni **(English Name: Japanese rock fish or Japanese sea perch;**
Japanese Name: Shiro-Mebaru)

Fig. 4-2 $^{134}{}_{Cs} > {}^{137}{}_{Cs}$
Kareius bicoloratus **(English Name: Stone flounder; Japanese Name: Ishigarei)**

Fig. 5 It may be possible to use *Ditrema temminkii* (English Name: Surfperch; Japanese-Name: UmitanagO) and *Cynoglossus joyneri* (English Name: Red tongue sole; Japanese Name: Akashita-Birame) for the accumulation or separation of specific nuclides 134_{Cs} and 137_{Cs}.

Fig. 5.1 $^{137}{}_{Cs} > {}^{134}{}_{Cs}$
***Ditrema temminkii* (English Name: Surfperch; Japanese-Name: Umitanago)**

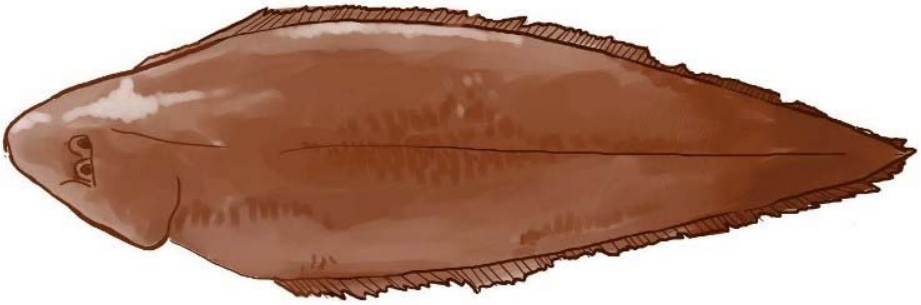

Fig. 5-2 $^{134}{}_{Cs} > {}^{137}{}_{Cs}$
***Cynoglossus joyneri* (English Name: Red tongue sole; Japanese Name: Akashita-Birame)**

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References

1. Japanese Governmental Off. Bull. On-Line (2012). http://www.gov-online.go.jp/useful/article/201204/3.html
2. Ministry of Education, C.S.S. & T., 2011, Monitoring information of environmental radioactivity level, Reading of environmental radioactivity level by prefecture [Fallout], March02011. Announce.: 1400 29-JUL-2011, Corrected: 28-SEP-2011, Add.: 14-DEC-2011, http://radioactivity.mext.go.jp/old/ja/monitoring_by_prefecture_fallout/2011/03/1060_03_gekkan_2.pdf
3. Nemoto Y, Shimamura S. and Igarashi S., 2012, Influence of radioactive substances on the marine organisms and fishing areas off Fukushima Prefecture. Nippon Suisan Gakkaishi. **78 (3)**, 514-519.
4. Ohnishi T., 2012, *Radiation research*, **177 (1)**, 1-14.
5. Katsura Hidemitsu, 2013, *Asian Journal of Chemistry*, **25 (13)**, 7613-7616.
6. Atkins W.R.G. and Warren F.J., 1953, *Journal of the Marine Biological Association of the United Kingdom*, **31**, 509-13.
7. Buscaino G., Buffa G., Sara G., Bellante A., Tonello A.J. Jr, Hardt FAS, Cremer M.J., Bonanno A., Cuttitta A. and Mazzola S., 2009, *Fisheries Science*, **75(3)**, 537-544.
8. Inazu T., Tsunoda A., Ohnishi S. and Matsubara, Y., 2011, Determination of radioactive substances in foods by Ge semiconductor detector, Kenkyu Hokoku - Kagawa-ken Sangyo Gijutsu Senta. **12**, 67-68.
9. Minatani T., Nagai H., Nakamura M., Otsuka K. and Sakai Y., 2012, *Journal of the Food Hygienic Society of Japan*, **53 (4)**, 177-82.
10. Katsura Hidemitsu, 2013, *Scientific Research and Essays (Academic Journals Inc*.). **8 (26)**, 1252-1257.
11. Choppin G.R., Liljenzin J.O. and Rydberg J., 2002, Radiochemistry and Nuclear Chemistry, 3rd edition, Butterworth-Heinemann (ISBN 978-0-7506-7463-8). Section:4.13
12. Thompson S.E., Burton C.A., Quinn D.J. and Ng, Y.C., 1972, Concentration factors of chemical elements in edible aquatic organisms. California Univ., Livermore. Lawrence Livermore Lab. UCRL-50564 Rev.1
13. Kalmun A.D.J., 1982, *Science*, **218 (26)**, 916-918.
14. Gerhard von der Emde, Stephen Schwarz, Leonel Gomez, Ruben Budell and Kirsty Grant, 1998, *Nature*, **395**, 890-894.
15. Murray R.W., 1962, *J. Exp. Biol.*, **39**, 119-128.
16. Wolfson, J. L., 1956, *Canadian Journal of Physics*, **34**, 256-64.
17. Hellstrom, S. and Brune, D., 1964, **Nukleonik**, **6(4)**, 174-8.

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Phosphate Loading and Foam Formation in Urban Lakes

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Abstract
Phosphorus (P) is the most vital nutrient that regulates primary production and determines the trophic state of freshwater bodies. However, the concern over dwindling stock coupled with the consequences of eutrophication of water bodies due to enrichment of nutrients has been increasing worldwide. P is a very essential macronutrient required to meet the global food demands and to ensure food security in the future. The present study (i) reviews the global phosphate demand, (ii) assesses the extent of pollution in Varthur lake in Bengaluru district of Karnataka, (iii) causal factors of foam formation in lakes and (iv) suggest measures to mitigate eutrophication. The sustained inflow of untreated or partially treated sewage to water bodies (lakes) has led to enrichment of nutrients. The physicochemical analysis of foam and lake water revealed that foam consists of higher concentrations of chemical contaminants and nutrients than the lake water. The external and internal loading of phosphates in lakes stimulated foam formation, profuse growth of macrophyte and eutrophic conditions. Biomonitoring revealed that only pollution tolerant phytoplankton and zooplankton dominated the lake. India has limited rock phosphate resources and is the largest importer of phosphates. Hence, there is an urgent need to (i) restrict phosphate use in detergents manufacturing and (ii) explore cost-effective viable alternatives in order to minimize the dependency on imports of mined phosphorus. This study will help different stakeholders to implement prudent management strategies to prevent urban lakes from eutrophication.

Keywords: *Lake, Eutrophication, Foam, Plankton, Phosphorus, Pollution, Water quality*

Introduction

Phosphorus is a non-renewable source and is a vital element in the Earth's crust forming the basis for all life. It occurs in pentavalent forms such as orthophosphate, polyphosphates, pyrophosphate, organic phosphonates, organic phosphate esters and phosphate diesters in aquatic ecosystems. Functions of phosphorus include: i) major constituent of the skeletal bones and teeth (as calcium phosphates), ii) crucial role in the energy transport system in cells, iii) essential for photosynthesis, iv) forms a part of structural component of cell membranes (phospholipids), v) forms a part of DNA and RNA structure, helps in cell division and development of new tissue, vi) helps in the synthesis of proteins, vitamins and is a component in enzymes etc.[1,2]

Demand for phosphorus for agricultural activities, medicinal and industrial uses varies depending on population, food habit, soil quality, etc. However, the flow of phosphorus to the environment had increased substantially over the decades with the increased use of phosphate fertilizers, manures and detergents with burgeoning population.

Global phosphate demand

Globally, phosphate rocks have been used for the manufacture of fertilizers, animal feed, detergents, pharmaceutical products, insecticides, beverages, toothpaste, matches, fireworks, military operations, bombs, etc.

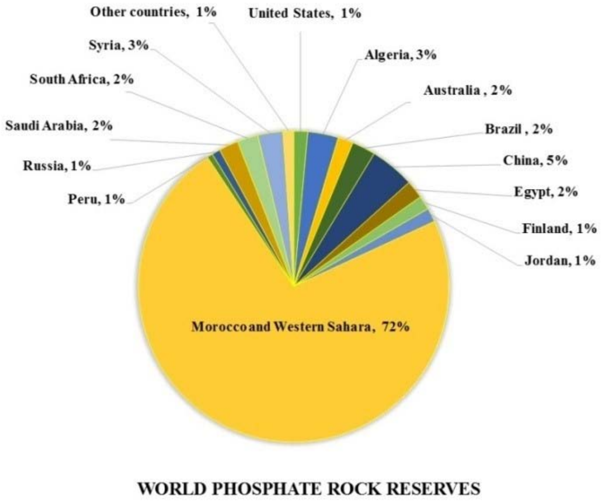

Fig. 1: Phosphate reserves (%) across many regions in the globe

Phosphate rocks/reserves occurs in few countries like United States, Algeria, Australia, Brazil, Canada, China, Egypt, Finland, Jordan, Morocco and Western Sahara, Russia, Peru, South Africa, Syria, Saudi Arabia and others. According to U.S. Geological Survey 2019[3], Morocco and Western Sahara has 50,000 million tonnes ~72% of world phosphate rock reserves (Figure 1). Phosphate reserves across countries are given in Figure 2, which indicates that most part of globe (India, Israel, Kazakhstan, Mexico, Senegal, Togo, Tunisia, Uzbekistan and Vietnam) has less than 1% of phosphate reserves and depend on imports.

Phosphorus is a very limited and non-renewable source. But, an increase in population, agricultural production, demand for more protein diet (like meat and dairy products), crop production, etc. have escalated the demand for phosphorus[4]. Factors such as income, price, prices of substitutes, technological change, consumer preferences, government activities, etc. need to be considered for assessing the demand for phosphate[5]. World consumption of phosphorus pentoxide (P_2O_5) projected to increase to 50.5 million tons in 2022 from 47.0 million tons in 2018. The countries such as Africa, India and South America would account for about 75% of the projected growth, whereas P_2O_5 consumption in U.S. remains as ~5 million tons per year[3]. According to IFASTAT 2019 data[6], the production, consumption and export of Grand total P_2O_5 is highest in China than other countries. Countries such as Brazil, followed by India and United States, are the largest importer of Grand total P_2O_5 (Figure 3).

P dependency in India

India has very limited reserves/resources of rock phosphates (RP) which are of low to medium grade quality and hence imports of P in the form of rock phosphate/phosphoric acid/direct fertilizers are necessary. India is the largest importer of RP (about 30% of global trade) in the world[7]. The total reserves/resources of rock phosphate account to 312.67 million tonnes (2015) and about 34% of it is in Jharkhand, 31% in Rajasthan, 19% in Madhya Pradesh, 8% in Uttar Pradesh and 8% in Uttarakhand, while states like Gujarat and Meghalaya has meagre quantities of resources[8]. In India, the demand for phosphatic fertilizer has increased gradually and as per the Fertiliser Association of India, the consumption of phosphorus pentoxide (P_2O_5) during 2008 to 2018 exceeded the production, necessitating imports (Figure 4).

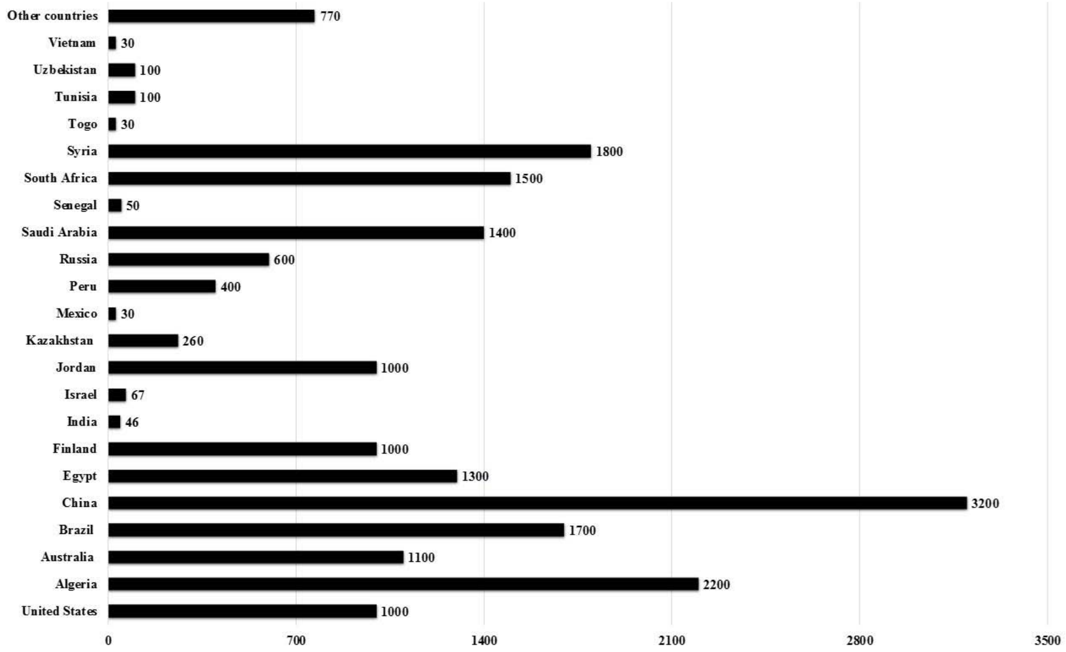

Fig. 2: Global phosphate reserves in million tonnes

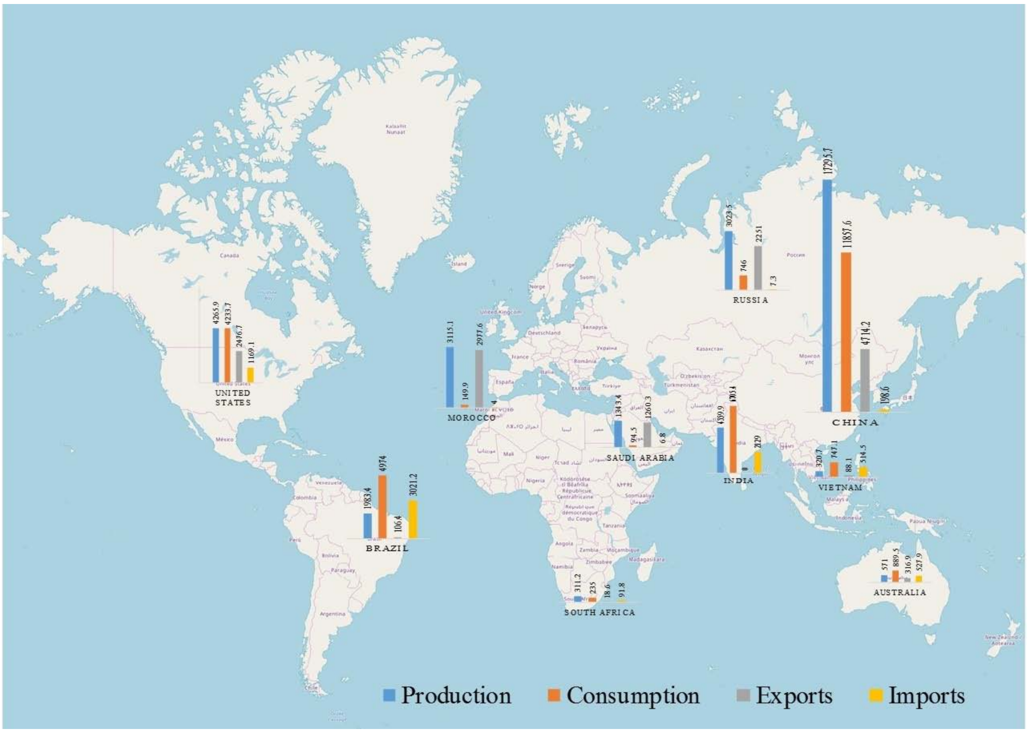

Fig. 3: Country wise production, consumption, export and import of total P_2O_5 (in 000 metric tonnes of nutrients)

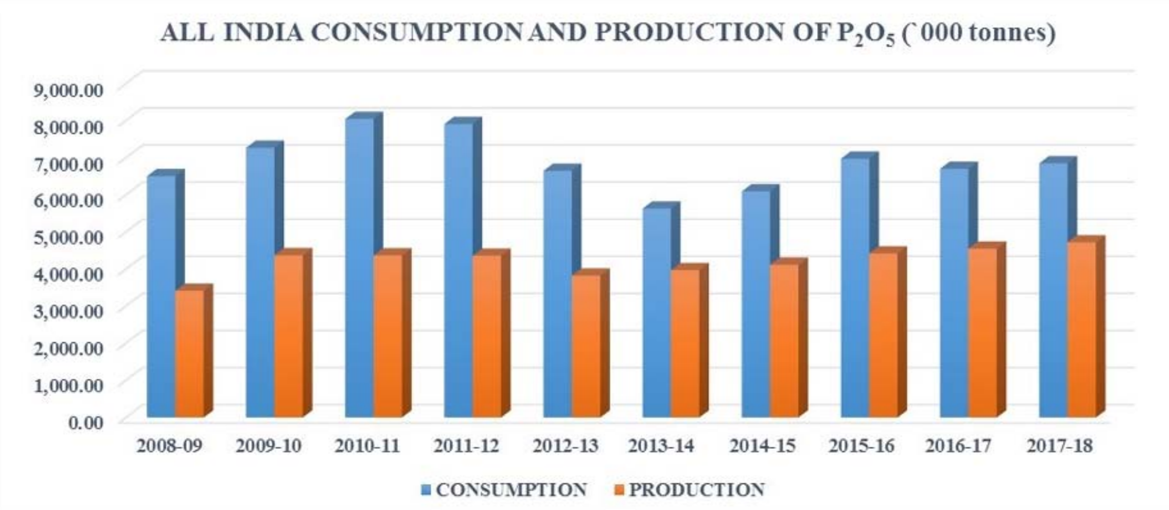

Fig. 4: Overall consumption and production of P_2O_5 ('000 tonnes) in India

Chemical fertilizers in India include Urea, Diammonium Phosphate (DAP), Single Super Phosphate (SSP), Muriate of Potash (MOP) and other complex fertilizers like Calcium Ammonium Nitrate (CAN) and various grades of NPK fertilizers. Indian Fertilizer Scenario, 2017[9] reported about 37 nitrogenous fertilizers (Urea, ASP and CAN) plants, 17 DAP and Complex Fertilizers plants and 105 Single Super Phosphate (SSP) fertilizer plants in India. As per the Department of Fertilizers, GoI[10], about 25% of Urea requirement, 90% of phosphate either as raw material or as finished fertilizers ((Diammonium Phosphate (DAP) or Monoammonium Phosphate (MAP) or Triple Superphosphate (TSP)) and potash requirement during the year 2017-2018 in India is completely met through imports. During 2016-17, the total production of urea, DAP and NP/NPK complex fertilizers was 24.33, 4.25 and 8.57 million tonnes respectively[8].

Phosphate fertilizer consumption in India includes both phosphoric acid based fertilizers and non-phosphoric acid based fertilizers. The non-phosphoric acid based fertilizers include phosphate in nitric acid based fertilizers and super phosphate. India is the third largest producer and consumer of fertilizers as per CARE Rating (2018)[11]. Recently, India has increased the domestic capacity and with easy availability of acid, the production of DAP has increased by 7.3% (38 to 46 LMT in FY16-18) whereas import of DAP had decreased by 3.8% during FY18. DAP is mainly imported from China (45%), Saudi Arabia (31%), USA (13%) and Jordan (5%).

The phosphate rock stocks are fast dwindling and there is no substitute for phosphorus, which is essential for agriculture and medicine, and hence it is necessary to minimize wasteful use of P for detergents manufacture apart from recovering from wastes. The main objective of the study is to assess (i) the water quality to understand the implication of sustained inflow of untreated sewage (rich in N and P), (ii) biomonitoring through assessment of plankton diversity, (iii) to understand the causal factors of pollution in Varthur lake leading to foam formation and (iv) to suggest remedial measures for lake protection.

Materials and Methods

Study area

Varthur lake (12°572 23.563 to 12°562 33.993 N, 77°432 08.713 to 77°442 41.723 E) is the second largest lake located towards the south of Bengaluru District in Karnataka, India and was built by the Ganga Kings over a thousand years ago to meet the local demand of domestic and irrigation water (Figure 5a). It covers a water-spread area of 190 ha and is the main source of water for irrigation for the nearby agricultural fields. The lake is located in Koramangala - Challaghatta (K&C) watershed and is a part of interconnected lakes (namely, Byappanahalli, Challaghatta, Agara, Bellandur, Haralur, Kasavanahalli, Kaikondanahalli, Doddanakundi, Vibhuthipura, Kundalahalli, Chinnappanahalli and Varthur) and canals that receive all the surface runoff, wastewater and sewage from the Bengaluru South taluk and finally drains into the Dakshina Pinakini River[12]. This had increased the pollution load of Varthur lake. The average annual rainfall of Bengaluru is 859 mm and temperatures vary from 14°C (minimum during December to January) to 33°C (maximum during March to May). There are two rainy periods, i.e. from June to September (south-west monsoon) and November to December (north-east monsoon).

Fig. 5a: Varthur lake - Sampling locations of water

Fig. 5b: Froth / foam formation in Varthur lake

Fertilizers and insecticides are being used in the lake catchment for cultivation of horticultural crops (Amaranthus, Coriander, Turnip, Palak, Spinach etc.), floriculture and agricultural crops (Maize, Paddy) and the runoff from the catchment with the nutrients reaches Varthur lake. Sustained inflow of untreated sewage, industrial effluents along with the nutrient rich catchment run-off has enhanced the nutrient content in the lake leading to eutrophication with the profuse growth of macrophytes covering the major part of the lake and foam/froth formation at outlets. Macrophytes in the lake include species such as *Eichhornia crassipes*, *Alternanthera philoxeroides*, *Colocasia esculenta*, *Cyperus* sp., *Ipomoea aquatica*, *Ludwigia* sp. and *Typha* sp. that occupied a large area of the lake. Heavy metals like copper, zinc, chromium, lead, nickel and cadmium were found to accumulate in lake sediments and in macrophytes in Varthur lake[13]. Decomposition of algae, fish and macrophytes releases a variety of organic compounds into the water body, which act as surfactants (foaming agents) with a hydrophilic end and hydrophobic hydrocarbon chain at the other end. Most surfactants originate from the detergents, oil and grease released from households or industries. These agents rise to the lake surface and interact with water molecules thus, reducing the surface tension of water. When the surface tension decreases, air mixes with the water molecules and foaming agents resulting in bubble formation. These bubbles aggregate together and forms foam in lakes. Foam generated in Varthur lake is sticky, white in color and spreads a foul smell to the surrounding area (Figure 5).

Water quality assessment

Water samples (V1 and V2 from two outlet points of the lake) and foam samples (V2) were collected from Varthur lake in clean disinfected bottles (Figure 5). In situ parameters like water temperature (WT), total dissolved solids (TDS), electrical conductivity (EC) and pH were measured on-site using handheld probes (Eutech: PCSTestr 35). Dissolved oxygen (DO) was determined on-site by Winkler's method[14]. The parameters like total alkalinity (TA), total hardness (TH), calcium (Ca), magnesium (Mg), chloride (Cl), chemical oxygen demand (COD), biochemical oxygen demand (BOD), sodium (Na), potassium (K), nitrate (N) and orthophosphate (OP) of water and foam samples collected from Varthur lake were analysed in the laboratory according to the standard protocols as per APHA[14].

Plankton identification

The plankton (phytoplankton and zooplankton) samples collected using standard plankton net (of mesh size 63 mm and 30 cm diameter) were transferred to clean sterile container and were preserved by adding 2 mL of 5% formalin. The planktons were identified microscopically according to the standard keys[15,16].

Results and Discussion

Water pollution in Varthur lake

Varthur lake series receives ~590 MLD (million liters per day) of untreated and partially treated sewage daily[17], which has sustained the level of nutrients (nitrogen, carbon and phosphorus) resulting in the pollution of the lake. TDS mainly consists of bicarbonates, carbonates, sulphates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron etc. and small amount of organic matter. TDS at V1, V2 and foam were 448 mg/L, 454 mg/L and 7000 mg/L respectively. EC was 749 µS at V1; 764 µS at V2 and 17000 µS in foams. The conductivity increases due to the presence of chloride, phosphate and nitrate in wastewater entering Varthur lake. pH indicates whether water is acidic or basic, ranging from 0 -14. pH values at V1, V2 and foam were 7.46, 7.35 and 6.98 respectively.

DO is the amount of oxygen dissolved in water. Hypoxic/ anoxic condition prevailed in Varthur Lake due to low dissolved oxygen levels, attributed to the high pollution/ organic load, extensive macrophyte cover and organic matter decomposition. BOD was higher at all sites i.e., 24.39 mg/L at V1, 60.98 mg/L at V2 and 650.41 mg/L in foam. COD at V1, V2 and foam were 40 mg/L, 88 mg/L and 1140 mg/L respectively. The higher values of BOD and COD indicate increase in organic pollution due to wastewater from household and industrial waste discharges. Varthur lake behaves as an anaerobic - aerobic lagoon[18]. In addition, surface foams may block aeration of lakes, increase decomposition rate (BOD), and hence deplete DO levels[19]. Similar instances of foam/froth formation are reported in lakes of Bengaluru[20].

Alkalinity indicates the acid-neutralizing capacity of water and it was recorded as 336 mg/L at V1 and V2 and as 12000 mg/L in foam. The hardness mainly depends on the presence of calcium and magnesium salts and is linked with bicarbonates, carbonates, sulphites, sulphates etc. Total hardness at V1, V2 and in foam were 206 mg/L, 224 mg/L and 13000 mg/L respectively. Calcium content was 57.72 mg/L at V1, 64.13 mg/L at V2 and 3607.2 mg/L in foam whereas magnesium was 15.10 mg/L at V1, 15.58 mg/L at V2 and 974.25 mg/L in foam. Ionic content in water increases due to water pollution. Chloride concentrations at V1, V2 and in foam were 117.86 mg/L, 122.12 mg/L and 3195 mg/L respectively. Sodium levels at V1, V2 and in foam were 169.5 mg/L, 161 mg/L and 770 mg/L respectively whereas potassium levels at V1, V2 and in foam were 35 mg/L, 34 mg/L and 230 mg/L respectively. Phosphorus and nitrogen are essential nutrients required for all living organisms. Orthophos-phate levels at V1, V2 and in foam were 1.263 mg/L, 0.881 mg/L, 74.59 mg/L respectively. The high values of phosphate are mainly due to agriculture runoff, waste-water and detergents. Nitrate levels at V1, V2 and in foam were 0.541 mg/L, 0.361 mg/L and 129.72 mg/L res-pectively. The major sources of nitrate are fertilizers, agricultural runoff and wastewater. Thus, foam samples collected from the lake had higher concentrations of all the physicochemical parameters compared to lake water samples (at V1 and V2). These results coincide with earlier studies as foams in lakes were enriched with organic and inorganic forms of phosphorus, carbon and nitrogen; chlorinated hydrocarbons; heavy metals and cations[21]. Both the natural and synthetic foams can collect and concentrate chemical contaminants[22]. Detergents can increase the ionic/chemical contents in water. Powder detergents add more chemical contamination than liquid detergents by increasing the concentration of TDS, chloride, sulphate, carbonate, bicarbonate and pH of wash water[23].

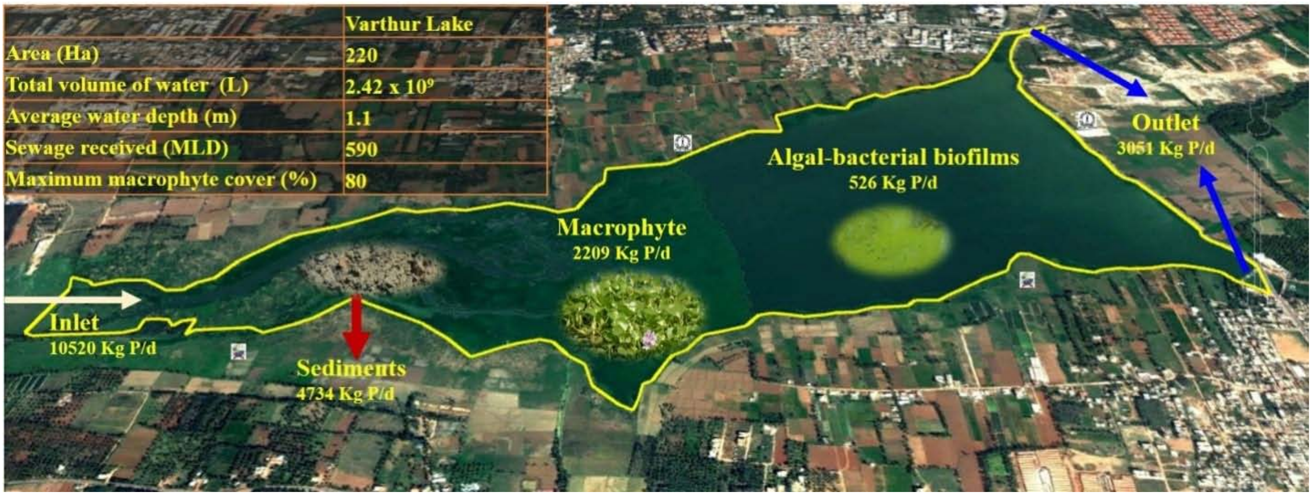

Fig. 6: Phosphorus content in Varthur lake

Varthur lake receives daily a considerable amount of untreated and partially treated sewage loaded with P, which gets trapped in sediments apart from assimilation by macrophyte, algal-bacterial biofilms etc. P uptake varies widely among biotic components. The foam formation is observed at outlet and is found to contain concentrated nutrients and ions (Figure 6).

Foam formation in lakes

White coloured foam is formed by the interactions between liquid phase, gas phase and surfactants in Varthur lake. Foams have low density and large surface area, exhibiting both solid and liquid like behavior[24]. Stable foams generated by the process of flotation involve three components (i) air bubbles, (ii) surfactants that reduce the surface tension and (iii) hydrophobic (bacterial) cells[25]. Foam in Varthur lake had total phosphate (TP) of >2 g/L and orthophosphate of 0.075 g/L which is due to the sustained inflow of sewage (containing detergents) and internal P loading from the lake sediments[26]. TP concentrations of uncontaminated surface waters range between 10-50 ìg P/L[27]. Even a concentration of phosphates (PO_4-P) greater than 0.5 ppm causes foam in surface water[28]. Phosphates are also responsible for the formation of white foam, which acts as a barrier to entry of oxygen and light in the water[29].

Surface-active agents of municipal wastewater include synthetic detergents, fats, oils, greases and biosurfactants. Synthetic detergents contain phosphates to soften water, increases pH and surfactant efficiency. Detergents cause foaming, eutrophication, limit oxygen production, reduce potable water sources and threaten aquatic life. Nature of the surfactants (whether, anionic/non-ionic synthetic detergents/biosurfactants) and their adsorption on interfaces, governed by electrostatic and steric repulsion determines the stability of foam[30]. The environmental risk associated with the use of surfactants depends on its final concentration in lake water[31] since it is toxic and persistent in nature[32].

The decomposition products of the phytoplankton, fulvic or humic acids, lipidic, proteic or colloidal substances present in water also act as surface-active compounds, producing foams both in marine and freshwater environments[33,34]. Even proteinaceous and carbonaceous matter from industrial and treatment plant effluents or from natural sources (plankton, higher plants and microorganisms) acts as a surface-active agent, reducing the surface tension and create foams[35]. Both surfactants and cells together form stable foams, whereas only surfactant (without bacterial cell) forms unstable foams in lakes. Foam formation never occurs in the absence of surfactants[36]. Surfactants have a polar, hydrophilic head group and a nonpolar, hydrophobic hydrocarbon tail group[37] and are of four types namely (i) anionic, (ii) cationic, (iii) amphoteric and (iv) nonionic, depending on the charge of their head group[38]. Surfactants form films on lakes and hinder evaporation of water and transport of gases across the aqueous interface[39]. *Skermania piniformis*, *Rhodococcus* sp., *Microthrix parvicella* and *Gordonia* sp. are foam-causing organisms growing on oil and hydrocarbons in wastewater[40]. Surfactants as well as foam are responsible for reducing oxygen levels[41], which affects aquatic life. Surfactants deteriorate water quality by creating foams in water bodies[42], which are harmful to fishes, vegetation and alsohuman beings. Foam transfers micro-contaminants and toxic metals into the food web, induces various chemical and physical interactions among components of foam and transports chemicals to the atmosphere through bubble breaking and wind-suspension processes[43].

Effect of pollution on aquatic organisms

The organisms such as primary producers (phytoplankton) and consumers (zooplankton) of foodweb, which inhabit the surface of lake, will be exposed to these contaminants. Among phytoplankton, Chlorophyceae members dominated Varthur lake indicating the presence of higher amounts of dissolved carbon content. The phytoplankton population comprised of *Chlorella* sp., *Monoraphidium* sp., *Dictyosphaerium* sp., *Chlamydomonas* sp., *Micracitinium* sp., *Scenedesmus* sp., *Pandorina* sp., *Schroederia* sp., *Pediastrum* sp., *Golenkinia* sp., *Oscillatoria* sp., *Chroococcus* sp., *Spirulina* sp., *Anabaena* sp., *Planktothrix* sp., *Merismopedia* sp., *Microcystis* sp., *Pinnularia* sp., *Nitzschia* sp., *Navicula* sp., *Amphora* sp., *Cyclotella* sp., *Aulacoseira* sp., *Synedra* sp., *Euglena* sp., *Phacus* sp., *Lepocinclis* sp. and *Trachelomonas* sp.

Zooplankton in Varthur lake includes *Brachionus quadridentatus, Brachionus plicatilis, Brachionus rubens, Brachionus calyciflorus*, *Brachionus diversicornis*, *Philodina* sp., *Brachionus angularis*, *Lecane luna*, *Platyias quadricornis*, *Cephalodella* sp., *Arcella* sp., *Vorticella* sp., *Paramecium* sp., Chironomid larvae, *Moina micrura*, *Chydorus sphaericus*, *Moina brachiata*, *Mesocyclops* sp., *Mesocyclops leuckarti* and *Microcyclops varicans*. Dominant are protozoa and rotifers, which indicates the deterioration of water quality with nutrient enrichments (eutrophic conditions).

Earlier, Varthur lake supported species like *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Clarias gariepinus*, *Oreochromis mossambica*, *Clarias batrachus*, *Heteropneustes fosslis*, *Mystus dittatus* and Minor carps[44]. The sustained inflow of untreated or partially treated wastewater has contributed to nutrient enrichments leading to the profuse growth of macrophytes, which has hindered the solar energy penetration in most part of the lake and affected the producers. This has led to the decline of native species of fish and the frequent mortality of *Clarias gariepinus* has been reported. However, the fish culture involving exotic species of fish has led to the dominance of exotic invasive species.

Phosphate increases primary productivity but several studies showed the adverse effects of detergent on aquatic life[45-47]. Even low concentrations (0.003 mg/L) of detergent effluent induce various toxicological effects and histological abnormalities in *Clarias gariepinus*, which depends on exposure time and toxicant concentration[48,49]. Wastewater from automobile service stations (depending on wastewater concentration and exposure period) are toxic to freshwater fish, *Clarias gariepinus*[50]. Life Cycle Assessment (LCA) of detergents indicates that during production and consumption stages, affects severely ecosystems. Thus, recovery of detergents would help in reducing the environmental impacts of laundering industry[51].

Phosphate loadings and accumulation in urban lakes

Phosphorus (P) plays a crucial role in the productivity of aquatic ecosystems. Various abiotic and biotic processes control P dynamics in sediments containing varied quantities of organic, inorganic and microbial P[52]. Phosphorus input to water bodies occurs in rural areas through agricultural run-off from adjacent fields with an increased use of phosphate-containing fertilizers and manure, while in urban localities through anthropogenic activities involving excessive use of laundry detergents and discharges from industries. The threshold values for anionic, cationic and non-ionic detergents that are detrimental for aquatic life are 3-12, 20 and 3-38 mg/L respectively[53]. Increase in phosphorus concentration promotes algal blooms and aquatic plant (macrophyte/ weed) growth that adversely affects the biodiversity, water quality, fish population as well as the recreational value of lakes. During algal bloom, luxury P uptake by algae will occur and thus P accumulates as inositol polyphosphate. If concentration of orthophosphate in lake water reduces, about 90 percent of polyphosphates within the algal cell are released enzymatically back into lake water within 24 hours. Similar P release (60% of OP) occurs under anaerobic conditions within 3 hours[54].

Suspended particulate matter (SPM) includes all suspended particles, both inorganic and organic in freshwater ecosystems[55]. Generally, litter in water decomposes rapidly than standing dead material[56]. Both macrophyte and periphyton plays an important role in P retention and nutrient turnover. During photosynthesis and at high pH, periphytons induce P precipitation with calcium and increases P adsorption near the sediment surface[57]. Among periphytons, P uptake rate was higher in epiphyton compared to metaphyton and epipelon[58]. The P content in lakes varies widely (Figure 7 and Table 1). The organic phosphorus compounds include inositol phosphates, monoesters, diesters and phosphonates[59]. In India, total input of P through wastewater (from detergents) is between 41,000 to 145,555 tonnes/annum. These detergent phosphates in the form of STPP (sodium tri-polyphosphates) and phosphate from human waste (feces and urine) reaches surface water bodies and along with the sustained inflow of untreated wastewater promotes eutrophication and froth formation. During pre-monsoon, high velocity wind coupled with the high intensity rainfall leads to the churning of the lake with upwelling of sediments and release of trapped phosphates, which contributes to the large-scale frothing.

Figure 8 illustrates pollution in urban lakes with the sustained inflow of phosphorus enriched municipal wastewater and its consequent effects. Various sources of P like untreated domestic and industrial sewage, detergents and fertilizers from agricultural fields reach urban lakes through run-off and leaching. These phosphates will stimulate the growth of phytoplankton and aquatic plants (macrophyte). Phytoplankton forms the base of the food chain, provides food, and transfers energy to higher trophic levels (zooplanktons, fishes, birds and humans). This leads to eutrophication with the extensive and dense growth of macrophytes creating anoxic condition in the underlying layers with malodor generation, which chokes fishes eventually leading to their death, reducing the overall biodiversity and productivity of lake ecosystems.

Table 1: Phosphorus content in urban lakes

Components	Functions	References
Macrophyte	Bioaccumulation of nutrients from lake water and sediments; influence the nutrients (P) recycling in lakes upon macrophyte decomposition	60
	Nutrient uptake and cycling, immobilize the sediment, controls resuspension of sediment and improve water transparency in lakes	61
Flocs	Suspended in lakes by means of shear forces or bioturbation, which affect the phosphorus concentration in the overlying water	62
Organic matter	Serves as an electron donor and induces changes in redox and pH after mineralization of organic matter	63
Dead biomass (standing and fallen)	Internal P loading in lakes	64
Litter from aquatic plants	Decays rapidly which firstly get converted to organic matter and finally to minerals	65
Periphytons	Prefer inorganic phosphorus and serve as a sink for P in wetlands	66
	Nutrient uptake and cycling	67
Suspended solids and sediments	Act as a sink for P in lakes	68

P is found as particulate organic P (POP), dissolved organic P (DOP), particulate inorganic P (PIP) and dissolved inorganic P (DIP). Phytoplankton can readily utilize dissolved inorganic phosphates (DIP) which, later are incorporated into cells in the form of organic molecules. Upon excretion, death and decomposition of aquatic inhabitants, DOP releases. In the sediment part, heterotrophs mediate transformations from POP to DOP to DIP to PIP. The DIP and PIP again released back to water column. Conversion of POP to DOP is facilitated by phytoplankton and prokaryotes. These conversions are mainly dependent on pH, alkalinity, temperature, redox potential, mineral concentration and oxic/anoxic conditions (Figure 8). DIP levels increases to 50-100 µg P/L due to agricultural runoff and to above 1000 µg P/L from municipal sewage[69]. The pollution increase with the sustained loading of P leads to accumulation in sediments.

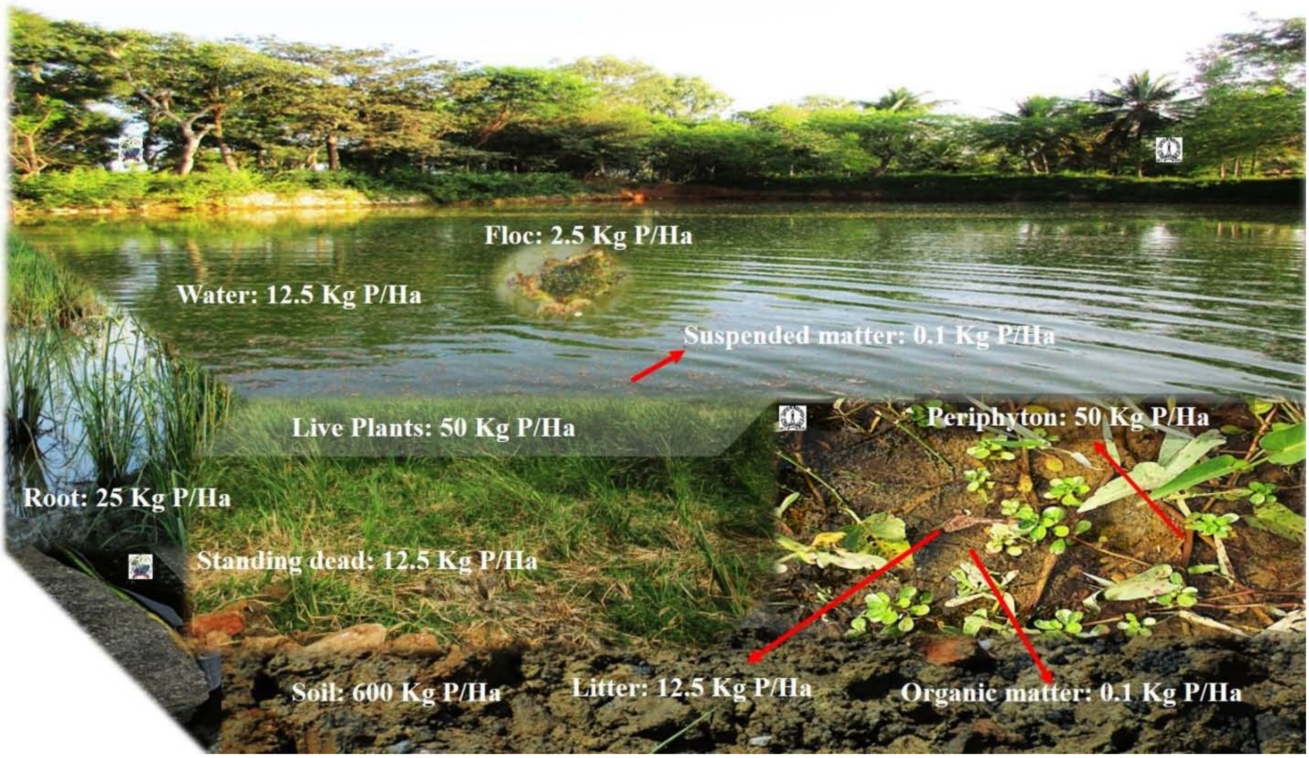

Fig. 7: Phosphorus dynamics in sewage fed urban lakes

Algae prefer non-apatite inorganic P for their growth and metabolism[70]. Dissolved organic phosphorus (DOP) can play an important role in biological and biogeochemical processes[71] and occurs commonly in agricultural, municipal and animal wastewaters[72]. Domestic and industrial wastewater contains increased level of P due to usage of detergents, soaps and cleaning materials[73], which may increase pollution load in lakes[74,75]. This will lead to algae bloom and massive growth of aquatic macrophytes[76] that threatens the freshwater resources and recreation[77]. Oxygen depletion due to increased decomposition of different aquatic macrophytes, weeds and phytoplankton leads to fish kill and loss of biodiversity in eutrophic lakes[78,79]. Approximately, 20% of phosphate rock is mined for making detergents, animal feeds and industries[80]. Thus, it is better to use green and eco-friendly detergents to avoid adverse effects on lakes[81].

The dynamics of phosphorus in sediments of lakes are shown in Figure 9. The external loading of nutrients from agricultural, horticultural and urban sources (wastewater) contributes to eutrophication. Inorganic orthophosphate (HPO_4^{2-} or H_2PO^{4-}) is the most bioavailable and mobile form of P exchanged between the lake sediments and water column. The bacterial community, algae, biofilms and aquatic macrophytes contribute to organic forms of P. Organic P is not available directly to aquatic organisms and thus the need to convert to inorganic orthophosphate. P transformations in sediments involve a series of processes (sorption/desorption; dissolution/precipitation; immmobilisation/ mineralisation) depending upon factors like temperature, pH, redox reactions and concentrations of available iron, calcium and aluminum.

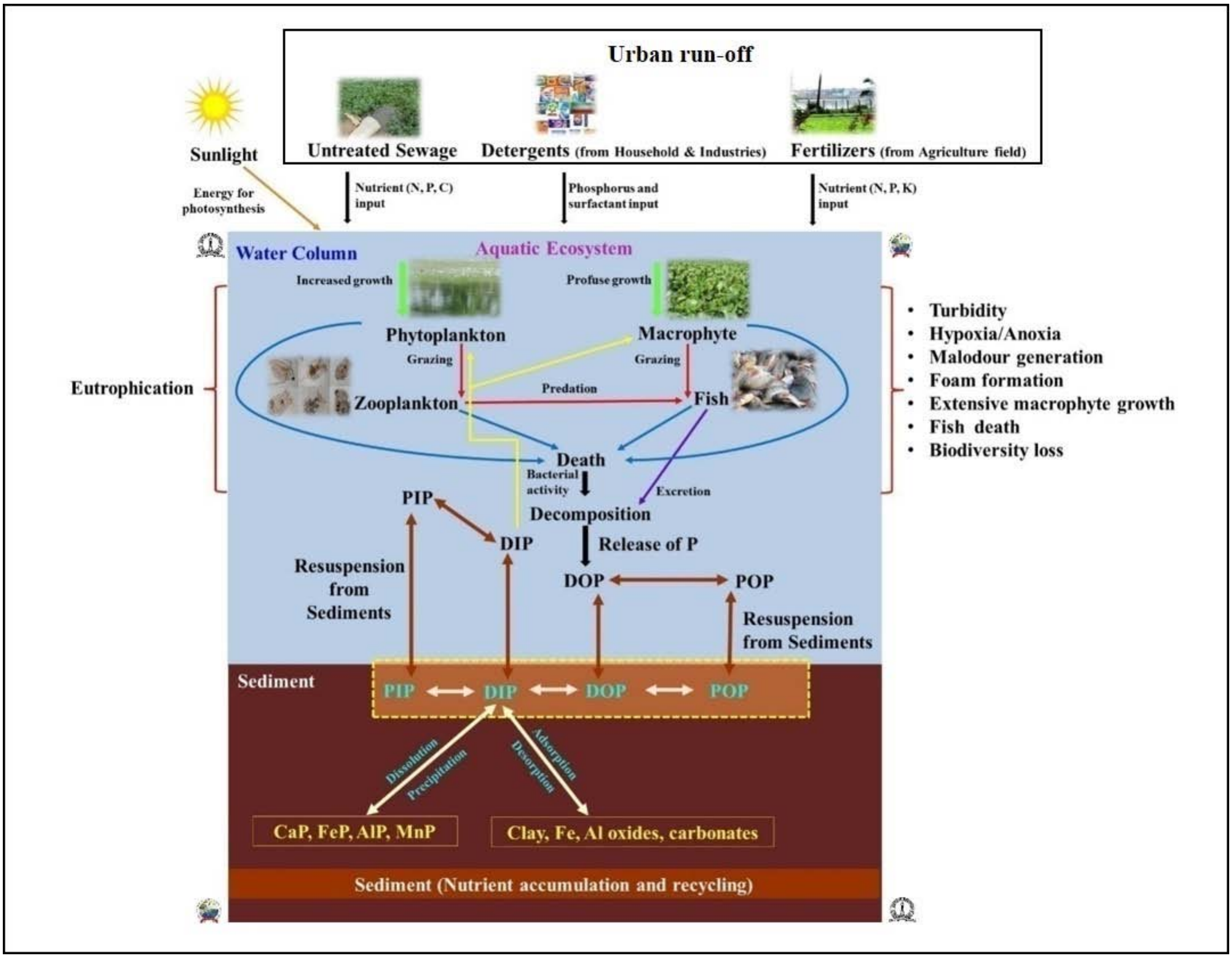

Fig.8: Sources of phosphorus and its adverse effects on lakes

Inorganic P can be either adsorbed (chemically bound) to suspended/settled sediments or desorbed (release of adsorbed P). During adsorption, phosphorus is bound to the sediment surface i.e., on clay surfaces or iron and aluminum oxides and hydroxides in sediments. While by desorption, the adsorbed phosphorus is released into the water. Orthophosphate remains adsorbed under aerobic conditions with high redox potential whereas gets desorbed under anaerobic conditions with low redox potential[54]. P gets precipitated with Ca (calcium), Fe (iron), Al (aluminium) and Mn (manganese) complexes (illustrated with equations below). Thus, ferric oxyhydroxides, calcium phosphate (apatite), aluminium oxyhydroxides, carbonates and clay are carriers of phosphates in lake sediments. These metal phosphates can release P back into water upon dissolution.

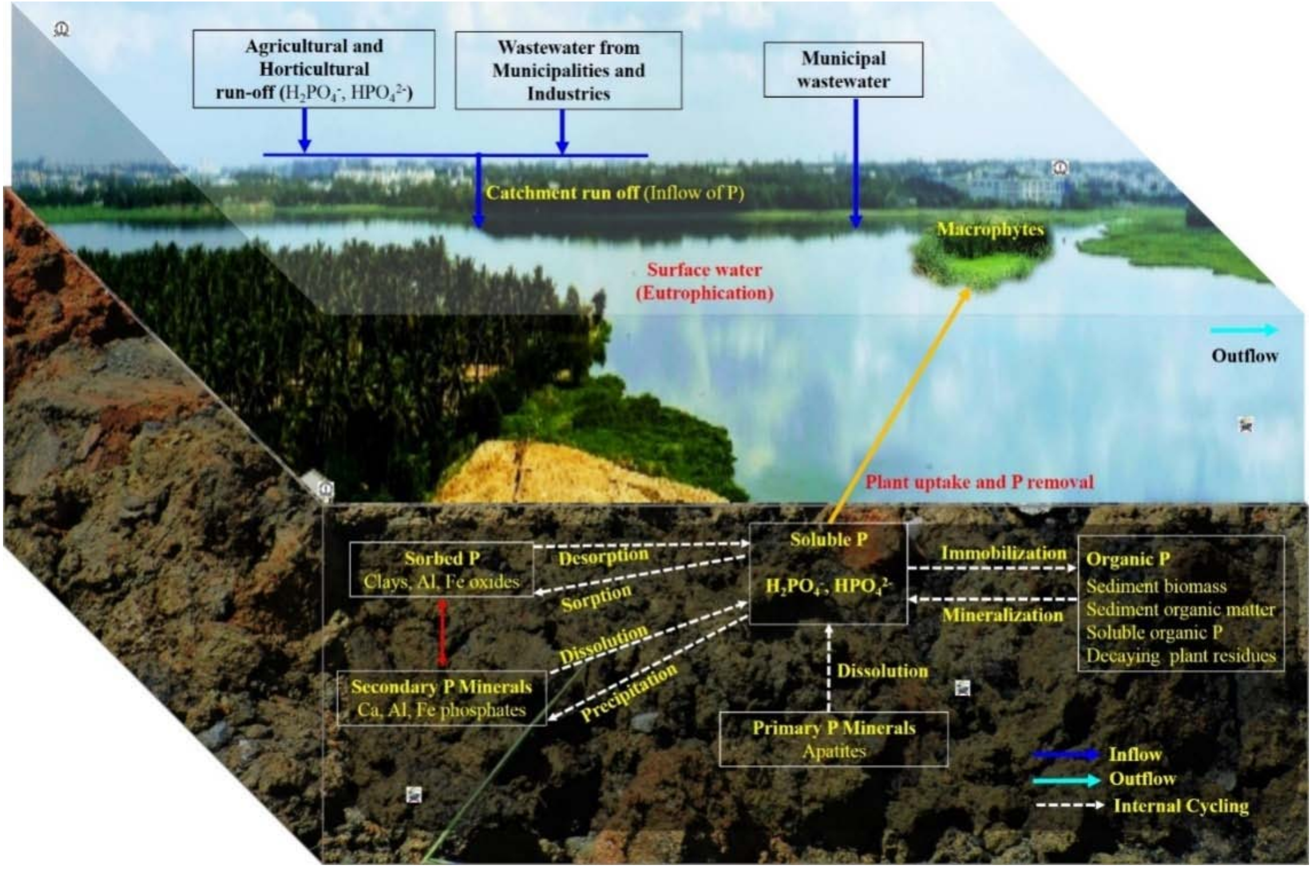

Fig. 9: Phosphorus cycling in lakes

$Ca(H_2PO_4)_2 + 2H_2O$ «$CaHPO_4 . 2H_2O + H_2PO_4^- + H^+$

$Ca(H_2PO_4)_2 + Ca_2^+ + 2OH^-$ «$2CaHPO_4 . 2H_2O$

$2CaHPO_4 + Ca_2^+ + 2OH^-$ «$Ca_3(PO_4)_2 + 2H_2O$

$2Ca(H_2PO_4)_2 + 2Ca_2^+ + 6OH^-$ «$Ca_4(HPO_4)_3 . 3H_2O + HPO_4^{2-} + 3H_2O$

$Ca_5(PO_4)_3OH + 7H^+$ «$5Ca^{2+} + 3H_2PO_4^- + H_2O$

$Al^{3+} + H_2PO_4^- + 2H_2O$ «$2H^+ + AlPO_4 .2H_2O$ (Variscite)

$AlPO_4 . 2H_2O + OH^-$ «$Al(OH)_3 + H_2PO_4^-$

$Fe^{3+} + H_2PO_4^- + 2H_2O$ «$2H^+ + FePO_4 .2H_2O$ (Strengite)

$3Fe^{2+} + 2PO_4^{3-} + 8H_2O$ « $Fe_3(PO_4)_2 .8H_2O$ (Vivianite)

$FePO_4 + H^+ + e^-$ « $Fe^{2+} + HPO_4^{2-}$

$Mg^{2+} + PO_4^{3-} + NH_4^+$ « $MgNH_4PO_4 .6H_2O$ (Struvite)

Organic phosphorus is converted into inorganic phosphorus with the help of microorganisms through mineralization process. Whereas, inorganic phosphorus is converted back to organic forms and are absorbed by the microbial cells through immobilization. The internal P loading from sediments to surface water increases the concentrations of P in water column even though there is a reduction in external loads (Figure 9).

Organic matter is a heterogeneous mix of decayed plant tissues and animal tissues, microbes (fungi and bacteria), humic substances, carbohydrates, lipids and amino acids. Organic matter competes with P for binding sites and inhibits the crystallization of Fe and Al oxides, thus, reducing the P sorption capacity[82]. Ionic strength affects phosphate sorption at the sediment-water interface[83]. Phosphate uptake and solubility in lake sediments is controlled by either redox potential/pH/both[84]. At high temperatures during summer, the pH at sediment-water interface increases whereas the redox potential decreases. Variations in redox potential also occur due to changes in DO (dissolved oxygen) and bacterial metabolism. Under anoxic conditions at the sediment-water interface, P release to overlying water is 7 to 10 times higher than under aerobic conditions[54]. Lastly, biological P uptake by benthic organisms also cause P removal from the lakes.

Phosphorus recovery from wastewater

The global phosphate demand can be met efficiently through recovering P from wastewater. Estimates indicate that phosphorus recovered from human wastes (urine and feces) could account for 22% of the total global phosphorus demand[85] as an individual excretes about 550 L urine/year, which is equivalent to 0.4 kg of phosphorus (P), 4 kg of nitrogen (N) and 0.9 kg of potassium (K) per year[86]. Urine is rich in nutrients and has high hygienic quality for utilizing as a fertilizer[87]. Urine contains ions like Na, K, NH_4, Ca, C1, PO_4, SO_4 and HCO_3[88].

Many studies have reported recovery of phosphorus from municipal wastewater, sewage sludge and sewage sludge ash[89-91]. Biomass ash derived from olive, sludge, meat and bone meal (MBM) and poultry litter has high P content (~5.4% by weight) useful for agricultural land[92]. P recovery from sewage sludge ash (SSA) is more promising than liquid phase and sewage sludge as it ensures high recycling rate, eliminates organic micropollutants, heavy metal decontamination, reduces gaseous emissions and energy demand[93,94]. Recovery of P from sludge is achievable by anaerobic digestion, wet chemical extraction (comprising either acid or alkaline dissolution) and incineration of sludge at high temperatures[95]. The overall P recycling efficiency from wastes was 51% in France, wherein the efficiency was 74.6% for food processing waste, 43.1% for household wastewater and 47.4% for municipal waste[96]. Recovering P from human discharge (a maximum of 3.7 Mt P) added to wastewater can satisfy major fraction of the global agricultural/fertilizer demand[97].

Phosphorus control and alternatives

In order to reduce the nutrient level in lakes, treated water from sewage treatment plants (STPs) should be allowed to pass through an integrated wetland model that consists of constructed wetlands and shallow algae pond as in Jakkur lake, Bengaluru[98]. Dredging of lakes will remove nutrient rich bottom sediments and reduce the internal phosphorus loading[99]. Phosphorus control measures include removal of phosphorus from municipal and industrial wastewater, ban of phosphorus in laundry detergents and other cleaning agents and control of agricultural and urban runoff[100]. Pollution prevention measures should be adopted by reducing phosphate use, reusing or removing phosphorus from wastewater through improved wastewater treatment plants which aids in the phosphorus recovery (by precipitating struvite) process. In addition, the use of locally recovered phosphorus can provide farmers with fertilizer as well as food security[101]. The replacement of sodium tripolyphosphate (STPP) as builder in detergents with Zeolite A helps to prevent eutrophication of surface waters. Since phosphate rocks are depleting, use of STPP need to be minimized as STPP is produced mainly from phosphate rock, sulphuric acid and sodium hydroxide/soda ash[102]. The internal loading of phosphate can be reduced by adopting different restoration methods such as dredging of lake sediments. P recycling (through urban wastewater treatment and food waste recycling) and use reduction (phosphorus substitution in beverages with alternatives, substitution of P in laundry detergents with zeolites, food wastage reduction, application of phosphorus solubilizing biofertilizing micro-organisms etc.) is expected to significantly improve the longevity of P resources.[103]

Restrictions on phosphate use in detergent manufacturing and ban on detergents containing phosphorous would lower the phosphate load. Entry of P rich detergents into aquatic environments needs to be restricted to avoid eutrophication[104]. The BIS (Bureau of Indian Standards)[105] has laid down the standards for eco-labelling of detergents (known as Ecomark) in India. The standards recommend that the product shall not contain phosphates and the need to replace phosphates with alternatives/ substitutes that are environment-friendly and biodegradable surfactants used for manufacturing of laundry detergent powders, and packaging material of the product must be recyclable, reusable or biodegradable. However, none of the detergent brands in the Indian market has opted for the Eco-mark or demonstrated environment-friendliness of a product or enlisted critical ingredients in terms of quantity (active ingredients, builders, soda ash, fillers and enzymes). In India, the expensive and popular brands of detergents still have high phosphates and STPP (sodium tripolyphosphate) compared to the cheaper detergents. This emphasizes the need for stringent environmental norms to mitigate wasteful use of phosphates in manufacturing detergents and ban on phosphate-based detergents to save fragile water bodies from eutrophication.

Conclusions

Poor water quality and frequent foam formation in Varthur Lake highlights of poor environmental status with nutrient enrichments due to the sustained discharge of partially treated or untreated sewage, industrial effluents, agriculture and floriculture run-off (non-point sources), solid waste dumping, etc. Foam with aerosols is being dispersed by winds has been affecting residents in the locality exposing them to health hazards and also traffic congestion. The surfactants are responsible for foam formation in lakes. Foam samples when compared to that of Varthur lake water have higher concentrations of chemical contaminants and nutrients. This underscores the need for preventing abuse of P use in detergents manufacturing as the global stock of P is limited and is required for manufacturing medicines and fertilizers (to sustain agricultural productivity). The current work underlines the need of P recovery from the lakes, which will be beneficial for different stakeholders to meet the food demand and to ensure food security. Measures like removal of phosphorus from municipal and industrial wastewater, adopting ways to recover P and ban of phosphorus in detergents and use of sustainable alternatives to P are essential to meet the burgeoning demand. There is an urgent need for enacting stringent legislations to regulate phosphate content in detergents and also ban of P based detergents in markets.

Recommendations

Different strategies need to be evolved to minimize eutrophication in water bodies through minimizing P in the environment, which help in saving waterbodies including Varthur lake in India and some of the suggestions are:

a) **Solutions at consumption level:**
  - ban of phosphorus use in the manufacture of detergents and other cleaning agents,
  - use of non-phosphate based builders like Zeo-

lite A to manufacture detergents,

- proper labeling of detergent packages (Ecolabel) including enlisting of all ingredients,
- awareness among customers to opt for detergents with minimum amount of polluting ingredients,
- stringent implementation of 'polluter pays' principle,
- minimize the use of phosphate fertilizers and use of sewage sludge ash as P source.

b) Measures to rejuvenate lakes include:

- Rejuvenation of lakes based on scientific principles,
- Dredging of lake removes nutrient rich bottom sediments, it can increase the storage capacity, aid in groundwater recharge, while reducing the internal nutrient loading,
- Restricting the entry of untreated solid and liquid waste/sewage directly into water bodies,
- Allowing only treated water to enter the lake through integrated wetlands to minimize the nutrient loadings,
- Creating constructed wetland of native species to treat partially treated or untreated wastewater,
- Adopting ways to control agricultural and urban runoff to lakes by creating buffer of riparian vegetation,
- Installation of aerators at different locations in the lake to enhance the dissolved oxygen levels and recreation services of water bodies,
- De-weeding at regular intervals to control the profuse growth of macrophytes and subsequent decay resulting to C, N and P inputs to water and sediments,
- Implementing pollution mitigation measures for reducing phosphorus (and nitrogen) loadings in the environment.

c) Recommendation for P recovery and reuse:

- Removal of phosphorus from municipal and industrial wastewater by passing through wastewater treatment plants to recover and reuse P and water,
- Recovery of phosphorus (and nitrogen) from domestic and industrial wastes to meet agricultural requirements,
- Extraction of microbial biosurfactants from wastes helps in industries, medicine, food processing industries, agriculture and phytoremediation.

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References

1. Cordell, D. and White, S., 2011, *Sustainability*, **3(10)**, 2027-2049.

2. Reynolds, C.S. and Davies, P.S., 2001, *Biological Reviews*, **76(1)**, 27-64.

3. U.S. Geological Survey, 2019, Mineral commodity summaries, U.S. Geological Survey, p. 200.

4. MacDonald, G.K., Bennett, E.M. and Carpenter, S.R., 2012, *Environmental Research Letters*, **7(4)**, 1-13.

5. Al Rawashdeh, R. and Maxwell, P., 2011, *Mineral Economics*, **24(1)**, 15-27.

6. https://www.ifastat.org/databases/plant-nutrition

7. Rao, A.S., Srivastava, S. and Ganeshamurty, A.N., 2015, *Current Science*, **108(7)**, 1253-1261.

8. https://ibm.gov.in/writereaddata/files/12102018174532Apatite_amp_Rock_Phospate2017.pdf

9. https://fert.nic.in/sites/default/files/2019-09/Fertilizers%20Scenario%202017.pdf

10. Government of India Ministry of Chemicals & Fertilizers, 2018, Annual Report 2017-2018. Department of Fertilizers.

11. http://www.careratings.com/upload/NewsFiles/Studies/Fertilizer%20Industry%20FY18%20update.pdf

12. Ramachandra, T.V., Asulabha, K.S., Sincy, V., Vinay, S., Aithal, B.H., Bhat, S.P. and Mahapatra, D.M., 2015, ENVIS Technical Report 93, CES, Indian Institute of Science, Bangalore 560012.

13. Ramachandra, T.V., Sudarshan, P., Vinay, S., Asulabha, K.S. and Varghese, S., 2020, *SN Applied Sciences*, **2(8)**, 1-14.

14. APHA, AWWA, WEF, 2005, Standard methods for the examination of water and wastewater. 21st Edition. D.C., Washington: American Public Health Association.

15. Altaff, K., 2004, A manual of zooplankton. University Grants Commission, New Delhi.

16. Desikachary, T.V., 1959, Cyanophyta. Indian Council of Agricultural Research, New Delhi.

17. Ramachandra, T.V., Mahapatra, D.M., Vinay, S., Sincy, V., Asulabha, K.S., Bhat, S.P. and Aithal, B.H., 2017, ENVIS Technical Report 116, CES, Indian Institute of Science, Bangalore 560012.

18. Mahapatra, D.M., Chanakya, H.N. and Ramachandra, T.V., 2011, *International Journal of Environment, Technology and Management*, **14(1/2/3/4)**, 84-102.

19. Minareci, O., Ozturk, M., Egemen, O. and Minareci, E., 2009, *African Journal of Biotechnology*, **8(15)**, 3568-3575.

20. Ramachandra, T.V., Asulabha, K.S., Sincy V., Bhat, S.P. and Aithal, B.H., 2016, ENVIS Technical Report 101, Energy & Wetlands Research Group, CES, Indian Institute of Science, Bangalore 560012.

21. Eisenreich, S.J., Elzerman, A.W. and Armstrong, D.E., 1978, *Environmental Science and Technology*, **12(4)**, 413-417.

22. https://www.epa.nsw.gov.au/-/media/epa/corporate-site/resources/epa/foam-chemical-contamination-in-waterway.pdf

23. Goel, G. and Kaur, S., 2012, *Journal of Human Ecology*, **38(1)**, 65-69.

24. Hill, C. and Eastoe, J., 2017, *Advances in Colloid and Interface Science*, **247**, 496-513.

25. Petrovski, S., Dyson, Z.A., Quill, E.S., McIlroy, S.J., Tillett, D. and Seviour, R.J., 2011, *Water Research*, **45(5)**, 2146-2154.

26. Ramachandra, T.V., Mahapatra, D.M., Asulabha, K.S. and Sincy, V., 2017, ENVIS Technical Report 108, CES, Indian Institute of Science, Bangalore 560012.

27. Edwards, A.C. and Wetzel, R.G., 2006, Encyclopedia of hydrological sciences.

28. Pena, B.S.D., Barranco, J.E. and Tovar-Castro, L., 2018, *International Journal of Current Research*, **10(11)**, 75008-75011.

29. Kogawa, A.C., Cernic, B.G., Domingos do Couto, L.G. and Salgado, H.R.N., 2017, *Saudi Pharmaceutical Journal*, **25(6)**, 934-938.

30. Heard, J., Harvey, E., Johnson, B.B., Wells, J.D. and Angove, M.J., 2008, *Colloids and Surfaces B: Biointerfaces*, **63(1)**, 21-26.

31. Lechuga, M., Fernandez-Serrano, M., Jurado, E., Nunez-Olea, J. and Rios, F., 2016, *Ecotoxicology and Environmental Safety*, **125**, 1-8.

32. Pedrazzani, R., Ceretti, E., Zerbini, I., Casale, R., Gozio, E., Bertanza, G., Gelatti, U., Donato, F. and Feretti, D., 2012, *Ecotoxicology and Environmental Safety*, **84**, 274-281.

33. Stefani, F., Salerno, F., Copetti, D., Rabuffetti, D., Guidetti, L., Torri. G., Naggi, A., Iacomini, M., Morabito, G. and Guzzella, L., 2016, *Hydrobiologia*, **767**, 249-265.

34. Schilling, K. and Zessner, M., 2011, *Water Research*, **45**, 4355-4366.

35. Ruzicka, K., Gabriel, O., Bletterie, U., Winkler, S. and Zessner, M., 2009, *Physics and Chemistry of the Earth*, **34(8-9)**, 565-573.

36. Blackall, L.L. and Marshall, K.C., 1989, *Journal of Industrial Microbiology*, **4(3)**, 181-187.

37. Ying, G.G., 2006, *Environment International*, **32(3)**, 417-431.

38. Ivankoviæ, T. and Hrenoviæ, J., 2010, *Archives of Industrial Hygiene and Toxicology*, **61(1)**, 95-110.

39. Olkowska, E., Ruman, M. and Polkowska, ¯., 2014, *Journal of analytical methods in Chemistry*, 1-15.

40. Soddell, J. and Seviour, R., 1996, *Water Science and Technology*, **34(5-6)**, 113-118.

41. Huang, X., Wu, T., Li, Y., Sun, D., Zhang, G., Wang, Y., Wang, G. and Zhang, M., 2012, *Journal of Hazardous Materials*, **219**, 82-88.

42. Jardak, K., Drogui, P. and Daghrir, R., 2016, *Environmental Science and Pollution Research*, **23(4)**, 3195-3216.

43. Szekielda, K.H., Kupferman, S.L., Klemas, V. and Polis, D.F., 1972, *Journal of Geophysical Research*, **77(27)**, 5278-5282.

44. Ramachandra, T.V., Alakananda, B, Rani A. and Khan M.A., 2011, *Journal of Environment Science & Engineering*, **53(1)**, 101-108.

45. Azizullah, A., Richter, P. and Hader, D.P., 2011, *Chemosphere*, **84(10)**, 1392-1400.

46. Warne, M.S.J. and Schifko, A.D., 1999, *Ecotoxicology and Environmental Safety*, **44(2)**, 196-206.

47. Pedrazzani, R., Ceretti, E., Zerbini, I., Casale, R., Gozio, E., Bertanza, G., Gelatti, U., Donato, F. and Feretti, D., 2012, *Ecotoxicology and Environmental Safety*, **84**, 274-281.

48. Ogundiran, M.A., Fawole O.O., Adewoye, S.O. and Ayandiran, T.A., 2010, *Agriculture and Biology Journal of North America*, **1(3)**, 330-342.

49. Nkpondion, N.N., Ugwumba, O.A. and Esenowo, I.K., 2016, *Journal of Environmental & Analytical Toxicology*, **6(36)**, 1-5.

50. Singru, P.C., Zade, S.B., Satyanarayan, S. and Sitre, S.R., 2017, *International Journal of Plant, Animal and Environmental Sciences*, **7(2)**, 100-106.

51. Giagnorio, M., Amelio, A., Grüttner, H. and Tiraferri, A., 2017, *Journal of Cleaner Production*, **154**, 593-601.

52. Condron, L. M. and Newman, S., 2011, *Journal of Soils and Sediments*, **11(5)**, 830-840.

53. Kundu, S., Coumar, M.V., Rajendiran, S., Ajay and Subba Rao, A., 2015, *Current Science*, **108(7)**, 1320-1325.

54. Snow, P.D. and Digiano, F.A., 1976. Mathematical modeling of phosphorus exchange between sediments and overlying water in shallow eutrophic lakes (Doctoral dissertation, University of Massachusetts).

55. He, Q., Qiu, Y., Liu, H., Sun, X., Kang, L., Cao, L., Li, H. and Ai, H., 2017, *Scientific Reports*, **7(1)**, 1-11.

56. Chimney, M. J. and Pietro, K. C., 2006, *Ecological Engineering*, **27(4)**, 301-321.

57. Zhao, Y., Chen, X., Xiong, X. and Wu, C., 2019, *Water*, **11(5)**, 1021.

58. Scinto, L. J. and Reddy, K. R., 2003, *Aquatic Botany*, **77(3)**, 203-222.

59. George, T.S., Giles, C.D. and Menezes-Blackburn, D. et al., 2018, *Plant Soil*, **427**, 191–208.

60. Wang, L., Liu, Q., Hu, C., Liang, R., Qiu, J. and Wang, Y., 2018, *Limnology*, **19(3)**, 355-366.

61. Pan, G., Yang, B., Wang, D., Chen, H., Tian, B. H., Zhang, M. L., Yuan, X.Z. and Chen, J., 2011, *Ecological Engineering*, **37(2)**, 302-308.

62. Kadlec, R. H., 2016, *Water*, **8(6)**, 1-36.

63. Gomez, E., Durillon, C., Rofes, G. and Picot, B., 1999, *Water Research*, **33(10)**, 2437-2447.

64. Jethwa, K. and Bajpai, S., 2016, *Journal of Chemical and Pharmaceutical Sciences*, **2**, 4-10.

65. Magee, P.A., 1993, USFWS Fish and Wildlife Leaflet, 1-7.

66. Lu, H., Yang, L., Zhang, S. and Wu, Y., 2014, *Plos one*, **9(1)**, 1-9.

67. Mariñelarena, A.J. and Di Giorgi, H.D., 2001, *Journal of Freshwater Ecology*, **16(3)**, 347-353.

68. Uusitalo, R., Yli-Halla, M. and Turtola, E., 2000, *Water Research*, **34(9)**, 2477-2482.

69. Wetzel, R.G., 2001. Limnology: Lake and River Ecosystems; Academic Press: San Diego, CA, USA, p.242-250.

70. Wang, C., Zhang, Y., Li, H. and Morrison, R. J., 2013, *Limnology*, **14(2)**, 147-157.

71. Wang, L., Liu, Q., Hu, C., Liang, R., Qiu, J. and Wang, Y., 2018, *Limnology*, **19(3)**, 355-366.

72. Lu, H., Yang, L., Zhang, S. and Wu, Y., 2014, *Plos one*, **9(1)**, 1-9.

73. Oteng-Peprah, M., De Vries, N.K. and Acheampong, M.A., 2018, *Journal of Environmental Management*, **206**, 498-506.

74. Köhler, J., 2006, *Journal of Business Chemistry*, **3(2)**, 15-30.

75. Banach, M. and Makara, A., 2011, *Journal of Chemical & Engineering Data*, **56(7)**, 3095-3099.

76. Vyas, A., Mishra, D. D., Bajapai, A., Dixit, S. and Verma, N., 2006, *Asian Journal of Experimental Science*, **20(2)**, 289-296.

77. Zhang, J., Guo, Y. and Wang, P., 2008, *Journal of Environmental Engineering*, **134(7)**, 585-589.

78. Kora, A.J., Rastogi, L., Kumar, S.J. and Jagatap, B.N., 2017, *Water Science*, **31(1)**, 24-33.

79. Jöbgen, A., Palm, A. and Melkonian, M., 2004, *Hydrobiologia*, **528(1-3)**, 123-142.

80. Richards, S., Paterson, E., Withers, P.J., and Stutter, M., 2015, *Journal of Environmental Management*, **150**, 427-434.

81. Siwayanan, P., Bakar, N.A., Aziz, R., Chelliapan, S. and Siwayanan, P., 2015, *Asian Social Science*, **11(9)**, 125-137.

82. Xu, G., Song, J., Zhang, Y., Lv, Y. and Han, G., 2020, *Marine Pollution Bulletin*, **150**, 1-9.

83. Wang, S., Jin, X., Bu, Q., Zhou, X. and Wu, F., 2006, *Journal of Hazardous Materials*, **128(2-3)**, 95-105.

84. Olila, O.G. and Reddy, K.R., 1997, *Hydrobiologia*, **345(1)**, pp.45-57.

85. Mihelcic, J.R., Fry, L.M. and Shaw, R., 2011, *Chemosphere*, **84(6)**, 832-839.

86. Jonsson, H., Stintzing, R., Vinneras, B. and Salomon, E., 2004, Guidelines on the Use of Urine and Faeces in Crop Production; EcoSanRes publication, Stockholm Environmental Institution (SEI): Stockholm, Sweden.

87. Heinonen-Tanski, H. and van Wijk-Sijbesma, C., 2005, *Bioresource Technology*, **96(4)**, 403-411.

88. Kirchmann, H. and Pettersson, S., 1994, *Fertilizer Research*, **40(2)**, 149-154.

89. Atienza-Martinez, M., Gea, G., Arauzo, J., Kersten, S.R. and Kootstra, A.M.J., 2014, *Biomass and Bioenergy*, **65**, 42-50.

90. _Roy, E.D., 2017, *Ecological Engineering*, **98**, 213-227.

91. Egle, L., Rechberger, H., Krampe, J. and Zessner, M., 2016, *Science of the Total Environment*, **571**, 522-542.

92. Tan, Z. and Lagerkvist, A., 2011, *Renewable and Sustainable Energy Reviews*, **15(8)**, 3588-3602.

93. Stemann, J., Peplinski, B. and Adam, C., 2015, *Waste Management*, **45**, 385-390.

94. Amann, A., Zoboli, O., Krampe, J., Rechberger, H., Zessner, M. and Egle, L., 2018, *Resources, Conservation and Recycling*, **130**, 127-139.

95. Melia, P.M., Cundy, A.B., Sohi, S.P., Hooda, P.S. and Busquets, R., 2017, *Chemosphere*, **186**, 381-395.

96. Senthilkumar, K., Mollier, A., Delmas, M., Pellerin, S. and Nesme, T., 2014, *Resources, Conservation and Recycling*, **87**, 97-108.

97. Kok, D.J.D., Pande, S., van Lier, J.B., Ortigara, A.R., Savenije, H. and Uhlenbrook, S., 2018, *Hydrology and Earth System Sciences*, **22(11)**, 5781-5799.

98. Ramachandra, T.V., Sincy, V., Asulabha, K.S., Mahapatra, D.M., Bhat, S.P. and Aithal, B. H., 2018, *Journal of Biodiversity*, **9(1&2)**, 81-102.

99. Ramachandra T.V., Sincy V. and Asulabha K.S., 2020, *Green Chemistry and Technology Letters*, **6(1)**, 14-26.

100. Ashley, K., Cordell, D. and Mavinic, D., 2011, *Chemosphere*, **84(6)**, 737-746.

101. Cordell, D., Rosemarin, A., Schroder, J.J. and Smit, A.L., 2011, *Chemosphere*, **84(6)**, 747-758.

102. Morse, G.K., Perry, R. and Lester, J.N., 1995, *Science of the Total Environment*, **166(1-3)**, pp.179-192.

103. Koppelaar, R.H.E.M. and Weikard, H.P., 2013, *Global Environmental Change*, **23(6)**, 1454-1466.

104. El-Gawad, H.S.A., 2014, *Water Science*, **28(1)**, 51-64.

105. Indian Standard (I.S: 4955-2001): Specification for Household Laundry Detergent Powders, Bureau of Indian Standards, Manak Bhavan, New Delhi.

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Fast Detection of Counterfeit Paracetamol by the Analysis of Blister Plastic Materials Using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC)

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Abstract
Counterfeit medicine is a global problem and needs stringent action upon detection. Early detection of counterfeit medicine will reduce fatal risk to the consumer. Fast laboratory testing is needed for effective surveillance and monitoring system of counterfeit medicines. In this study, Fourier Transform Infrared (FTIR) and Differential Scanning Calorimetry (DSC) are used for quick detection of counterfeit medicines through their blister packaging materials. Original and falsified paracetamol blister pack were characterised using FTIR and DSC. The FTIR spectra for the fake and original paracetamol plastic packaging shows the same functional groups. DSC thermograms of the counterfeit medicine show a distinct difference from the original due to the composite of the packaging material used. This study confirmed that identification of counterfeit medical product could be done rapidly by comparing the DSC thermograms of their packaging material and the original product.

Keywords: *Counterfeit Medicine, DSC, FTIR, Fast Detection, Blister packaging*

Introduction

Counterfeit medicines is a vast global issue that affects developing countries and developed countries alike requiring effective regulatory systems and market control. Conservative estimates in the last decade indicate that 10 per cent of all pharmaceutical items taken globally are counterfeit. In some parts of Africa and Asia, this figure may well exceed 50% [1].

All categories of medicines can be and have been counterfeited. These include expensive lifestyle medicines such as drugs for treating erectile dysfunction, fat-lowering or sleeping pills, antibiotics, anticancer drugs and medication for lowering hypertension or cholesterol. Popular and cheap medicines are also vulnerable to be counterfeited, such as painkillers and anti-histamines [2]. The World Health Organization (WHO) defines a counterfeit medicine as a medicine that is deliberately and fraudulently mislabeled with respect to its identity and/or source and may include but not limited to medicines containing no active ingredient, the wrong amount of active ingredient, the wrong active ingredient, high levels of contaminants and fake packaging.

In May 2017, WHO had to adopt "Substandard and Falsified (SF) medical products" as a new definition to replace the old "substandard/spurious/falsely-labelled/ falsified/counterfeit (SSFFC)" terminology". Counterfeit is considered falsified in the latest terminology. Falsified is defined as "Medical products that deliberately/ fraudulently misrepresent their identity, composition or source" [3].

Falsified medicine is manufactured with the intention to trick and cheat medical professionals, patients and consumers by imitating authentic medicines. Due to the similarity, often, the users are not able to differentiate and distinguish between the authentic and the falsified medicine[4]. This widespread of falsified medicines require efforts to find rapid methods of identification to monitor market penetration to protect consumer, protect profits for the pharmaceutical industries, and prevent the development of drug resistance as a result of incomplete dosing schemes [5].

More advanced analytical methods should be applied as the counterfeiters become more professional and well-resourced. Collating and organising analytical facts leading to detection and investigation is described as forensic analysis. Forensic analysis can use quick detection methods that can be developed and deployed in order to speed up the detection of counterfeit medicines. While quick and efficient evaluation of falsified medicines is a research problem, many papers have been published about the identification of counterfeit tablets and pills[6]. Differentiating between counterfeit and genuine products is now hardly a problem given the diversity of analytical tools that have been tested like chromatography and spectroscopy[7, 8].

However, most techniques are time-consuming and require enormous pattern preparation or sample destruction. Therefore, it is vital to have an analytical approach to classify genuine and counterfeit medicine samples at once from blister packaging analyses, allowing the tablet to continue to be intact in its physical form [9]. Blister packaging is shaped by a forming film and a lidding cloth. The forming film is the packaging substance that incorporates the product in deep drawn pockets, made of a polymeric material and a coating agent. The lidding material is a hard temper aluminium, print primer and a sealing agent, supplying the base upon which the final blister packaging is built. The blister film is the most critical packaging material used in this study to identify and further investigate falsified medicinal products.

Two analytical instruments useful in the forensics analysis of polymer film in the medicine blister packaging are FTIR and DSC. Both instruments will be used in this study to characterise the blister film of authentic and falsified paracetamol products. Attenuated total reflectance (ATR) is an FTIR sampling technique that became popular because it does not require samples to be added in KBr pellets or undergo pretreatment; sample is characterised through direct contact with the crystal surface to obtain spectroscopic data. ATR-FTIR has been successfully applied to analyse polymorphic contents of bulk pharmaceutical materials[10] and powder mixtures and to assess dentifrice adulteration[11]. Several recent publications have used the aforementioned techniques to analyse spectroscopy data in forensic cases including counterfeit medicines[12].

Differential Scanning Calorimetry (DSC) is known to have more advantages than other techniques in determining material purity. DSC measures the temperatures and heat flow associated with the thermal transition in material as a function of time and temperature [13]. It can provide qualitative and quantitative information about the physical and chemical changes that involve endothermic or exothermic processes[14]. This concept can be applied to detect counterfeit drugs by a comparison of thermograms of the authentic medicines and counterfeit drugs. Hence, DSC could be used as a fast counterfeit drug detector. DSC has proven to be useful in detecting fake medicines and the purity of drug components[15]. DSC analysis was also fundamental in food packaging industries to determine the quality of plastics being used[16]. Combining all the above advantages, DSC may be useful in the fast detection of counterfeit medicines in the markets by investigating their packaging materials through thermal analysis.

Paracetamol (acetaminophen, N-acetyl-p-amino-phenol, $C_8H_9NO_2$) is one of the widely available non-prescription medicines Cases involving counterfeited paracetamol have been increasingly reported in the literature[17]. Paracetamol is frequently need as a self medication for mild acute or continual pain that is not relieved by non-pharma cological approaches like ice or heat pack. It is available in various forms like tablets, capsules, oral solution, oral suspension and suppositories[18].

Materials and Methods

FTIR analysis was used to identify the type of plastic used in the packaging materials. Authentic paracetamol samples were taken in five different batches from the licenced pharmacy, and five falsified samples were taken from the exhibits seized by the enforcement agency. FTIR analysis was done using the Bruker ALPHA FTIR Routine Spectrometer. Polystyrene calibration film (0.038 mm) was used to calibrate the spectrometer to ensure that an accurate wavelength was obtained throughout the whole experiment. We employed the Attenuated Total Reflectance (ATR) technique to analyse the samples.

The plastic packaging material for authentic and counterfeit paracetamol was cut into small pieces using stainless steel scissors and was placed in the centre of the diamond crystal surface of the FTIR. Uniform and constant pressure was applied directly onto the sample on the surface by rotating the pressure device until it stopped at maximum. This was done to ensure that high-quality spectra were obtained. The spectra were obtained in the spectral region of 4000-500 cm^{-1} and were done in 8 scans. The spectra were then analysed using OPUS software. Identification was made by comparing the spectra with Bio-Rad Spectral Database (Bio-Rad. California, USA). The spectra obtained for the authentic and suspected counterfeit packaging materials were analysed and compared to determine counterfeiting. The fake and original paracetamol product were further analysed using DSC.

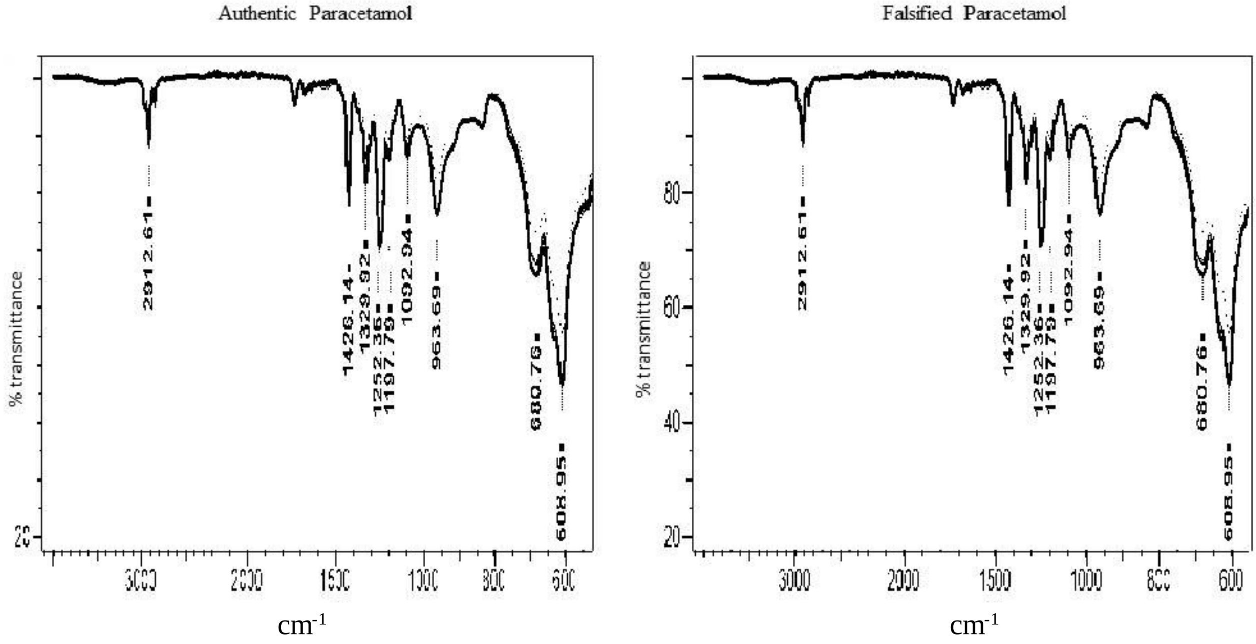
Thermal analysis was done using the Perkin Elmer DSC 6000 connected to a chiller (Intracooler SP, Perkin Elmer, U.S.) and a thermal analysis gas station (Perkin Elmer, U.S.) to control the flow of the purge gas, nitrogen, at a flow rate of 20 mL min^{-1}. Indium and zinc (Perkin Elmer, U.S.) were used to calibrate the DSC.

The plastic packaging material was cut into small pieces and placed in the centre of the DSC pan. An amount of about 1 to 1.5 mg was weighed using a microbalance (Mettler Toledo UMT2), placed in an aluminium pan (sealed pan, Kit No. 0219-0062, Perkin Elmer, U.S.) and sealed. The DSC was programmed to scan the sample by heating from 0°C to 400° C at 10°C min^{-1}.

The onset of melting temperature, peak temperature, end of melting temperature, and energy taken for the melting process (ΔH) was determined using the Pyris Manager software.

Results and Discussion

Assessing the ATR-FTIR spectra for the counterfeit paracetamol plastic blister and the authentic paracetamol plastic blister shows that they are almost the same (Figure 1). **Table 1** shows the ATR-FTIR spectra of the counterfeit and authentic paracetamol blisters. The ATR-FTIR spectra identified distinctive main bands at 502.67 cm^{-1}, 608.95 cm^{-1} and 680.76 cm^{-1}due to C-Cl bond stretching, 963.69 cm^{-1} due to the rocking of CH_2 group, 1252.36 cm^{-1} and 1329.92 cm^{-1} due to C-H deformation corresponding to a CH-Cl group and 1426.14 cm^{-1} due to CH_2 deformation (wagging). The IR spectra peak at 2912.61 cm^{-1} was due to C-H bond stretching. Therefore, these results suggest the presence of PVC in the chemical composition of the film of the blister packaging. Further comparison with the spectral library revealed that the polymer used in the production of both plastic blishfers was polyvinyl chloride (PVC). This shows that the counterfeit paracetamol blister uses the same material as the authentic paracetamol blister pack.



Authentic Paracetamol

Falsified Paracetamol

Figure 1: ATR-FTIR spectra of blister packaging for (X) Authentic Paracetamol and (Y) for counterfeit paracetamol.

Table 1: Infrared characteristics and wavenumbers of authentic and counterfeit paracetamol

Authentic Paracetamol			**Counterfeit Paracetamol**		
Wave Number (cm^{-1})	**Functional Group**		**WaveNumber (cm^{-1})**	**Functional Group**	
502.67	C-CI	Stretch	502.67	C-CI	Stretch
608.95	C-Cl	Stretch	608.95	C-Cl	Stretch
680.76	C-Cl	Stretch	680.76	C-Cl	Stretch
963.69	CH_2	Rock	963.69	CH_2	Rock
1092.94	C-C	Stretch	1092.94	C-C	Stretch
1252.36	CH	Bend	1252.36	CH	Bend
1329.92	CH_2	Bend	1329.92	CH_2	Bend
1426.14	CH_2	Bend	1426.14	CH_2	Bend
2912.61	CH_2	Stretch	2912.61	CH_2	Stretch

DSC analysis was further done on the paracetamol plastic blister and the authentic paracetamol plastic blister, demonstrating a distinct difference between the genuine and counterfeit paracetamol plastic blisters (Figure 2). The DSC thermograms for both paracetamol plastics consist of two peaks with similar pattern. The authentic paracetamol exhibits two peaks corresponding to the melting of the polymer at 255.92±1.06°C and a distinct exothermic peak at 293.41±2.12°C. The DSC thermogram for the counterfeit paracetamol plastics blister also consisted of two peaks but at 267.48±1.23°C for melting point and exothermic peak at 308.03±2.11°C. These results suggested that the plastics blisters of counterfeit paracetamol are made of a different quality PVC from that of the original blister. It was explained by a difference of only a few degrees in T_m° (Figure 2) which can be significant in establishing basic crystallisation mechanisms. Despite the importance of this melting temperature, it's direct experimental determination has proved to be very elusive [19]. The onset, peak, end of melting, and enthalpy change of fusion (ΔH) for the DSC analysis are presented in **Table 2**.

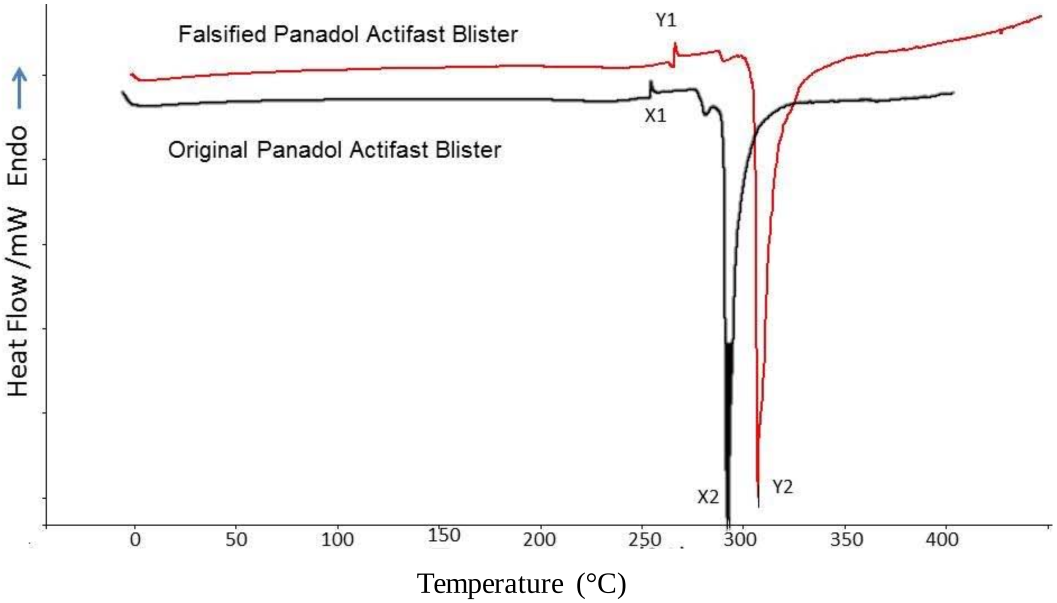


Figure 2: Comparison of the DSC thermograms of the authentic paracetamol blister (X) and the counterfeit paracetamol blister (Y).

Table 2: The onset, peak, end of melting, and enthalpy change of fusion of the original paracetamol blister and the counterfeit paracetamol blister.

	Product		The onset of melting (°C)	The peak of melting (°C)	End of melting (°C)	ΔH (J/g)
	Authentic Paracetamol Blister (X)	X1	254.52±2.73	255.91±1.06	256.682±1.2	7.03862±1.74
		X2	289.70±2.29	293.40±2.12	298.35±2.08	1168.51±198.83
	Counterfeit Paracetamol Blister (Y)	Y1	267.09±1.34	267.47±1.23	268.77±1.22	6.5214±1.31
		Y2	304.03±4.0	308.02±2.11	313.74±1.56	1254.20±219.37

DSC has the upper hand to understand the crystallinity and melting point of a polymer. This is particularly valuable as PVC has a broad melting point[20]. Although the same polymer was submitted to DSC analysis, it can distinguish between both polymers using their melting points, their heats of fusion, and crystallisation points. PVC is also known to have the ability to adjust its elasticity and hardness significantly through the addition of plasticiser[21]. This also may alter the melting point of the blister. The counterfeiter may use PVC with different compatibiliser in their product[22]. It can be concluded that another quality of PVC was used to produce the plastic blisfer of the counterfeit paracetamol. It may be obtained from a different supplier or by using a different compatibiliser from the legal manufacturer of the authentic paracetamol.[23].

Conclusions

This work demonstrated that FTIR is a powerful analytical tool for the chemical characterisation of blister packaging to identify authentic and counterfeit samples of paracetamol blister film. However, DSC has demonstrated its capability to detect counterfeit paracetamol products by differentiating the melting point of the polymer used in both samples. DSC allows us to distinguish between different PVC qualities based on their thermal transitions despite having a similar FTIR spectrum. By forensic analysis using both FTIR and DSC, the competent authority could receive vital information to expand their investigation of the source of the counterfeit medicinal products. Identifying the various substances can provide leads for investigators. This will provide support to law enforcement and other members of the criminal justice and legal systems to successfully investigate and adjudicate these crimes.

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References

1. Ozawa, S., et al., 2018, *JAMA Network Open*, **1**, e181662-e181662.
2. World Health Organization, WHO Global Surveillance and Monitoring System for substandard and falsified medical products. 2017.
3. World Health Organization, 2018.
4. Bolla, A.S., A.R. Patel and R. Priefer, 2020, *International Journal of Pharmaceutics*, **587**, 119702.
5. Almuzaini, T., I. Choonara and H. Sammons, 2013, *BMJ open*, **3**.
6. Jung, C.R., et al., 2012, *Forensic Science International*, **216**, 92-96.
7. de Veij, M., et al., 2007, *Journal of Raman Spectroscopy*, **38**, 181-187.
8. Guillemain, A., K. Dégardin, and Y. Roggo, 2017, *Talanta*, **165**, 632-640.
9. Dégardin, K., et al., 2018, *Forensic Science International*, **291**, 144-157.
10. Miloudi, L., et al., 2018, *Analyst*, **143**, 2377-2389.
11. Ortiz, R.S., et al., 2013, *Forensic Science International*, **226**, 282-289.
12. Pereira, T.M., et al., 2014, *Analytical Methods*, **6**, 2722-2728.
13. Roy, S., Riga, A.T. and Alexander, K.S., 2002, *Thermochimica Acta*, **392-393**, 399-404.
14. Clas, S.-D., Dalton, C.R. and Hancock, B.C., 1999, *Pharmaceutical Science & Technology Today*, **2**, 311-320.

15. dos Santos, M.K., et al., 2019, *Journal of Pharmaceutical and Biomedical Analysis*, **166**, 304-309.

16. Lord, A.W., 2014, Encyclopedia of Analytical Science 3rd edition ed, Academic Press, p. 451-464.

17. Barstis, T. and M. Lieberman, 2013, *Chemistry & Industry*, **77**, 28-30.

18. Bhargava, R., S.-Q. Wang, and J.L. Koenig, 1999, *Macromolecules*, **32**, 8989-8995.

19. Mandelkern L., S.G.M., Mathieu P.J.M., 1984, *Analytical Calorimetry*.

20. Wypych, G., 2020, PVC Formulary (Third Edition), ChemTec Publishing, p. 5-45.

21. Chen, J., et al., 2018, *IOP Conference Series: Materials Science and Engineering*, **292**, 012008.

22. Kollár, M. and G. Zsoldos, 2012, *Journal of Thermal Analysis and Calorimetry*, **107**, 645-650.

23. Najafi, V. and H. Abdollahi, 2020, *European Polymer Journal*, **128**, 109620.

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Reaction Kinetics of the Controlled Oxidation of Perfumery Cyclic Alcohols using an Inorganic Salt in Acidic Medium

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Abstract

The quantitative aspects of the oxidation of alcohols by organic oxidants have been extensively reported but there are very few reports of the kinetic and thermodynamic investigations of the oxidation of alcohols. Further, inorganic oxidants have rarely been used for oxidation of alcohols.

This paper reports the kinetics of the controlled oxidation of Cyclic alcohols, Cyclopentanol and Cyclohexanol to the corresponding ketones using $KBrO_3$ *in acidic medium. The oxidation was studied under first order kinetic conditions with respect to the inorganic oxidant. The progress of the oxidation was monitored by iodometric titration of the unreacted oxidant at regular time intervals. Ionic strength has no effect on the oxidation in dilute solution. From the variation of reaction rate with temperature (308-318K), the thermodynamic activation parameters of the oxidation were evaluated and interpreted.*

The sequence of oxidation rates of the Cyclic alcohols has been explained on the basis of their ring size and stability, steric factors and structural characteristics. Suitable reaction mechanisms have been suggested on the basis of the formation of halic acid, $HBrO_3$ *during the oxidation reaction.*

Keywords: *Cyclic alcohols,* $KBrO_3$*, Controlled oxidation, kinetics, ionic strength, halic acid, ring size and stability, steric factors, thermodynamic activation parameters, reaction mechanism.*

Introduction

The quantitative aspects of the oxidation of alcohols to the corresponding carbonyl compounds has been reported in literature.[1-7] But there are few reports of the kinetic and thermodynamic studies of the oxidation of alcohols.[8-16] This paper reports the kinetics of the controlled oxidation of the cyclic alcohols, Cyclopentanol and Cyclohexanol using the inorganic oxidant, $KBrO_3$ in acidic medium. These alcohols are used in the manufacture of perfumes and fragrances.

The effects of alcohol and oxidant concentrations, ionic strength and temperature on the oxidation rate of the cyclic alcohols have been studied in detail. From the dependence of oxidation rate with temperature, the thermodynamic activation parameters of the oxidation reaction have been determined and interpreted in terms of the molecular dynamics of the oxidation process. A suitable reaction mechanism has been suggested for the oxidation of the cyclic alcohols under study.

Materials and Methods

Cyclopentanol and Cyclohexanol were procured from S.H. Kelkar & Co., Mumbai and used after distillation. All other chemicals and reagents used were of Analar Grade. The oxidation was carried out under first order kinetic conditions with respect to the oxidant ie.[$KBrO_3$] << [alc.].The unreacted oxidant was estimated iodometrically at regular time intervals during the reaction and the first order rate constants(k) were determined from the linear plots of log (unreacted oxidant) vs. time. Analar Grade K_2SO_4 was used to determine the effect of ionic strength (μ) on oxidation rate of alcohols as per the Bronsted-Bjerrum equation, $\log k = \log k_0 + 1.02\, Z_A Z_B\, \mu^{1/2}$ in the range $\mu = 0.05\text{-}0.25$ mol dm^{-3}. The oxidation was studied in the temperature range, 308-318K and from the Arrhenius plots of logk vs.T^{-1}, the Energy of Activation and other thermodynamic activation parameters were evaluated and interpreted.

Results and Discussion

The cyclic alcohols, Cyclopentanol and Cyclohexanol were oxidized to the corresponding ketones by $KBrO_3$ in acidic medium.

Effect of alcohol and oxidant concentrations on oxidation rate of cyclic alcohols

For both alcohols under study, the oxidation rate increased with [alc.]but decreased with [$KBrO_3$] (Table 1).

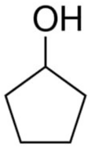
Reaction mechanism of oxidation of cyclic alcohols

In acidic medium,$KBrO_3$ rapidly forms the halic acid,$HBrO_3$ which is a strong acid and strong oxidant.[17,18].The oxidation of alcohol results in the formation of the corresponding hypohalite ion, OBr^-.

$$RR'CHOH + BrO_3^- + 2H^+ \rightarrow RR'C{=}O + OBr^- + 2H_2O$$

The oxidation product, ketone was identified by 2,4-dinitrophenyl hydrozone test and confirmed by TLC. The oxidation rates follow the sequence :

Cyclopentanol > Cyclohexanol



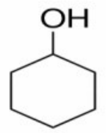


Table 1 : Rate constant data for the oxidation of cyclic alcohols using $KBrO_3$ in acidic medium

[alc.]=0.1M [H_2SO_4]=1M Temperature=308K

[alc.]x10^1 mol dm^{-3}	[$KBrO_3$] x 10^3 mol dm^{-3}	Cyclopentanol k x 10^2 s^{-1}	Cyclohexanol k x 10^2 s^{-1}
0.25	5.00	0.69	1.15
0.50	5.00	1.84	1.61
0.63	5.00	2.53	2.30
0.75	5.00	3.15	2.99
0.88	5.00	5.29	3.45
1.00	5.00	8.52	4.37
1.00	2.50	3.20	6.67
1.00	5.00	2.93	5.98
1.00	10.00	2.07	4.83
1.00	15.00	1.84	4.37
1.00	20.00	1.61	3.22
1.00	25.00	1.61	1.84

5.7 and 8 membered rings in cyclic alcohols are more reactive than 6 membered rings and consequently are more susceptible to oxidation.[14,19-21] Hence Cyclopentanol is oxidized faster than Cyclohexanol.

Effect of ionic strength on oxidation rate of cyclic alcohols

The effect of ionic strength on oxidation rate of alcohols was studied using K_2SO_4 in the range μ=0.05-0.25 mol dm^{-3} (Table 2).The graphs of log k vs.$\mu^{1/2}$ were found to be straight lines parallel to the $\mu^{1/2}$ axis indicating that the oxidation rate is independent of ionic strength. This observation confirms the involvement of a non ionic species (alcohol) in the oxidation reaction and is in accordance with reaction mechanism suggested for the oxidation of the cyclic alcohols under study.

Table 2 :Effect of Ionic Strength on the oxidation rate of Cyclic alcohols

[alc.]=0.1M, [$KBrO_3$]=0.005M ,[H_2SO_4]= 0.5M, Temp.=308K

K_2SO_4	Cyclopentanol	Cyclohexanol
μ mol dm^{-3}	k x 10^2 s^{-1}	k x 10^2 s^{-1}
0.05	5.75	2.10
0.10	5.75	2.30
0.15	5.98	2.20
0.20	5.95	2.60
0.25	5.98	2.80

Thermodynamic study of oxidation of cyclic alcohols

The oxidation was studied at different temperatures in the range 308-318K.From the effect of temperature on oxidation rate of cyclic alcohols, the thermodynamic activation parameters were determined from the Arrhenius plots of log k vs.T^{-1}(Table 3).

The important inferences from the thermodynamic study are :

1) The oxidation rates of alcohols increase with temperature but are inversely proportional to Energy of Activation (E).
2) The equilibrium constant (K*) for the formation of the activated complex from the reactant molecules increases with temperature hence K* is a function of temperature.
3) For a given alcohol and oxidant, Energy of Activation (E) is constant at all temperatures indicating that the site of oxidation ie –OH bond is the same at all temperatures.
4) The negative values of Entropy of Activation (“ S*) indicate a reorientation of solvent molecules due to the formation of a rigid activated complex[22] during the reaction. This results in the curtailment of the vibrational and rotational motions of the reacting system and decrease in its entropy. The entropy loss can be explained by a model in which the water molecules are held to the –OH bond which is site of oxidation of alcohols.

Table 3 : Thermodynamic activation parameters of Cyclic alcohols

Cyclopentanol

Temp.K	k x 10^2 s^{-1}	E kJ mol^{-1}	K*x 10^{15}	Δ H* kJ mol^{-1}	ΔG* kJ mol$^{=1}$	ΔS* kJ K^{-1} mol^{-1}
308	1.15	86.56	1.79	86.97	84.00	-0.0098
311	1.61	86.56	2.49	86.97	83.98	-0.0096
313	2.99	86.56	4.59	85.91	83.96	-0.0063
318	3.45	86.56	5.21	86.97	83.92	-0.0096

Cyclohexanol

308	2.30	97.48	3.58	85.19	95.02	-0.0325
311	3.68	97.48	5.68	84.83	95.00	-0.0327
313	5.29	97.48	8.11	84.45	94.98	-0.0337
318	8.52	97.48	12.90	84.58	94.94	-0.0326

Conclusions

For $KBrO_3$ oxidant in acidic medium,the oxidation rates follow the sequence:
Cyclopentanol > Cyclohexanol.
Ionic strength has no effect on the oxidation of cyclic alcohols. The oxidation is accompanied by decrease in entropy of activation.

References

1. Corey, E.J. and Suggs, J.W., 1975, *Tetahedron Lett.,* **16,** 2647.
2. Corey, E.J. and Boyer, D.L., 1978, *Tetrahedron Lett.,***19,** 240.
3. Corey, E.J. and Schmidt, G., 1978, *Tetrahedron Lett.,* **20,** 399.
4. Piancatelli, G., Scettri, A. and Auria, M.D., 1982, *Synthesis,* 345.
5. Barret, R. and Daudon, M., 1990, *Tetrahedron Lett.,* 4871.
6. Waldemar Adam et. al., 1994, *J. Org. Chem.,* **59(26),** 8281.
7. Maqdziak, M., Rodriquez, A.A., Van De Water, R.W. and Pettur, T.R., 2002, *Org. Lett.,* **4(2),** 285.
8. Banerjee, K.K., 1988, *J. Chem. Soc., Perkin Trans.,* **2,** 547.
9. Abdul Jameed, A., 1997, *J. Indian Chem. Soc.,* **74,** 405.
10. Mucientes, A. et al., 1999, *Intl. J. Chem. Kinetics,* **34,** 1.
11. Choudhary, K., Sharma, P.K. and Bannerji, K.K., 1999, *Intl. J. Chem. Kinetics,* **31,** 469.
12. Asim, K. Das, 2000, *J. Indian Chem. Soc.,* **77,** 223.
13. Prabhu, D.V., 1997, *J. Indian Chem. Soc.,* **84,** 1175.
14. Prabhu, D.V., 2008, *Oriental J. Chem.,* **24(1),** 163
15. Prabhu, D.V., Tandel, M.A. and Parbat, H.A., 2014, *Asian J. Chem.,* **26(19),** 6669.

    DOI:10.14233/ajchem.2014.17431
16. Prabhu, D.V. and Chetana Rana, 2017, *Rasayan J. Chem.,* **10(2),** 385.

    DOI:10.7324/RJC.2017.1021649
17. Ananda, S. and Gopalan, R., 1985, *J. Indian Chem. Soc.,* **62,** 216.
18. Shukla, K.S., Mathur, P.C. and Bansal, O.P., 1973, *J. Inorg. Nucl. Chem.,* **35,** 1301.
19. Brown, H.C. and Herstein, H., 1952, *J. Am. Chem. Soc.,* **74,** 2929.
20. Kuivila, H.G. and Becker, B.T., 1952, *J. Am. Chem. Soc.,* **74,** 5329.
21. Brown, H.C., Fletcher, R.S. and Johannesen, R.B., 1957, *J. Am. Chem. Soc.,* **79,** 212.
22. Eichhorn, G.L. and Trachtenberg, I.M., 1952, *J. Am. Chem. Soc.,* 5185.

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Efficiency Evaluation of Stable Cyanide Complex Conversion and its Application for Evaluating Some Cyanide Contaminated Wastewater Resource in Hanoi City, Vietnam

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Abstract

Cyanide compounds are listed as one of the toxic pollutants that are usually released into the environment. In this paper, we present the evaluation results of the conversion efficiency of stable cyanide complexes using a specialized distillation system. The experiments performed show that the optimal distillation time for complexes of $[Fe(CN)_6]^{4-}$ *and* $[Fe(CN)_6]^{3-}$ *was 60 minutes, conversion efficiencies of* $[Fe(CN)_6]^{4-}$ *and* $[Fe(CN)_6]^{3-}$ *were 100 ± 0.08 % and 84 ± 0.02 %, respectively, and the average of the recovery rate of complexes* R_{TB} *was 90 ÷ 100 %. We further used the above-derived optimal experimental conditions to determine the cyanide concentration in metal plating wastewater in Thanh Oai district, Hanoi city, Vietnam.*

Keywords: *Stable cyanide complexes conversion, recovery rate, wastewater, metal plating, Hanoi city, Vietnam*

Introduction

In recent years, together with industrial development, environmental pollution has increased at an alarming rate. Due to typical characteristics of a developing country's industry, e.g., without general planning, using obsolete processing technologies, loose environmental regulations, and other reasons, most industrial wastes are disposed directly to the environment without processing, causing pollution. Water pollution has become one of the greatest concerns. Besides the destruction of the natural environment, polluted water contains several parameters above permissible limits which affect directly humans and other living things[1,2].

Cyanide compounds are widely used as an important material in industries like textiles, plastics, paints, photography, electroplating, agriculture, medicine, and metallurgy[3,4]. Cyanide compounds have been listed as one of the toxic pollutants that are usually released into the environment. Humans are in close contact with cyanide in their existence through food, drink, smoking, medicines and products that contain cyanide[5,6].

Cyanide quantity in effluents ought to be restricted to a minimum. According to Vietnam's national standards, the permissible limits of cyanide in domestic water for irrigation, and waterways are 0.005 mg/L and 0.02 mg/L respectively[7,8].

In water environment, cyanide exists in solution as stable complex with heavy metals and partly in a free state. The cyanide determination in environmental samples requires distillation to convert cyanide in complex solutions into a free state[9,10].

Therefore, we have tried to measure the conversion efficiency of stable cyanide complexes to determine cyanide in metal plating wastewater samples in Thanh Oai district, Hanoi, Vietnam.

Materials and Methods

pH HM-25R meter was procured from TOA Japan and Balance Analytical Model GP 150 - 3P was procured from Sartorius Germany with accuracy of ±0.1 mg. The cyanide distillation equipment (KCM) was procured from Behr Company, Germany. All glass equipment was cleaned carefully using a mixture of concentrated sulfuric acid and potassium dichromate.

Chemicals - Standard cyanide solution with different concentrations: 1000 mg/L, 100 mg/L and 10 mg/L;
Analytical CN^- solution, 2 mg/L concentration;
Chloramine T, 1% solution ;
Pyridine-barbituric acid reagent was prepared as per procedure in literature;

Complex solutions of $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$ and acid and base solutions with different concentrations for pH adjustment.

Research on the conversion efficiency of CN^- - contained complexes

The complex of CN^- with metals is usually sustainable. Thus, the study of the biodegradable complex to transform into HCN vapour is very important[8,11]. Since the samples of wastewater from metal plating include disproportionately large iron complexes which are sustainable (complexation constants β are large)[12,13]., we focussed our experiment on the two sustainable complexes of the irons viz. $[Fe(CN)_6]^{4-}$ ($\beta_6 = 10^{36.9}$) and $[Fe(CN)_6]^{3-}$ ($\beta_6 = 10^{43.9}$).

The flowchart of the conversion efficiency of CN^- contained complexes research process is illustrated in Figure 1.

The distillation time of stable cyanide complex solutions was set at t = 10, 20, 30, 40, 60, 75, and 90 minutes. The distillation efficiency (H) was calculated as follows:

$$H = \frac{m_{tn}}{m_{lt}} \times 100\%$$

where m_{tn} is the amount of the distilled CN^- and m_{lt} is the amount of the theoretical CN^- in the distillation flask.

The optimal distillation time to achieve the highest distillation efficiency was determined based on its evaluation at different times.

CN^- was determined by photometric method based on the oxidation of cyanide by Chloramine T to generate CNCl at pH < 8 (to avoid the hydrolytic reaction). Then, CNCl combines with pyridine - barbituric reagent generating a pink complex with maximum absorption at 580nm[8,10]. The standard-line equation was $A = (2.511 \pm 0.072)*C_{CN-} + (0.018 \pm 0.009)$[14]. The correlation coefficient $R^2 = 0.9985$ satisfies $0.99 \le R^2 \le 1$.

To evaluate the efficiency of stable cyanide complexes, we re-determined the CN^- concentrations in $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$ complexes distilling at the selected optimal time, i.e., t = 60 minutes.

The recovery rate of complex solution was calculated according to the following equation:

$$R\% = \frac{C_{m+c} - C_m}{C_c} \times 100$$

where R% is the recovery rate, C_{m+c} is the concentration of the substance in an additional standard sample, C_m is the concentration of the substance in the sample and C_c is the additional standard concentration.

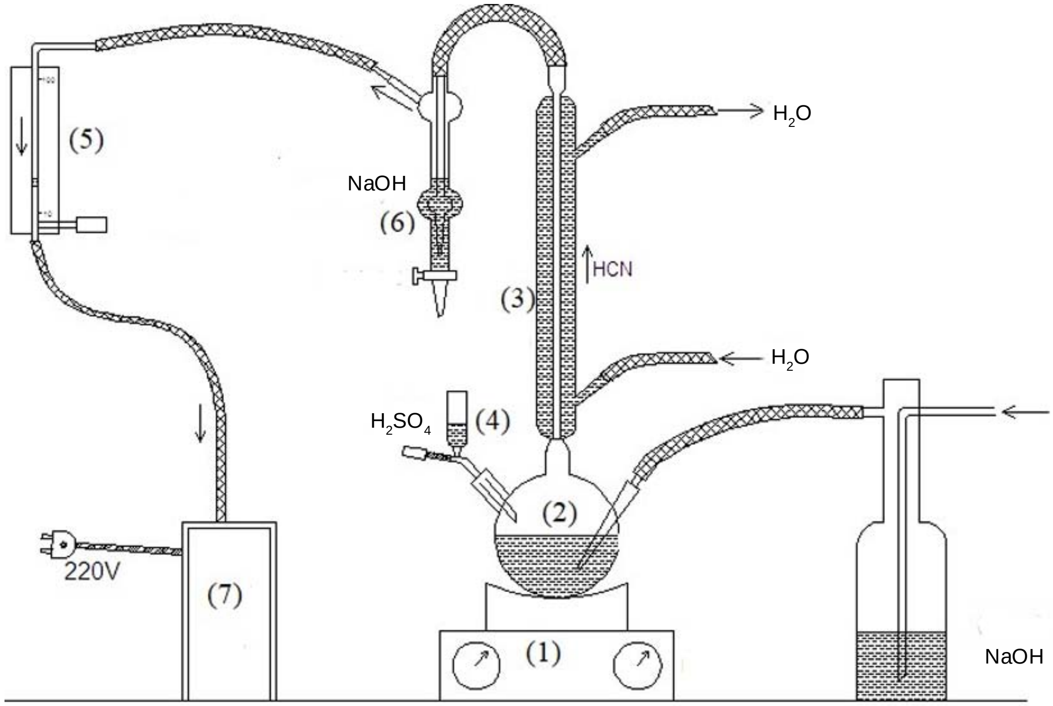
The general recovery rate was calculated by repeating the above experiments multiple times and taking the average recovery rates[15,16].

The waste-water samplings in a private metal-plating operation in Thanh Oai district, Hanoi city were done based on national standards and technical regulations such as TCVN 6663-1:2011 (ISO 5667-2: 1991), TCVN 6663-3:2008; TCVN 5999:1995 and QCVN 40:2011/ BTNMT[7]. Samplings in Thanh Thuy trade village, Thanh Oai district, Hanoi city, Vietnam (14 positions) are presented in Table 1.

Table 1. Wastewater sampling locations in Thanh Oai district, Hanoi city

Sample Notation	Sampling Location	Coordinates
TO1	At the disposal gate of washing tanks of zinc plating workshop in Rua Ha hamlet	20º52’34,58’’N 105°48'51.75"E
TO2	At the waste-water culvert gate of zinc plating workshop in Rua Ha hamlet	20°52'34.65"N 105°48'30.48"E
TO3	At the 500-meter spot of wastewater culvert gate of zinc plating workshop in Rua Ha hamlet	20°52'34.58"N 105°48'28.70"E
TO4	At the disposal gate of the electrical tank of electrical plating workshop in Rua Thuong hamlet	20°52'49.39"N 105°48'42.05"E
TO5	At the wastewater culvert gate of electrical plating workshop in Rua Thuong hamlet	20°52'49.15"N 105°48'41.78"E
TO6	At the 800-meter spot of wastewater culvert gate of electrical plating workshop in Rua Thuong hamlet	20°52'46.97"N 105°48'44.35"E
TO7	At the disposal gate of washing tanks of plating workshop at the beginning position in Rua Ha hamlet	20°52'32.85"N 105°48'32.49"E
TO8	At the wastewater culvert gate of plating workshop at the beginning position in Rua Ha hamlet	20°52'32.99"N 105°48'32.41"E
TO9	At the culvert gate near the Rua Ha bridge and school towards Ao Sen field (this position has many mechanical manufacturing factories)	20°52'31.13"N 105°48'31.33"E
TO10	Wastewater from washing tanks of plating workshop near Dong Bai	20°52'34.55"N 105°48'38.71"E
TO11	At the culvert gate of plating workshop near Dong Bai	20°52'34.84"N 105°48'39.10"E
TO12	At the culvert gate in the middle of the village (this position has many mechanical manufacturing operations).	20°52'31.10"N 105°48'33.80"E
TO13	Wastewater at the culvert gate at the crossroads between Rua Thuong and Rua Ha hamlet (Households are concentrated here and there are fewer manufacturing activities here).	20°52'38.88"N 105°48'37.22"E
TO14	Wastewater at the culvert gate of the industrial zone at the entrance of Thanh Thuy trade village.	20°52'19.41"N 105°48'16.25"E

Complex solutions and environmental samples were distilled through a specialized distillation system as shown in Figure 1.



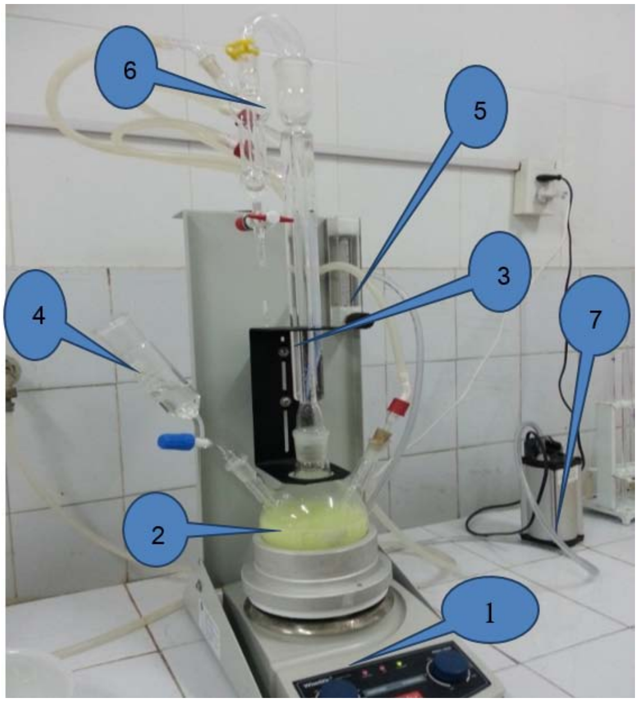


Fig. 1. Cyanide distillation system

(1) Electric heater

(2) 500 mL- Distillation flask

(3) Water-cooled condenser

(4) Funnel for H_2SO_4

(5) Flowmeter

(6) Absorption vaze with NaOH

(7) Vacuum pump MVP200

250 mL of sample solution was taken in a 500 mL distilling flask and 5 mL of 1.25 M NaOH solution was taken in a residual flask for absorption.

The stopcock was unlocked to add 25 mL of 9 M H_2SO_4 solution into a distilling flask to acidify the sample and release Cyanide in the form of HCN vapor.

The solution was boiled in a distillation flask and HCN vapor was absorbed in an absorption flask.

Results and Discussion

Evaluation results of CN^- concentrations at different times

The results of the determination of $CN^–$ concentrations in solution at different distillation times of complex solutions of $[Fe(CN)_6]^{4–}$ and $[Fe(CN)_6]^{3–}$ at different values (*t* = 10, 20, 30, 40, 60, 75, and 90 minutes) are presented in Table 2. From the results obtained, 60 minutes was selected as the optimal distillation time.

Table 2. Evaluation results of the distillation time for complexes $[Fe(CN)_6]^{4–}$ and $[Fe(CN)_6]^{3–}$

No.	Distillation time (minutes)	$[Fe(CN)_6]^{4-}$			$[Fe(CN)_6]^{3-}$		
		m_{lt} $CN^–$ weight in distillation tank (mg)	m_{tn} Determined CN^- weight (mg)	Efficiency H (%)	m_{lt} CN^-weight in distillation tank (mg)	m_{tn} Determined CN^- weight (mg)	Efficiency H (%)
1	10	0.125	0.064	51.20	0.125	0.051	40.80
2	20		0.077	61.60		0.064	51.20
3	30		0.095	75.60		0.081	64.80
4	40		0.107	85.60		0.091	72.80
5	60		0.122	97.80		0.103	82.40
6	75		0.122	97.80		0.103	82.40
7	90		0.122	97.80		0.103	82.40

Evaluation results of $CN^–$ concentrations in complexes after distillation

Table 3 shows the results of the $CN^–$ concentration re-determination in complexes of $[Fe(CN)_6]^{4–}$ and $[Fe(CN)_6]^{3–}$ after the distillation at its optimal time (with seven experiments).

Table 3. The CN^- concentration redetermination in $[Fe(CN)_6]^{4–}$ and $[Fe(CN)_6]^{3–}$ after distillation

Samples	Theoretical C^o_{CN} (mg/L)	$C_{CN–}$ (mg/L)	Average C_{CN-} (mg/L)	H (%)
$[Fe(CN)_6]^{4-}$ (0.5 mg/L)	0.025	0.022	0.025 ± 0.002	100 ± 0.08
		0.027		
		0.024		
		0.028		
		0.025		
		0.026		
		0.023		
$[Fe(CN)_6]^{3-}$ (0.5 mg/L)	0.025	0.021	0.021± 0.001	84 ± 0.02
		0.019		
		0.020		
		0.025		
		0.017		
		0.021		
		0.022		

For $[Fe\ (CN)_6]^{3-}$ solution, the concentration 0.021 mg/L (only 84%) is perhaps due to a larger stable constant $\beta_6 = 10^{43.9}$ than that of $[Fe(CN)_6]^{4-}$ complex ($\beta_6 = 10^{31.9}$).

The recovery rate of cyanide in solution

We repeated the distillation experiment six times for CN^- samples with pre-defined concentrations to get the recovery rate of complex solutions of $[Fe(CN)_6]^{4-}$and $[Fe(CN)_6]^{3-}$ as shown in Tables 4 and 5.

Table 4. The recovery rate of $[Fe(CN)_6]^{4-}$ solution

	Sample + 0.01 mg CN^-/L	Recovery R%	Sample + 0.02 mg CN^-/L	Recovery R%	Sample + 0.04 mg CN^-/L	Recovery R%
1	0.033	110	0.040	90	0.066	110
2	0.038	110	0.046	95	0.063	90
3	0.034	100	0.046	110	0.061	92.5
4	0.037	90	0.050	110	0.061	82.5
5	0.035	90	0.048	110	0.064	95
6	0.033	80	0.042	85	0.068	107.5
R_{TB}%		**96.7**		**100**		**96.3**

Table 5. The recovery rate of the $[Fe\ (CN)_6]^{3-}$ complex solution

	Sample + 0.01 mg CN^-/L	Recovery R%	Sample + 0.02 mg CN^-/L	Recovery R%	Sample + 0.04 mg CN^-/L	Recovery R%
1	0.030	90	0.037	80	0.063	105.0
2	0.028	90	0.040	105	0.060	102.5
3	0.029	90	0.041	105	0.059	97.5
4	0.033	80	0.042	85	0.061	90.0
5	0.025	80	0.039	110	0.056	97.5
6	0.032	110	0.042	105	0.055	85.0
R_{TB}%		**90.0**		**98.3**		**96.3**

Tables 4 and 5 indicate that the average recovery rates R_{TB} varied from 90 to 100% and satisfied the value as prescribed by AOAC[16]. This confirms the liberation of CN^- as HCN by distillation. This fact is fundamental to determining the cyanide concentration in the environmental samples.

Determination of the cyanide concentration in metal-plating waste-water in Thanh Oai district, Hanoi city

We conducted experiments to determine the cyanide concentration in ten waste-water samples of a private metal plating operation in Thanh Oai district, Hanoi city. The cyanide concentrations were compared with C_{max} which was indicated in the National Technical Regulation on Industrial Waste-water QCVN 40:2011/BTNMT[7] (C_{max} = 0.108 mg/L). The results are presented in Table 6.

Table 6. Results of the CN^- concentration determination in wastewater samples in Thanh Oai *district, Hanoi city*

Location	The first stage sample		The second stage sample	
	C_{CNtb} (mg/L)	Statistic C_{TB} (mg/L)	C_{CNtb} (mg/L)	Statistic C_{TB} (mg/L)
TO1	0.362	0.362±0.006	0.352	0.352 ± 0.004
TO2	0.100	0.100±0.008	0.079	0.079 ± 0.005
TO3	0.048	0.048±0.004	0.052	0.052 ± 0.004
TO4	0.118	0.118±0.003	0.126	0.126 ± 0.004
TO5	0.098	0.098±0.006	0.076	0.076 ± 0.004
TO6	0.052	0.052±0.004	0.048	0.048 ± 0.003
TO7	0.257	0.257±0.004	0.259	0.259 ± 0.006
TO8	0.246	0.246±0.005	0.231	0.231 ± 0.005
TO9	0.063	0.063±0.005	0.048	0.048 ± 0.003
TO10	0.505	0.505±0.001	0.498	0.498 ± 0.003
TO11	0.362	0.362±0.006	0.344	0.344 ± 0.006
TO12	0.032	0.032±0.005	-	-
TO13	-	-	0.052	0.052±0.005
TO14	-	-	-	-

As shown in Table 6, the cyanide concentration in the wastewater samples under study varied from 0.032 to 0.362 mg/L. 71.4 percent (10/14) of sampling locations surpass the cyanide concentration permissible limit. The analytic results in 14 locations also show that the average concentrations in the first and the second stages of analysis were 0.160 mg/L and 0.155 mg/L respectively. While the average concentration value for the whole year was 0.157 mg/L.

Cyanide concentration in wastewater samples over three years varied from 0.018 to 0.456 mg/L. Among 14 sampling samples, 10 sites had cyanide concentration from 0.1112 to 0.456 mg/L. These concentrations exceed the permissible limit by 1.04 to 4.22 times. The average cyanide concentration over three years was 0.1821 which exceeded the allowed limit by 1.69 times. The cyanide concentration decreased gradually from waste-water samples (near plating and washing tanks of the plating operation) namely TO1, TO7, TO10, and TO11 to waste-water samples taken at the culvert gates. Two samples TO13 and TO14 had very low cyanide concentration. These samples were taken from wastewater canals far away from the source; therefore they were more diluted and had lower cyanide concentration. The analytic results in two seasons (dry season and rainy season) were quite similar. However, since the canal was shallow in the dry season, the cyanide concentration in this season was slightly greater than that in the rainy one.

Conclusions

Thanks to a specialized cyanide distillation, we determined the optimal distillation time for complexes of $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$ (60 minutes), during which the conversion efficiencies of $[Fe(CN)6]^{4-}$ and $[Fe(CN)6]^{3-}$ reached 100 ± 0.08% and 84 ± 0.02%, respectively. Besides, the average recovery rate of complexes is as per the requirements of AOAC. Based on the results, we confirmed that the stable cyanide complexes could be converted almost completely and the CN^- ion in complexes could be released nearly entirely in the form of HCN gas.

We also determined the cyanide concentration in metal-plating waste-water samples of a private operation in

Thanh Oai district, Hanoi city, Vietnam. The results showed that the cyanide concentration varied from 0.018 to 0.456 mg/L, while the average concentration in 3 years was 0.1821 mg/L. It is worth noting that 71.4 percent of waste-water samples (10/14 samples) had the cyanide concentration exceeding 1.04 to 4.22 times the permissible limit.

References

1. Le Huy Ba., 2006, Environmental Toxicology, Ho Chi Minh City National University Publishing House, p.26-28.

2. World Health Organization Geneva, 2004, Hydrogen cyanide and cyanides: human health, Concise International Chemical Assessment Document, **61**.

3. Agency for Toxic Substances and Disease Registry (ATSDR), 1993, Toxicological Profile for Cyanide. The US. Dept. of Health and Human Services. Public Health Service. TP-**92**/09.

4. Agency for Toxic Substances and Disease Registry (ATSDR), 2006, Toxicological Profile for cyanide, PB2007-**100674** U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA, p.153-159.

5. Chandra H., Grupa B.N., Bhargave S.H., Clerk S.H. and Mahendra, P.N., 1980, *Journal Anal. Toxicol.*, **4**, 61- 165.

6. Blanc P., Hogan M., Mallin K., Hryhrczuk D., Hessl S. and Bermard B., 1985, *JAMA*, **253**, 367-371.

7. Ministry of Resources and Environment, 2011, National technical regulation on industrial water quality. QCVN **40**: 2011/BTNMT.

8. APHA-AWWA-AEF, 1998, Standard Methods for Examination of water and wastewater, 20th Edition.

9. Ministry of Science, 1999, The issuance of technological procedures for the disposal and reuse of cyanide. Decision 1971/1999-QD-BKHCNMT.

10. Perkin Elmer, 1995, Water and Environmental Analysis (According to US EPA Regulations).

11. Cincinnati, OH, 1983, Methods for chemical analysis of water and waste, method **335.2**. Cyanide, total, environmental monitoring systems laboratory (EMSL).

12. Mundder, T., Botz, M. and Smith, A., 2001, The Chemistry and Treatment of Cyanidation Wastes, second edition, Mining Journal Books, London, UK.

13. Mohammd Sarwar, Farhat Jamshaid and Usman Alikhan 1996, *Jour. Chem. Soc. Pak.*, **18(1)**.

14. Bui Thi Thu and Dao Van Bay, 2014, *Journal of Science of Hanoi National University of Education*, **59(4)**, 45-53.

15. Doerffel Dozent, 2008, Statistics in Analytical Chemistry, Vietnam National University Publishing House.

16. Tran Cao Son, 2010, Method verification in chemical and micro-organism analysis, National Institute of Food Control, Science and Technics Publishing House, p.39-59.

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A Comparative Study of the Transition Metal Ion Catalyzed Oxidation of Perfumery Alcohols by Kinetic Methods

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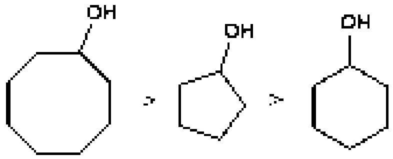
Abstract
Oxidation of alcohols is an industrially important reaction. The controlled oxidation of the cyclic alcohols, Cyclopentanol, Cyclohexanol and Cyclooctanol to the corresponding ketones and the primary acyclic alcohols, Geraniol, Nerol and Citronellol to the corresponding aldehydes was studied using Ce(IV) in acidic medium.

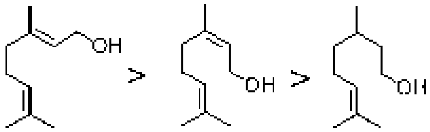
The N-haloamine,Sodium-N-chloro-p-toluenesulphonylamide was used in alkaline medium to oxidize1) acyclic primary alcohols, Geraniol, Nerol and Citronellol to aldehydes and 2)secondary cyclic alcohols, Borneol and Isoborneol to the corresponding ketones. All these alcohols are used in the manufacture of perfumes, fragrances and cosmetics. Cyclohexanol is an important feedstock in the polymer industry for the manufacture of nylon and plasticizers. The oxidation rates were determined using first order kinetic methods in which [oxidant]<< [alcohol] and the progress of the reaction was monitored by titrimetric estimation of the unreacted oxidant at regular time intervals during the course of the reaction.

The observed sequences of oxidation rates of alcohols are:

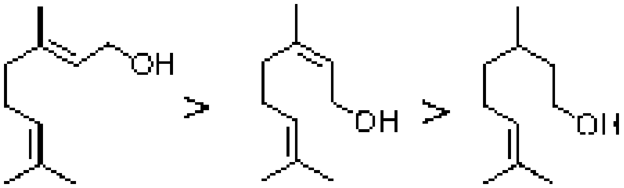
1) *Cyclooctanol > Cyclopentanol>Cyclohexanol using Ce (IV) as oxidant in acidic medium and in the micellar phase using Sodium dodecyl sulphate surfactant,*



2) *Geraniol> Nerol > Citronellol using Ce (IV) as oxidant in acidic medium,*



3) *Borneol> Isoborneol using N-haloamine as oxidant in alkaline medium,*



4) Geraniol > Nerol > Citronellol using N-haloamine as oxidant in alkaline medium.

The sequences of oxidation rates of alcohols have been explained on the basis of their steric factors, structural and isomeric characteristics, ring size and ring stability.

Environmentally hazardous and expensive Os(VIII),Cr(VI) and Ru(III) ions have been used on a large scale to catalyze organic reactions. We have used relatively less hazardous and cheaper transition metal ions, Mn(II),Co(II),Ni(II),Cu(II) and Zn (II) to catalyze the oxidation of perfumery alcohols. For each alcohol, the sequence of catalytic efficiencies of the transition metal ions has been determined based on Irving-Williams order of stability constants of metal complexes formed during the oxidation reaction[1].But some deviations from the expected sequence of catalytic efficiencies have been observed. Suitable reaction mechanisms have been suggested for the oxidation of alcohols in the presence and absence of transition metal ion catalysts. The reaction mechanism suggested for the transition metal ion catalyzed oxidation of alcohols indicates the formation of transient complexes involving hypervalent M(III) ions formed due to the their low third ionization enthalpies.

Keywords: *Perfumery alcohols, oxidation, kinetic methods, transition metal ion catalysts, reaction mechanism, hypervalent metal ions*

Introduction

The quantitative aspects of the conversion of alcohols to carbonyl compounds has been extensively reported[1-3].N-halogen compounds have been widely used as oxidizing agents in organic reactions[4-6].

This paper discusses the comparative rates of oxidation of the following types of perfumery alcohols:

1)a) Cyclic alcohols-Cyclopentanol, Cyclohexanol and Cyclooctanol and b)Primary acyclic alcohols, Geraniol, Nerol and Citronellol using Ce(IV) in acidic medium. Due to the low solubility of the alcohols, the oxidation was studied in micellar phase using Sodium dodecyl sulphate (SDS).

2)a) Primary acyclic alcohols, Geraniol, Nerol and Citronellol and b)Secondary cyclic alcohols, Borneol and Isoborneol using the N-haloamine, Sodium-N-chloro-p-toluenesulphonylamide in alkaline medium.

The oxidation was carried out using first order kinetic methods with respect to the oxidant ie [ox.] << [alc.] and the progress of the reaction was monitored titrimetrically. The sequences of oxidation rates of alcohols have been explained on the basis of their steric factors, isomeric and structural features, ring size and stability. Using similar methods, transition metal ions of the first series have been used to catalyze the oxidation of alcohols and the sequence of their catalytic efficiencies has been determined for each alcohol under study. Suitable reaction mechanisms have been suggested for the oxidation of alcohols under study, in the presence and absence of transition metal ion catalysts.

Materials and Methods

The perfumery alcohols were procured from E. Merck, Schuchalt, Germany and S.H. Kelkar & Co. Ltd., Mumbai. Some of the alcohols were distilled before use. Analar Grade AR (BDH) metal salts were used for the catalytic studies. All other chemicals and reagents used were of Analytical Grade (AR).

The solutions of alcohol and oxidant in required amounts were allowed to equilibrate in a previously adjusted thermostat having accuracy +/- 0.1^{0}C.After the temperature equilibrium was attained, the solutions were mixed to initiate the reaction. Aliquots of the reaction mixture were withdrawn at regular time intervals, the reaction was quenched using ice and the unreacted oxidant was estimated titrimetrically. Unreacted Ce(IV) was estimated by titration against standard Ferrous ammonium sulphate in sulphuric acid using Ferroin as an indicator. The unreacted N-haloamine was estimated iodometrically.

The first order rate constants (k) were determined from the linear plots of log (unreacted oxidant] versus time. For each alcohol, the rate of oxidation increased with [alc.].

An identical procedure was followed to study the catalytic effect of transition metal ions on the rate of oxidation of alcohols in the concentration range,

[M(II)]=0.25-2.50 x 10^{-3}mol dm^{-3}. For each metal ion catalyst, the oxidation rates of alcohols increased with [M(II)].

Results and Discussion

1) Oxidation of Cyclic and Primary acyclic alcohols by Ce(IV) in acidic medium

The rate constants of oxidation of i)Cyclic alcohols, Cyclopentanol, Cyclohexanol and Cyclooctanol and ii) Primary acyclic alcohols, Geraniol, Nerol and Citronellol by Ce(IV) in acidic medium are shown in Tables 1 and 2 respectively. For all the alcohols studied, the rate of oxidation increased with alcohol concentration.

Table 1 : Rate constant data for the oxidation of Cyclic alcohols by Ce(IV) in acidic medium and in micellar phase using Sodium dodecyl sulphate

[H_2SO_4] = 1mol dm^{-3} [SDS]=0.05 mol dm^{-3} Temperature = 303K

[alc.] x 10^1 mol dm^{-3}	[ox.] x 10^2 mol dm^{-3}	k x 10^4 s^{-1} Cyclopentanol	k x 10^4 s^{-1} Cyclohexanol	k x 10^4 s^{-1} Cyclooctanol
0.25	0.50	0.46	0.48	4.86
0.50	0.50	0.73	0.54	6.38
0.63	0.50	0.77	0.56	6.60
0.75	0.50	1.17	0.59	7.20
0.88	0.50	1.13	0.62	7.75
1.00	0.50	1.29	0.73	8.29

The sequence of oxidation rates is Cyclooctanol > Cyclopentanol >Cyclohexanol

Table 2: Rate constant data for the oxidation of Primary acyclic alcohols by Ce(IV) in acidic medium

[H_2SO_4] = 1 mol dm^{-3} Temperature = 303

[alc.] x 10^1 mol dm^{-3}	[ox.] x 10^2 mol dm^{-3}	k x 10^3 s^{-1} Geraniol	k x 10^3 s^{-1} Nerol	k x10^3 s^{-1} Citronellol
0.25	0.50	2.80	2.01	0.32
0.50	0.50	3.11	2.57	0.35
0.63	0.50	3.47	3.00	0.39
0.75	0.50	3.76	3.50	0.42
0.88	0.50	4.30	3.66	0.45
1.00	0.50	4.85	4.37	0.58

The sequence of oxidation rates is Geraniol > Nerol >Citronellol.

2) Reaction mechanism of oxidation of alcohols by Ce(IV) in acidic medium

For a secondary alcohol RR' CHOH, the reaction mechanism suggested is:

$$RR'CHOH + Ce(IV) \overset{K}{\leftrightarrow} \underset{\text{Complex}}{[RR'CHOH\text{-}Ce]^{IV}} \text{ ....(i)}$$

$$[RR'CHOH\text{-}Ce]^{IV} \underset{\text{slow}}{\overset{k}{\rightarrow}} RR'C^{*}OH + Ce(III) + H^{+} \text{ ....(ii)}$$

where RR'C*OH is a free radical generated during the course of the reaction.

$$RR'C^{*}OH + Ce(IV) \overset{\text{fast}}{\rightarrow} RR'C^{+}OH + Ce(III) \text{ ....(iii)}$$

$$RR'C^{+}OH \overset{\text{fast}}{\rightarrow} \underset{\text{ketone}}{RR'C{=}O} + H^{+} \text{ ....(iv)}$$

Net reaction : $RR'CHOH + 2Ce(IV) \rightarrow RR'C = O + 2\ Ce(III) + 2H^{+}$

In the case of a primary alcohol, RCH_2OH, the product of oxidation is an aldehyde, RCHO.

The products of oxidation of alcohols viz aldehyde and ketone were identified by 2,4-Dinitrophenylhydrozone test and confirmed by TLC.

In aqueous H_2SO_4 medium, Ce(IV) has been found to oxidize alcohols through complexation followed by generation of free radicals[7-9].

The oxidation of oxidation rates of Cyclic alcohols follows the sequence :

Cyclooctanol > Cyclopentanol > Cyclohexanol. This as per expectation as 5, 7 and 8 membered rings in cyclic alcohols are more reactive than 6 membered rings (as in Cyclohexanol)[10-12]. 6 membered rings have least strain and hence are least susceptible to oxidation.

The oxidation of the primary acyclic alcohols follows the sequence:

Geraniol > Nerol > Citronellol. The reaction rates of oxidation of alcohols are consistent with respect to their steric effects on the oxidation. Geraniol has the least hindered α-hydrogen hence its oxidation is fastest whereas Citronellol has the most hindered α-hydrogen and is therefore least susceptible to oxidation.

3) Transition metal ion catalyzed oxidation of Cyclic alcohols and Primary acyclic alcohols by Ce(IV) in acidic medium

1) The transition metal ion catalyzed oxidation of the Cyclic alcohols, Cyclooctanol, Cyclopentanol and Cyclohexanol by Ce(IV) in acidic medium was studied in the micellar phase using metal ions in the concentration range $[M(II)] = 0.50\text{-}2.50 \times 10^{-3}$ mol dm^{-3}.

For all cyclic alcohols studied, the rate constant of oxidation increased with [M(II)] (Table 3).

Table 3: Rate constant data for the transition metal ion catalyzed oxidation of Cyclic alcohols by Ce(IV) in acidic medium in micellar phase

[alc.]=0.2 mol dm^{-3} [Ce)IV)]=0.05 mol dm^{-3} [H_2SO_4]= 2.0 mol dm^{-3}
[SDS]=0.05 mol dm^{-3} Temperature=303K

$[M(II)] \times 10^{3}$ mol dm^{-3}	$k \times 10^{4}$ s^{-1} Ni(II)	$k \times 10^{4}$s^{-1} Cu(II)	$k \times 10^{4}$s^{-1} Zn(II)
Cyclopentanol			
In absence	0.90	0.90	0.90
0.50	0.92	0.94	0.96
1.00	0.95	1.00	1.07
1.50	1.05	0.85	1.21

[M(II)] x 10^3 mol dm^{-3}	k x 10^4 s^{-1} Ni(II)	k x $10^4 s^{-1}$ Cu(II)	k x $10^4 s^{-1}$ Zn(II)
2.00	1.18	0.84	1.06
2.50	1.33	0.80	1.06
Cyclohexanol			
In absence	0.36	0.36	0.36
0.50	0.40	0.46	0.32
1.00	0.48	0.56	0.37
1.50	0.57	0.67	0.39
2.00	0.64	0.79	0.42
2.50	0.72	0.90	0.55
Cyclooctanol			
In absence	0.23	0.23	0.23
0.50	0.49	1.96	2.01
1.00	0.75	2.16	2.32
1.50	1.01	2.19	2.69
2.00	1.26	2.60	2.72
2.50	1.32	2.84	2.69

The sequences of oxidation rates of Cyclic alcohols are:

Cyclopentanol : Zn(II) > Ni(II) > Cu(II) (Table 3)

Cyclohexanol : Cu(II) > Ni(II) >Zn(II) (Table 3)

Cyclooctanol : Zn(II) > Cu(II) > Ni(II) (Table 3)

2) The transition metal ion catalyzed oxidation of the primary acyclic alcohols,Geraniol,Nerol and Citronellol by Ce (IV) in acidic medium was studied in the metal ion range [M(II)]=0.25-0.50 x10^3mol dm^{-3}. For all alcohols studied, the oxidation rate increased with [M(II)] (Table4).

Table 4: Rate constant data for the transition metal ion catalyzed oxidation of Primary acyclic alcohols by Ce (IV) in acidic medium

[alc.] = 0.1 mol dm^{-3} [Ce(IV)] = 2.5 x 10^{-3} mol dm^{-3} [H_2SO_4] = 1.0 mol dm^{-3}

Temperature = 303K

[M(II)] x 10^3	k x $10^3 s^{-1}$	k x $10^3 s^{-1}$	k x 10^3 s^{-1}	k x $10^3 s^{-1}$	k x $10^3 s^{-1}$
mol dm^{-3}	Mn(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
Geraniol					
In absence	4.85	4.85	4.85	4.85	4.85
0.25	5.67	5.39	5.11	5.10	4.88
0.30	6.52	5.67	5.68	5.39	5.04
0.35	6.75	6.34	6.03	5.83	5.42
0.40	7.83	6.78	6.50	6.39	5.60
0.45	8.36	7.26	7.04	6.86	5.79
0.50	9.52	7.99	7.36	7.10	6.31

$[M(II)] \times 10^3$ mol dm^{-3}	k x $10^3 s^{-1}$ Mn(II)	k x $10^3 s^{-1}$ Co(II)	k x $10^3 s^{-1}$ Ni(II)	k x $10^3 s^{-1}$ Cu(II)	k x $10^3 s^{-1}$ Zn(II)
Nerol					
In absence	4.34	4.34	4.34	4.34	4.34
0.25	4.63	4.53	4.65	4.44	4.58
0.30	5.22	4.90	5.18	5.00	4.88
0.35	5.79	5.37	5.79	5.39	5.35
0.40	6.71	5.81	6.36	5.82	5.67
0.45	7.61	6.70	6.72	6.21	6.02
0.50	8.64	7.89	7.38	6.69	6.46
Citronellol					
In absence	3.53	3.53	3.53	3.53	3.53
0.25	4.08	3.69	3.77	3.89	3.75
0.30	4.35	4.21	4.15	4.48	3.94
0.35	4.86	4.87	4.50	4.82	4.31
0.40	5.31	5.05	4.71	5.30	4.76
0.45	5.68	5.52	5.08	5.69	5.06
0.50	6.39	5.71	5.24	5.83	5.83

The sequences of oxidation rates of primary acyclic alcohols are:

Geraniol : Mn(II) > Co(II) > Ni(II) > Cu(II) > Zn(II) (Table 4)

Nerol : Mn(II) > Co(II) > Ni(II) > Cu(II) > Zn(II) (Table 4)

Citronellol : Mn(II) > Co(II) > Cu(II) > Ni(II) > Zn(II) (Table 4)

4) Reaction mechanism of the transition metal ion catalyzed oxidation of alcohols by Ce(IV) in acidic medium

Mn, Co, Ni and Cu form the hypervalent ions, Mn(III),Co(III),Ni(III) and Cu(III) respectively as their third ionization enthalpies are comparatively small unlike Zn whose energy of solvation cannot suffice to make the 3+ chemical state stable.

a) Reaction mechanism in presence of Mn(II),Co(II),Ni(II) and Cu(II) ions

$$RR'CHOH + M(II) \overset{K}{\leftrightarrow} \underbrace{RR'CHOH.M(II)}_{\text{Complex } C_1} \text{ ....(i)}$$

$$RR'CHOH.M(II) + Ce(IV) \xrightarrow[\text{slow}]{k} \underbrace{RR'CHOH.M(III)}_{\text{Complex } C_2} + Ce(III) \text{ ....(ii)}$$

The electron transfer takes place slowly[13].

$$\text{Complex } C_2 \xrightarrow{\text{fast}} RR'C^{*}OH + H^{+} + M(II) \text{ ....(iii)}$$

where RR'C*OH is a free radical generated during the course of the reaction[7-9].

$$RR'C^{*}OH + Ce(IV) \xrightarrow{\text{fast}} RR'C^{+}OH + Ce(III) \text{ ....(iv)}$$

$$RR'C^{+}OH \xrightarrow{\text{fast}} \underbrace{RR'C{=}O}_{\text{ketone}} + H^{+}$$

Net reaction : $RR'CHOH + 2Ce(IV) \rightarrow RR'C = O + 2H^{+} + 2Ce(III)$

In the case of a primary alcohol, RCH_2OH the oxidation product is an aldehyde, RCHO.

b) Reaction mechanism in presence of Zn(II) ions

A similar reaction mechanism can be given for the oxidation of alcohols in the presence of Zn(II) ions except that Zn will always be in the divalent state (Zn (II)) during the oxidation reaction.

The catalytic efficiency of metal ions is inversely proportional to the stability of their complexes formed as short lived intermediates during the course of reaction.

The stability is generally dependent on the charge density of the metal ions involved in addition to many other factors. Thus, the stability order for the complexes of the metal ions understudy is expected to be Cu(II) > Zn(II) > Ni(II) > Co(II) > Mn(II)[14,15]; and the catalytic efficiencies of the metal ions are in reverse order i.e. Mn(II) > Co(iI) > Ni(II) > Zn(II) >Cu(II).

However these generalizations are approximate guides to metal ion behavior[16] and literature survey shows some discrepancies in the sequence of catalytic efficiencies of metal ions.

In our study, we have observed some differences between the experimentally observed sequence of catalytic efficiencies of metal ions and the theoretically expected sequence (Table 3 (All cyclic alcohols) and Table 4 (All Primary acyclic alcohols).

5) Oxidation of Primary acyclic and secondary cyclic alcohols by Sodium -N-chloro-p- toluenesulphonylamide in alkaline medium

The rate constants of oxidation of 1) Primary acyclic alcohols, Geraniol,Nerol and Citronellol and 2)Secondary cyclic alcohols, Borneol and isoborneol by Sodium –N-chloro-p-toluenesulphonylamide in alkaline medium are shown in Tables 5 and 6 respectively. The oxidant is represented as TsNClNa where Ts is the tosyl group,H_3C-C_6H_5-SO_2- For all the alcohols studied, the oxidation rates increased with alcohol concentration.

Table 5: Rate constant data for oxidation of Primary acyclic alcohols by Sodium-N-chloro-p-toluenesulphonylamide (TsNClNa) in alkaline medium

[NaOH]=0.05 mol dm^{-3} Temperature=303K

[alc.] x 10^1	[TsNClNa]x 10^2	k x 10^4s^{-1}	k x 10^4s^{-1}	k x 10^4s$^{=1}$
mol dm^{-3}	mol dm^{-3}	Geraniol	Nerol	Citronellol
0.25	0.50	4.57	3.08	1.32
0.50	0.50	5.52	3.24	2.03
0.63	0.50	5.14	3.75	3.45
0.75	0.50	5.60	3.89	3.52
0.88	0.50	5.94	4.27	4.00
1.00	0.50	6.30	4.69	4.64

The sequence of oxidation rates of Primary acyclic alcohols is
Geraniol > Nerol >Citronellol

Table 6 : Rate constant data for oxidation of Secondary cyclic alcohols by Sodium-N-chloro-p-toluenesulphonylamide (TsNClNa) in alkaline medium

[NaOH]=0.05 mol dm^{-3} Temperature=303K

[alc.] x 10^1	[TsNClNa]x 10^2	k x 10^4s^{-1}	k x 10^4s-^{1}
mol dm^{-3}	mol dm^{-3}	Borneol	Isoborneol
0.25	0.50	2.45	1.27
0.50	0.50	7.37	1.38
0.63	0.50	10.00	2.04
0.75	0.50	12.70	2.30
0.88	0.50	14.90	3.24
1.00	0.50	22.10	3.90

The sequence of oxidation rates of secondary cyclic alcohols is Borneol > Isoborneol

6) Reaction mechanism of the oxidation of alcohols by Sodium –N-chloro-p-toluenesulphonylamide (TsNClNa) in alkaline medium

In aqueous medium, TsNClNa acts as a strong electrolyte and ionizes as follows:

$TsNClNa \rightarrow TsNCl^- + Na^+$

In alkaline medium, the reaction mechanism suggested is

$$TsNCl^- + H_2O \overset{K_1}{\leftrightarrow} TsNHCl + OH^- \text{ ....(i)}$$

$$RCH_2OH + TsNHCl \overset{K_2}{\leftrightarrow} Complex \text{ ....(ii)}$$

$$Complex \xrightarrow[\text{slow}]{k} RCHO + TsNH_2 + HCl \text{ ....(iii)}$$

In the case of a secondary alcohol, the corresponding ketone is formed.

The oxidation products ie aldehyde and ketone are identified by 2,4 -Dinitrophenylhydrozone test and confirmed by TLC.

The sequence of oxidation rates of Primary acyclic alcohols under study is Geraniol > Nerol > Citronellol. Geraniol has the least hindered α-hydrogen atom hence is oxidized fastest whereas Citronellol has the most hindered α-hydrogen atom hence is least susceptible to oxidation.

The relative rates of oxidation of the Secondary cyclic alcohols, Borneol and Isoborneol are consistent with respect to their steric effects on the oxidation.Borneol has less hindered α-hydrogen than Isoborneol hence undergoes faster oxidation than Isoborneol.

Conclusions

The sequences of oxidation of the perfumery alcohols studied are as follows:

i) Cyclic alcohols: Cyclooctanol > Cyclopentanol > Cyclohexanol with Ce(IV) as oxidant in acidic medium,
ii) Primary acyclic alcohols: Geraniol > Nerol >Citronellol with Ce)IV) as oxidant in acidic medium.

Less hazardous and less costly transition metal ions have been effectively used to catalyze the oxidation of alcohols and the sequence of their catalytic efficiencies has been determined for each alcohol.

In the case of Sodium-N-chloro-p-toluenesulphonylamide as oxidant in alkaline medium, the oxidation rates follow the sequences:

i) Primary acyclic alcohols:Geraniol > Nerol >Citronellol,
ii) Secondary cyclic alcohols: Borneol > Isoborneol.

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References

1. Hudlicky, M., 1990, Oxidations in Organic Chemistry, ACS Monographs, p.186.

2. Lee, S.V., Madin, A., Trost, B.M. and Fleming, I., (Eds.), 1991, Comprehensive Organic Synthesis, Vol. 7, Pergamon Press, Oxford, p.251.

3. (a) Corey, E.J. and Suggs, J.W., 1975, *Tetrahedron Letts.*, 2647.

   (b) Corey, E.J. and Boyer, B.L., 1978, *Tetrahedron Letts.*, 240.

   (c) Corey, E.J. and Schmidt, G., 1979, *Tetrahedron Letts.*, 299.

4. Singh, A.K., Jain, B., Negi, R., Katre, Y., Singh, S.P. and Sharma, V.K., 2009, *Transition Met. Chem.*, **4**, 521-528.

5. Gunasekaran, S. and Venkatasubramanian, N., 1983, *Proc. Indian Acad. Sc. (Chem. Sc.)*, **92(1),** 107-112.

6. Jagdeesh, R.V. and Puttaswamy, 2008, *J. Phy. Org. Chem.*, **21(10),** 844-858.

7. Richardson, H., 1965, Oxidation in Organic Chemistry (Part1), Wiberg K. B., (Ed.) Academic Press, New York, p.244.

8. Mino, G., Kaizerman, S. and Rasmussen, F., 1959, *J. Am. Chem. Soc.*, **81**, 1494.

9. Duke, F.R. and Forist, A.A., 1949, *J. Am. Chem. Soc.*, **71**, 2790.

10. Brown, H.C. and Herstein, H., 1952, *J. Am. Chem. Soc.*, **74,** 2929.

11. Kuivila, H.G. and Becker, B.T., 1952, *J. Am. Chem. Soc.*, **74**, 5329.

12. Brown, H.C., Fletcher, R.S. and Johannesen, R.B., 1957, *J. Am. Chem. Soc.*, **79**, 212.

13. Asim K. Das, 2000, *J. Ind. Chem. Soc.*, **77**, 225.

14. Irving, H. and Williams, R.J.P., 1953, *J. Am. Chem. Soc.*, 3192.

15. Mellor, D.P. and Maley, L., 1947, *Nature*, **158**, 370, 1948**,** *Nature*, **161**,436.

16) Willims, R.J.P., 1956, *J. Chem. Soc.*, **8.**

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Role of Ethylene in Horticulture – A Review

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Abstract

The phytohormone ethylene plays a central role in plant physiological and developmental processes, such as germination, growth, flower initiation, senescence of leaves and flowers, organ abscission, degreening of citrus, and fruit ripening. To understand the production, action and role of ethylene in horticultural crop production, many researches have been conducted. Therefore, the objective of this review is to compile relevant finds on role of ethylene in Horticulture. Ethylene is a major signal, mediating responses to a range of biotic and abiotic stresses. It strongly influences nearly every development stage in plant growth, from germination to fruit ripening and senescence. The sources of ethylene can be ripening fruit, germinating seeds, exhaust from internal combustion engines/heaters, smoke (including cigarettes), welding, rotting vegetation, natural gas leaks, and manufacturing plants of some kinds. Inspite of its importance ethylene also has detrimental effects on horticulture commodity. The major problems are loss of green tissue of leafy vegetables and cucumber, postharvest loss of fruit, lettuce russet spotting (dark brown spotting of the mid-ribs of lettuce leaves), bitter tasting carrots, sprouting of potatoes and toughening of asparagus and turnips; yellowing and abscission (dropping) of leaves in Brassicas; softening, pitting, and development of off-flavor in peppers, summer squash, and watermelons; browning and discoloration in eggplant pulp and seed; discoloration and off-flavor in sweet potatoes; and increased ripening and softening of mature green tomatoes; and some common flowers that are ethylene sensitive include carnations, delphiniums, freesia, gypsophila and Geraldton wax. Thus in order to minimize the bad effects of ethylene, one should know thoroughly the production and action of ethylene.

Keywords: *Abiotic, Biotic, Ethylene, and Phytohormone*

Abbreviations

ACC	1-Aminocyclopropane-1-Carboxylic Acid
ACO	ACC Oxidase
ACS	ACC Synthase
1-MCP	1-Methylcyclopropene
MTA	5´-Methylthioadenosine
MAPKKK	Mitogen-Activated Protein Kinase Kinase Kinase
PG	Polygalacturonase
PHYA	Phytochrome A
PSY	Phytoene Synthase
SAM	*S*-Adenosyl-L-methionine

Introduction

Ethylene and other plant growth regulators (PGRs) are important chemicals in agricultural production. Plant growth regulators are now used worldwide on a diversity of crops each year. The plant hormone, Ethylene (C_2H_4) strongly influences nearly every development stage in plant growth, from germination to fruit ripening and senescence. Moreover, its critical role in post-harvest physiology of agricultural products has also been well documented. Obviously, a compound with so many different effects may be useful in many ways to modify plant growth and development as required by growers. However, many factors including its gaseous nature and some negative effects on plant growth, restrict the extensive practical usefulness of C_2H_4.[5]

Ethylene has been used since the times of the ancient Egyptians, who would use them to stimulate ripening in figs (wounding stimulates ethylene production by plant tissues). The ancient Chinese would burn incense in closed rooms to enhance the ripening of pears. In 1864, it was discovered that gas leaks from street lights led to stunting of growth, twisting of plants, and abnormal thickening of stems. In 1901, a Russian scientist named Dimitry Neljubow showed that the active component was ethylene. Sarah Doubt discovered that ethylene stimulated abscission in 1917. It wasn’t until 1934 that Gane reported that plants synthesize ethylene. In 1935, Crocker proposed that ethylene was the plant hormone responsible for fruit ripening as well as senescence of vegetative tissues.[37]

Ethylene (C_2H_4) is colorless flammable gas, simplest alkene (a hydrocarbon with carbon-carbon double bonds) with a faint “sweet and musky” odour when pure (https://en.wikipedia.org/wiki/Ethylene). It is produced by higher plants, bacteria, and fungi and influences many aspects of plant growth and development.[1] It is used in fruit ripening, germination of seed, degreening of citrus and eliminating sour taste of fruit. It can also cause damage in crops. Examples of damage might include yellowing of vegetables, bud damage in dormant nursery stock, or abscission in ornamentals (leaves, flowers drop off). Often two of the important items to be known are if a crop naturally produces a lot of ethylene; and if it is responsive to ethylene. Responsiveness will depend on the crop, the stage of plant development, the temperature, the concentration of ethylene, and the duration of exposure[18, 28] (http://postharvest.tfrec.wsu.edu/pages/PC2000F, 2010).

The sources of ethylene can be ripening fruits, germinating seeds, exhaust from internal combustion engines/heaters, smoke (including cigarettes), welding, rotting vegetation, natural gas leaks, and manufacturing plants. There are also several anti-ethylene chemicals. Silver thiosulfate (STS) is used on flowers. Amino-ethoxyvinyl-glycine (AVG, trade name ReTain) blocks ethylene synthesis. It is applied preharvest. The fruit (plant) will not produce much ethylene, so there is ethylene response. The ethylene blocker 1-methylcyclo-propene (1-MCP, trade name EthylBloc) blocks ethylene by binding to its receptor. It is applied on plants. The fruit (plant) may still produce some ethylene, but there is no response to ethylene.[36]

A lot of research has been done in recent years in the elucidation of ethylene biosynthesis and perception and transduction pathways and consequently in the understanding of the role of ethylene in Agriculture.

Ethylene Production

Ethylene Production by Biosynthesis

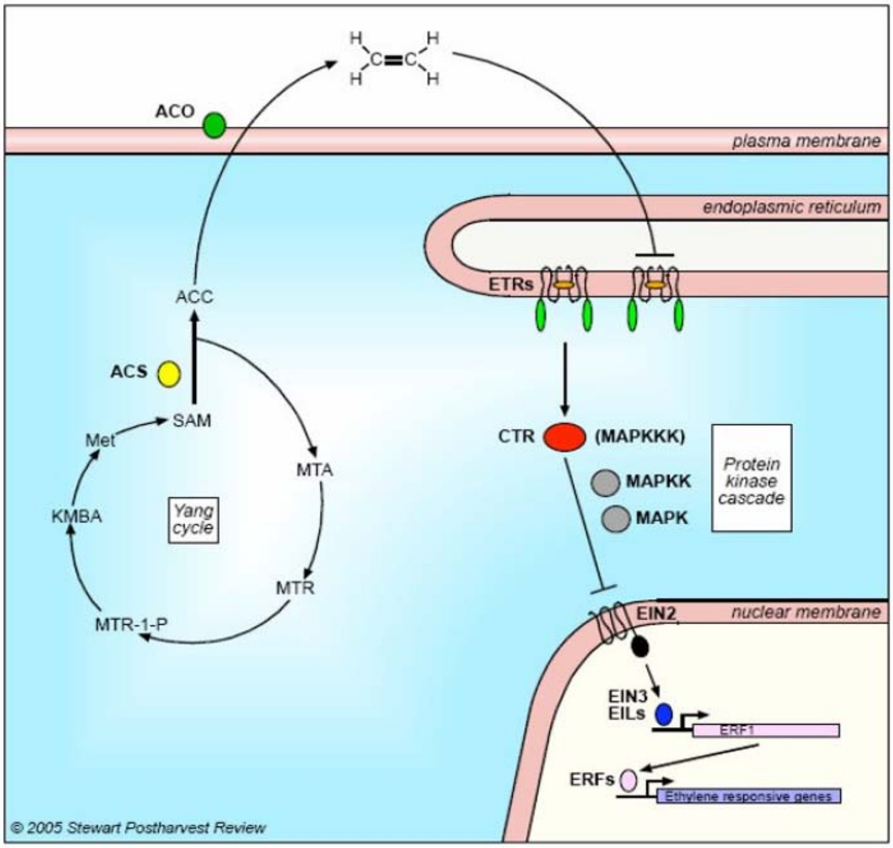
Ethylene is produced from all parts of higher plants, including leaves, stems, roots, flowers, fruits, tubers, and seeds. Ethylene production is regulated by a variety of developmental and environmental factors. During the life of the plant, ethylene production is induced during certain stages of growth such as germination, ripening of fruits, abscission of leaves, and senescence of flowers.

Ethylene production can also be induced by a variety of external aspects such as mechanical wounding, environmental stresses, and certain chemicals including auxin and other regulators. The pathway for ethylene biosynthesis is named the Yang cycle after the scientist Shang Fa Yang who made key contributions to elucidating this pathway.[8, 39]

Ethylene is biosynthesized from the amino acid methionine to *S*-adenosyl-L-methionine (SAM, also called Adomet) by the enzyme Met Adenosyltransferase. SAM is then converted to 1aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS). The activity of ACS determines the rate of ethylene production, therefore regulation of this enzyme is the key for the ethylene biosynthesis. The final step requires oxygen and involves the action of the enzyme ACC-oxidase (ACO), formerly known as the ethylene forming enzyme (EFE). Ethylene biosynthesis can be induced by endogenous or exogenous ethylene. ACC synthesis increases with high levels of auxins, especially indole acetic acid (IAA) and cytokinins.[8]

Ethylene is produced in the following sequence of steps in plants:

Methionine → S-Adenosylmethionine (SAM) → 1-AminoCyclopropane-1-Carboxylic acid (ACC) → ethylene (Seid, 2014).



Abbreviations: ACO, ACC Oxidase: ACS, ACC Synthase: MAPKKK. Mitogen-Activated Proein Kinase Kinase Kinase

Fig. 1. Pathways of the biosynthesis and signal transduction of ethylene [8]

The above figure illustrates the processes involved in ethylene synthesis and action after binding to a receptor which causes the release of a “second messenger” which interacts directly or indirectly with the cell nucleus stimulating the expression of the genes necessary to bring about the effect.[8]

Factors affecting ethylene production and action

Genotype (species and cultivar): Flowers produce high levels of ethylene just before they fade and wither away e.g. fading flowers of Vanda orchids are recorded to produce 4300. mL ethylene/kg/h.[6] Tables 1 and 2 show typical ethylene production rates of a range of horticultural commodities and low levels at which exogenous ethylene can have an effect on horticultural commodities respectively.

Table 1: Rates of Ethylene production[6]

Class	**μL ethylene/kg/h at 20^0C**	**Commodities**
Very low	0.01-0.1	Cherry, citrus, grape, jujube, strawberry, pomegranate leafy vegetables root vegetables potatoes and cut flowers
Low	0.1-1.0	Blueberry, cucumber, okra, peppers, persimmon, pineapple and raspberry
Moderate	1.0-10.0	Banana, fig, honeydew melon, mango and tomato
High	10.0-100.0	Apple, apricot, avocado, cantaloupe, feijoa, kiwifruit, nectarine, papaya, peach, pear and plum
Very high	>100.0	Cherimoya, passion fruit, sapota and mammee apple

Table 2: Threshold for ethylene action on various fruits[31]

Fruit	**Threshold conc. (ppm)**
Avocado	0.1
Banana- (var.Gros Michel – (var.Lacatan)	0.1-1 0.05
(var.Silk fig)	0.2-0.25
Honeydew melon	0.3-1
Lemon -(var.Fort Meyers)	0.1
Mango (var.Kent)	0.04-0.4
(var.Valencia)	0.1
Tomato (var. VC-243-20	0.5

1) **Physiological age:** Ethylene production and response of climacteric fruits depends on their physiological age. The following figure indicates ethylene production by papaya fruit at different stages.

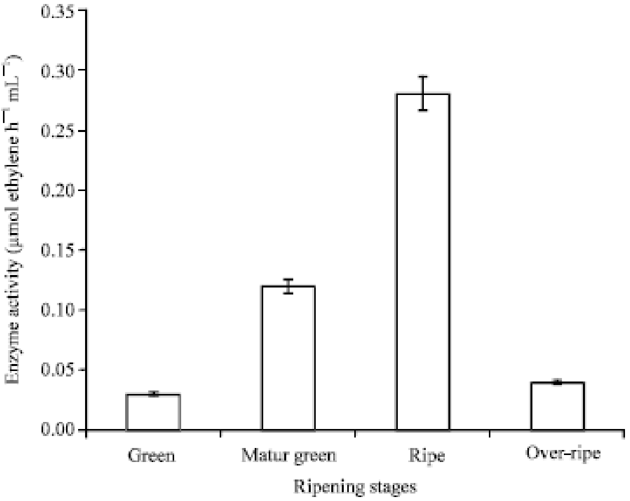


Fig 2: Ethylene production during the ripening stages of papaya *(Carica papaya)*[19]

2) Temperature: It has also a great effect on rates of ethylene production. Though it is different from crop to crop, mostly peak ethylene production is at ~25°C and is inhibited above 30°C.

Table 3: Temperature effects on ethylene production by stone fruit

Fruit	µL C_2H_4/kg.h			
	0^0C	**5^0C**	**10^0C**	**20^0C**
Nectarine	0.01-5	0.02-10	0.05-50	0.1-160
Peach	0.01-5	0.02-10	0.05-40	0.1-140
Plum	0.01-5	0.02-15	0.04-60	0.1-200

3) Atmospheric composition: One of the effects of changing atmospheric composition is to alter the synthesis of and reaction to ethylene. Low levels of oxygen inhibit ethylene synthesis and high levels of carbon dioxide inhibit the effect of ethylene by binding to ethylene receptors. When the level of oxygen is reduced (<8%), ethylene production rates and action are reduced. While elevated O_2 levels stimulate ethylene production and action. By constrast, CO_2 competitively inhibits ethylene action, consequently, it can also inhibit autocatalytic ethylene production. CO_2 injury can induce elevated ethylene production.[3]

4) Exogenous ethylene: Ethylene exposure induces climacteric fruits to initiate autocatalytic ethylene production[18].

5) Other hydrocarbons: Propylene, carbon monoxide, acetylene, etc. can enhance ethylene production by fruits and mimic ethylene action.[34]

Table 4: Gases that may mimic ethylene effects[34]

Compound	Relative Activity: Moles/unit effectiveness
Ethylene	1
Propylene	130
Vinyl chloride	2370
Carbon monoxide	2900
Acetylene	12500
1-Butene	140000

6) Stresses: Physical damage, diseases, fumigation, irradiation, etc. are all stresses that stimulate ethylene production.[9, 17]

7) Growth regulators: May reduce or stimulate ethylene production depending on the growth regulator.[38]

8) Inhibitors: Biosynthesis inhibitors (AVG, AOA) action inhibitors (CO_2, Ag , Ag+, MCP)[16, 36]

Commercial use of Ethylene for Horticultural Crops

Ethylene has a wide variety of potential agricultural uses. This is true, at least in part, because of the numerous ways ethylene-regulated plant processes may be manipulated. It is possible to apply ethylene in gas condition to stimulate the plant to synthesize the gas. Plant functions in which ethylene plays a regulatory role may be promoted or inhibited by manipulating either the synthesis or action of endogenous ethylene. There are also means of removing ethylene from plants or plant products.[5]

The varied effects of ethylene include growth inhibition, growth promotion, root initiation, flower initiation and prevention, modification of flower sex, fruit growth stimulation, fruit ripening, storage product hydrolysis, pigment synthesis promotion and inhibition, latex secretion promotion, modification of flavor, fruit degreening, participation in expression of plant disease symptoms, promotion of leaf, flower, and fruit abscission[5] and dehiscence, release of seed and bud dormancy and release of apical dominance. Additional effects of less apparent agricultural value include: modification of geotropic behavior, tissue proliferation and formative growth, leaf epinasty, leaf movement, inhibition and respiratory changes. In addition, CEPA, an ethylene substrate, has been shown to promote tillering and stem stiffness of small grains and modest yield increase in some crops. Ethylene is a natural regulator of some of these processes when applied to the plant (http://www.actahort.org/

members/showpdf?booknrarnr=34_3). It can stimulate lateral branching in potted plants (azaleas and geraniums) at low concentration.[18]

Ethylene for Seed Germination and Root Formation

During germination, ethylene appears to have two roles. Ethylene can break seed dormancy in some species. It can also reduce the time required for radical emergence in non-dormant seeds. These two activities are probably related to making the seeds less sensitive to abscisic acid, which keeps the seeds dormant.[1] For some seeds, like lettuce, the actual role of ethylene in seed germination is obscure and is used to overcome dormancy due to high temperature.[27] In some cases, ethylene has been shown to be essential for germination, while in other instances it is not needed.

Production of ethylene is initiated by certain factors, such as chilling, light, and imbibition that promote germination, and many species, such as wild oat (*Avena fatua* L.)[4] and Virginia-type peanut (*Arachis hypogaea* L.)[21] manufacture ethylene during germination.[24] Thus, dormancy may be broken in some species by exposure to ethylene during early imbibition.[24] Ethylene (1-100 ppm) was applied to lettuce seeds during imbibition to braek the dormancy that had been caused by heating the seeds at 97°C for 30 hours before imbibition. During the time before radical emergence in the lettuce seeds, ethylene was required, but in the first part of imbibition, ethylene was not needed. At the time of radical protrusion in nondormant lettuce seeds, there was a rapid increase in ethylene production which peaked at 24 hours and then decreased.[13] For most seeds, ethylene production increases with radical emergence, peaks soon after, and then declines.[24]

Ethylene also has effect on germination of lettuce. Abeles (1986) found that there was a limited 5°C range in which thermo inhibition in lettuce seeds can be surpressed by ethylene. Any temperature greater than 30°C did not permit ethylene to initiate germination.[1] Thermoinhibition in lettuce seeds occurs when the endosperm cannot be pierced by the embryo at temperatures greater than 25°[1]. The same effect was found in a study by Dunlap and Morgan (1977) in which ethylene had no effect on lettuce seed germination at 36°C, but it did at 32°C. Prevention of ethylene production by the seeds did not impact thermoinhibition.[1] However, in lettuce seeds, the transformation of ACC to ethylene was hindered by high temperatures[22]. In the temperature range of 20 to 25°C, lettuce seeds may only need to manufacture a small amount of endogenous ethylene for germination.[27] On the other hand, ethylene was necessary when lettuce seeds were subjected to a stressful environment, such as 35°C, while undergoing imbibition; at this high temperature, ACC was not detected[15]. ACC synthesis had less of an effect on hindering the formation of ethylene than warm, stressful temperatures did, and warm, stressful temperatures prevented ethylene production altogether.[27]

In Arabidopsis, ethylene has a remarkable effect on root development: it inhibits elongation and promotes radial expansion. Similar effects are obtained with ACC, the ethylene precursor. Treatments with ethylene and ACC are effective in inducing root hairs.[30] The response is rapid: decrease in cell elongation and the induction of root hairs can be observed after incubation of minutes; with longer incubation times, ethylene affects the overall root shape, resulting in decreased root length and increased width. Arabidopsis seedlings grown in the presence of ethylene have shorter and thicker roots. A similar effect was observed when inoculating some legumes with particular strains of *Rhizobium*; in this case it was called a *tsr* phenotype (for thick short roots); this linked ethylene with nodulation. Indeed, ethylene insensitive mutants are hypernodulant.[29] Although in Arabidopsis the effect of treatment with ethylene and ethylene inhibitors is already detectable one or two days after germination, notable differences are observed also days later; when the seedlings are treated with ethylene, roots are shorter; when treated with norbornadiene, an inhibitor of ethylene action, roots are longer. Longer roots may be the result of three different, though not completely independent processes: increased cell division, reduced cell death, and increased cell expansion.[11]

Impatiens seeds treated with the ethylene biosynthetic precursor 1-aminocyclopropane- 1-carboxylic acid (ACC) had reduced times to radical protrusion and seedling emergence. Sixty hours after imbibition, 68% more seeds showed radical protrusion on media containing 0.1 or 1.0 mM ACC compared to seeds germinated in water alone.[23] As the following table indicates, speed of germination is increased as ethylene and ethylene precursor are used for stratification of Echinacea species (*E. simulate).*[41]

Table 5: Three day and final germination percentage of *E. simulata* seeds stratified for either 1 day at 25°C or 35°C or 7 days at 5°C exposed to water, 5 mM ACC, or 1 mM ethephon.[41]

Treatment	**3-day**	**12-day**
1 day at 25°C		
Water	40b	48b
ACC	61ab	61ab
Ethephon	52ab	54ab
1 day at 35°C		
Water	42b	42b
ACC	45b	45b
Ethephon	47b	47b
7 days at 5°C		
ACC	50b	55ab
74a	74a	74a
Ethephon	55ab	55ab

ACC had a promotive effect on adventitious rooting cutting of two tomato cultivars namely NR (mutant none ripe) and wild-type.[8] Cuttings were treated with a range of ACC concentrations to determine if applied ethylene affected adventitious rooting. Wild-type cuttings treated with 30 mg g21 ACC produced approximately 22% more adventitious roots than control wild-type cuttings. NR cuttings treated with 10 mg g21 ACC produced approximately 41% more adventitious roots than control NR cuttings. NR cuttings never produced as many adventitious roots as wild-type cuttings at any applied ACC concentration (Table 5).

Table 6: Number of adventitious roots of wild-type and NR vegetative tomato cuttings treated with various concentrations of ACC Data were taken 3 weeks after the onset of propagation

[ACC] (mg/g)	**No. of Adventitious Roots**	
	NR tomato	**NR Wild type**
0	26.0 -6 3.1	54.6 -6 2.4
1	31.0- 6 2.8	55.5- 6 4.3
3	33.4- 6 2.6	56.8- 6 3.4
10	38.0 -6 2.6	60.0- 6 4.3
30	35.4 -6 2.3	69.9- 6 6.5
100	36.0- 6 2.5	57.3- 6 4.6
300	25.9 -6 2.2	51.5- 6 4.2
1000	21.3 -6 3.3	50.1- 6 3.8

Ethylene involved in plant tissue culture

Ethylene produced by plant tissues grown *invitro* may accumulate in large quantities in the culture vessels, particularly from rapidly growing non-differentiated callus or suspension cultures, and hence is likely to influence growth and development in such systems. Research into this aspect of tissue culture has been sparse, although it has grown recently with the increasing importance of *invitro* regeneration. This review deals with the measurement and relevance of the accumulated ethylene, and the influence of both exogenous and endogenous ethylene on the different types of tissue culture systems. The relationships between ethylene and other growth regulators in tissue culture growth and development are also discussed. Although in some cases its influence seems negligible, in many types of tissue culture, ethylene may act either as a promoter or inhibitor depending on the species used. Thus, ethylene has an important influence on many aspects of invitro regeneration, but it is also clear that we cannot at present describe a specific role or roles for ethylene in tissue culture which can be applied at a general, species-wide level. If its effects are to be enhanced or diminished in order to improve the efficiency and range of plant tissue culture, then more research is needed to clarify what its fundamental role might be in in vitro growth and development.[26]

Ethylene as ripening and senescence hormone

The ripening of fleshy fruits represents the unique coordination of developmental and biochemical pathways leading to changes in color, texture, aroma, and nutritional quality of mature seed-bearing plant organs. The gaseous plant hormone ethylene plays a key regulatory role in ripening of many fruits, including some representing important contributors of nutrition and fiber to the diets of humans. Examples include banana, apple, pear, most stone fruits, melons, squash, and tomato.[7] Ethylene can be explosive if it reaches high concentrations, so it has to be used cautiously. Several commercial liquid products release ethylene (ethephon, trade name Ethrel). These are only used preharvest.[32] The most commonly known use of ethylene is to trigger ripening in some crops, such as bananas and avocados. The application of ethylene at a controlled rate means that these products can be presented to the customer as "ready to eat". For avocados, this is a significant benefit as the consumer can now purchase a pipe avocado rather than buying a hard fruit that may take several days to ripen.[18] Table 7 shows beneficial postharvest applications.

Table 7: Ripening conditions for some fruit using ethylene[40]

Fruit	Temperature (ºC)	Ethylene (ppm)	Treatment time (hrs)
Avocado	18 – 21	10	24 – 72
Banana	15 – 21	10	24
Kiwifruit	18 – 21	10	24
Persimmon	18- 21	10	24
Tomato	13- 22	10	continuous

Although controlled ripening is the major postharvest use of ethylene, it can also be applied pre-harvest to promote postharvest benefits. The chemical Ethephon produces ethylene and is applied. In the field the use of Ethephon can have several benefits such as fruit thinning (apples, cherries), fruit loosening prior to harvest (nuts), colour development (apples), degreening (citrus), and flower induction (pineapples). All these responses are the result of the plant being exposed to fairly low concentrations of the plant growth regulator ethylene.[18] Ethylene is thought of as the aging hormone in plants. In addition to causing fruit to ripen, it can cause plants to die. It can be produced when plants are injured, either mechanically or by disease. Ethylene will cause a wide range of effects in plants, depending on the age of the plant and how sensitive the plant is to ethylene. Ethylene effects include fruit ripening, loss of chlorophyll, abortion of plant parts, stem shortening, abscission of plant parts, and epinasty (bending of stems).[7]

Negative Impacts of Ethylene and Remedies in some Horticultural crops

The negative impacts of ethylene on horticultural crops resulted in huge postharvest losses which can discourage all involved groups especially in the postharvest chain. Thus, action should be taken in order to minimize this evil effect of ethylene.[33] The major reason for the loss of shelf life is that ethylene exposure increases the rate of deterioration as the product ages.[18] In green tissues such leafy vegetables and cucumbers, ethylene stimulates the loss of chlorophyll or green colour and also increases the susceptibility of the product to rots.[28] Lettuce is also susceptible to ethylene. It suffers from a distinctive disorder, called russet spotting as a result of exposure to ethylene. The symptoms of russet spotting are dark brown spotting of the mid-ribs of lettuce leaves. The quality of carrots can also be reduced as a result of ethylene exposure. In response to ethylene, carrots produce bitter tasting compounds called isocoumarins. It has been shown that concentrations of ethylene as low as 0.5 ppm can cause bitterness in carrots within 2 weeks of storage.[31] Ethylene can also promote sprouting of potatoes and toughening of asparagus and turnips; yellowing and abscission (dropping) of leaves in Brassicas); softening, pitting, and development of off-flavor in peppers, summer squash, and watermelons; browning and discoloration in eggplant pulp and seed; discoloration and off-flavor in sweet potatoes; and increased ripening and softening of mature green tomatoes.[37]

A very obvious effect of ethylene is the loss of flowers or leaves from sensitive ornamental plants. Some common flowers that are ethylene sensitive include carnations, delphiniums, freesia, gypsophila and Geraldton wax.[28]

The damage resulting from ethylene exposure could easily be minimized if there was a greater awareness of the potential harm and the simple measures that can be used to prevent damage. Sensitive plants should be treated with an ethylene inhibitor, such as silver thiosulfate. Preventing ethylene buildup around the product is often the simplest method of reducing the damaging effects of ethylene. For example, products that are sensitive to ethylene are better handled with electric forklifts rather than gas powered ones. It is also important to remove over ripe or rotting fruit from the storage and handling area, as they are also sources of ethylene. For maximum shelf life and quality, green leafy vegetables should not be stored or transported in trucks where there are mixed loads containing ripening fruit such as apples, pears, mangoes, tomatoes or bananas. For ethylene sensitive products, it is important to avoid storing them along with products that produce high levels of ethylene.[28] Increasing the ventilation rate of the storage area, assuming that the outside air is ethylene free is another way of reducing the level of ethylene around fresh produce.[18]

Using ethylene scrubbers in storage areas to remove ethylene in the air is also another remedy for problems caused by ethylene. The traditional method had been to use potassium permanganate or Purafil. Potassium permanganate reacts with ethylene to produce carbon dioxide and water. In order to scrub the air efficiently it is best to spread the potassium permanganate over as larger a surface area as possible either in trays or within highly permeable bags. For cut flowers it is possible to pulse the stems with a solution of silver thiosulfate. The flowers take the solution up through their stems and this protects them from the effects of ethylene. There is another relatively new compound called EthylBlock. It is a gaseous compound that inhibits the effects of ethylene. It is effective at very low concentrations, as low as 10 parts per billion (ppb). The active ingredient is 1- MCP (1-methylcyclopropene). Use of 1-MCP on bananas prevents premature ripening. The ventilation rate of the storage area should be increased assuming that the outside air is ethylene free.[18]

Summary

Ethylene, hydrocarbon gas, well known as a growth hormone, is biologically active in trace amounts (as little as 10 nL). It promotes ripening, leaf and flower senescence and abscission, acceleration of respiration and modification of leaf and fruit pigments, onset of epinastic curvatures, and root initiation, and it causes loss of geotropic sensitivity. It also controls plumular expansion and maintains the plumular hook, which facilitates the emergence of germinating seedlings through the soil, a process vital to successful seed germination. In pea *(Pisum sativum* L), exogenous ethylene exaggerates the curvature of the apical hook, inhibits stem elongation, and prevents a normal geotropic response, effects known as the “triple response. Ethylene is produced in response to a plethora of abiotic and biotic stresses including flooding, wounding, viruses, bacteria, fungi, insects, and nematodes other plant hormones such as IAA, ABA, cytokinins, and methyl jasmonate; and small metabolites such as carbohydrates, orthophosphate, and polyamines.[12] Because of its profound effects on plant growth and development, the biosynthesis of ethylene, it’s action, and the control of its action by chemical, physical, and biotechnological means have been intensively investigated. Thanks to new tools available in biochemistry and molecular genetics, parts of the ethylene biosynthesis, perception, and signal transduction reactions have been elucidated. This knowledge has been applied to enhance the quality of a number of agronomically important crops.[14, 35]

Although of tremendous use to the horticulture commodity, ethylene has enormous detrimental effects. For instance: rotting of flowers, fruit and vegetable, sprouting of some seeds and quality deterioration of most horticultural commodities. To overcome these challenges, we should have greater awareness of the potential harm and the simple measures that can be used to prevent damage. Measurement can be inhibiting its production or action, scavenging, and treating the commodity to maintain commodities quality and quantity.

References

1. Abeles, F.B., 1986, Role of ethylene in *Lactuca sativa* cv ‘Grand Rapids’ seed germination. *Plant Physiol.*, **81**, 780-787.

2. Abeles, F.B., Morgan, P.W. and Saltveit, M.E., **1992**, Ethylene in Plant Biology. 2nd ed. Academic Press, Inc., San Diego, CA.

3. Adel A. Kader and Mikal E. Saltveit. n.d. Atmospheric Modification. University of California, Davis, California, U.S.A.

4. Adkins, S.W. and J.D. Ross. 1981, Studies in wild oat seed dormancy. I. The role of ethylene in dormancy breakage and germination of wild oat seeds (*Avena fatua* L.). *Plant Physiol.*, **67**, 358-36.

5. Arshad M, Frankenberger WT Jr. 2002, Ethylene: agricultural sources and applications. New York: Kluwer Academic/ Plenum Publishers.

6. Chris B. Watkins, Jacqueline F. and Nock., 2012, Production Guide for Storage of Organic Fruits and Vegetables. Department of Horticulture, Cornell University.

7. Cornelius S. Barry, James J. and Giovannoni, 2007, Ethylene and Fruit Ripening. Boyce Thompson Institute for Plant Research, Ithaca, New York.

8. David A. Brummell., 2005, Stewart Postharvest Review. Regulation and genetic manipulation of ripening in climacteric fruit. An international journal for reviews in postharvest biology and technology. New Zealand Institute for Crop & Food Research, Food Industry Science Centre, Batchelar Road, Palmerston North, New Zealand.

9. David G. Clark*, Erika K. Gubrium, James E. Barrett, Terril A. Nell, and Harry J. Klee., 1999, Root Formation in Ethylene-Insensitive Plants 1. Environmental Horticulture Department and Horticultural Sciences Department, University of Florida, Gainesville, Florida

10. Dunlap, J.R. and Morgan, P.W., 1977, Reversal of induced dormancy in lettuce by ethylene, kinetin, and gibberellic acid, *Plant Physiol.*, **60**, 222-224.

11. Emilio Cervantes, 2006, Ethylene in Seed Germination and Early Root Development.

12. Fluhr, R. and Mattoo, A.K., 1996, Ethylene-biosynthesis and perception. *Crit. Rev. Plant Sciences.*, **15**, 479-523.

13. Fu, J.R. and Yang, S.F., 1983, Release of heat pretreatment-induced dormancy in lettuce seeds by ethylene on cytokinin in relation to the production of ethylene and the synthesis of 1-aminocyclopropane-1-carboxylic acid during germination. *J. Plant Growth Regul.*, **2**, 185-191

14. Giovannoni, J., 2001, Molecular biology of fruit maturation and ripening. Annu. Rev. Plmjt *Physiol. Plant Mol. Biol.*, **52**, 725-749.

15. Huang, X.L. and Khan, A.A., 1992, Alleviation of thermo inhibition in preconditioned lettuce seeds involves ethylene, not polyamine biosynthesis, *J. Amer. Soc. Hort. Sci.*, **117**, 841-845.

16. Figueroa1, M. T. Colinas2, J. Mejía2 and F. Ramírez2, 2005, Postharvest Physiological Changes in Roses of Different Vase Life. Facultad de Agronomía, Universidad de Concepción Casilla. 537, Chillán, México.

17. Jean-Claude Pech, Mondher Bouzayen and Alain Latche. n.d. Physiological, Biochemical, and Molecular Aspects of Ethylene Biosynthesis and Action.

18. Jenny Jobling, 2000, Postharvest Ethylene: A critical factor in quality management. Sydney Postharvest Laboratory Information Sheet. www.postharvest.com.au.

19. Golden, O.J. Williams and Dunkley, H.M., 2014. Ethylene in Postharvest Technology: A Review. *Asian Journal of Biological Sciences,* **7**, 135-143.

20. Ketring, D.L. and Morgan. P.W., 1971, Physiology of oil seeds. II. Dormancy release in Virginia-type peanut seeds by plant growth regulators, *Plant Physiol.*, **47**, 488-492.

21. Ketring, D.L. and Morgan, P.W., 1972, Physiology of oil seeds. IV. Role of endogenous ethylene and inhibitory regulators during natural and induced after ripening of dormant Virginia-type peanut seeds. *Plant Physiol.*, **50**, 382-387

22. Khan, A.A. and Prusinski, J., 1989, Kinetin enhanced 1-aminocyclopropane-1-carboxylic acid utilization during alleviation of high temperature stress in lettuce seeds. *Plant Physiol.*, **91**, 733-737.

23. Dutt, S. Kester and Geneve, R., 2004, Elevated Levels of Ethylene During Germination Reduces the Time to Emergence in Impatiens. Department of Horticulture University of Kentucky Lexington Kentucky 40546, U.S.A.

24. Matilla, A.J. 2000. Ethylene in seed formation and germination. *Seed Sci. Res.*, **10**, 111-126.

25. Muhammad ArshadAffiliated withUniversity of Agriculture, William T. and Frankenberger, 2002, The Plant Hormone, Ethylene

26. Biddington, 1992, The influence of ethylene in plant tissue culture.

27. Nascimento W.M., 2003, Ethylene and lettuce seed germination. *Sci. Agric.*, **60**, 601-606.

28. Optimal Fresh, 2001, The Fruit, Vegetable & Fresh Produce Storage Expert System. CSIRO Publishing, Collingwood Vic., Australia.

29. Penmetsa R.V., Cook, D., 1997, A legume ethylene-insensitive mutant hyperinfected by its rhizobial symbiont. *Science,* **275**, 527-530.

30. Pitts, R.J., Cernac, A., Estelle, M., 1999, Auxin and ethylene promote root hair elongation in Arabidopsis. *Plant Journal,* **16**, 553-560

31. Reid, M., 1992, Ethylene in Postharvest Technology. In. Postharvest Technology of Horticultural Crops. Publication 3311 University of California p. 97 – 108.

32. Roisin McGarry, Jocelyn A. Ozga, and Dennis M. Reinecke, 2005, The effect of Ethephon on Saskatoon (Amelanchier alnifolia Nutt.) Fruit Ripening. Department of Agriculture, Food and Nutritional Science, University of Albert, Canada.

33. Seid Hussen, 2014, Ethylene as a Postharvest "Evil" and its Remedies in some Horticultural Crops.

34. Stanley P. Burg and Ellen A. Burg. N.d. Ethylene Action and the Ripening of Fruits. Ethylene influences the growth and development of plants and is the hormone which initiates fruit ripening.

35. Stepanova, A.N. and Ecker, J.R., 2000, Ethylene signaling: from mutants to molecules. *Cur. Opin. Plant Biol.,* **3**, 353-360.

36. Susan Lurie, 2005, Regulation of ethylene biosynthesis in fruits by aminoethoxyvinylglycine and 1-methylcyclopropene. Agricultural Research Organization, Bet Dagan 50250, Israel.

37. Suslow, T.V., 2000, Post-harvest handling for organic crops. Organic Vegetable Production in California Series. Pub. 7254. University of California Davis. Available online at: http://ucanr.org/freepubs/docs/7254.pdf.

38. Trivellini, A.1, Ferrante, A.2*, Lucchesini, M.1, Mensuali-Sodi, A.3,Vernieri, P.1, Tognoni, F.1 and Serra, G.3., 2007, Ethylene and abiscisic acid interaction during hibiscus (*Hibiscus rosa-sinensis* L.) flower development and senescence.

39. Wang KLC, Li H. and Ecker J.R., 2002, Ethylene biosynthesis and signaling networks. *Plant Cell,* **14**, S131–S151.

40. Wills, McGlasson, Graham and Joyce, 1998, Postharvest. An introduction to the physiology and handling of fruit, vegetables and ornamentals, 4th Edition, UNSW Press, Sydney.

41. Wood Laura Anne. 2007, Relationship between Ethylene and Seed Dormancy Release in Echinacea Species. University of Kentucky Master's Theses. Paper 465. http://uknowledge.uky.edu/gradschool_theses/465.

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Crown Ethers, Cryptands and Calixarene in the Solvent Extraction Separation of Elements

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Abstract

The macrocyclic, macrobicyclic polymers and newly synthesized supramolecules have opened up a new era in separation science. Such compounds include crownethers, cryptands and Calix[n]arenes. The mechanism of the complexation with metal ions is different with availability of hole or cavity in the centre of ligand. Further with existence of upper and lower rim in calix[n]arene, several derivatives useful for extractive separations were synthesized. The stereochemistry of metal complexes with these compounds is most interesting. This is followed by an account of synthesis of miscellaneous compounds using template effector with Friedel and Craft reactors. The chemistry of the coordination complexes of metals is most challenging on account of principles of solvent extraction which lay stress on the importance of extraction equilibrium. This is followed by an account of special features of the extractions with crown cryptands and Calixavenes. An exhaustive coverage is presented on solvent extrachem separation of main group, transition and the inner transition elements. The systematic study of analysis of S-block alkali and alkaline earths is the most significant contribution in chemistry of macrocyclic compounds. A rapid view of modern methods of analysis is also included.

1) What are Supramolecules?

With the early discovery of organic ligands like dimethyldioxime 8 Hydroxyquinoline or dithizone, newer methods were developed for the quantitative analysis of metals resulting in the formation of linear or opened structures. However, with the discovery of Macrocyclic ligands, Chemistry became most challenging for alkali metals. Further, with the discovery of supra-molecular compounds, the chemistry of their coordination complexes became more fascinating for transition elements. Such compounds not only included Crown ether, cryptands but also Calix[n] arenes, and Calix[n] resorcinarene. Furthermore, applications of atomic and molecular spectroscopic methods such as plasma emission or molecular luminance for their structure elucidation proved to be most interesting.

With the discovery of Crown ether by Pederson and Cram, the macrocyclic compounds and cryptands had opened up a novel field of complexation. Guitz contributed to the theme of synthesis of cyclic three dimensional structure compounds called Calix[n] arene and Calix[n] resorcinarene. They initiated research work in the synthesis of novel compounds by interaction of catechol and dichloroether to discover novel compounds called supramolecules. The first crown ether discovered was DB18C6. Further work by Cram and Lehn led to the synthesis of Cryptands which were bridged structures. The novelty of these compounds was that they have a bridge in their center. In crown ethers, if the size of hole matched with ionic size of the metal, they formed complexes.

For instance, the hole in center of DB18C6 matches with the ionic size of metal like Potassium to form stable complexes $[K^+$ DB1866]° The subsequent work led to the discovery of Calix[n]arene and Calix[n]resorcinarene. These were three dimensional structures with cone in the center of the molecule. With the variation in size of either hole or cone in center of metal, complexes were synthesized. They had reasonable stability. Initially compounds like crown ether or cryptands were termed as macrocyclic or macro bicyclicpolyethers, but with the discovery of calix(n)arene, the cyclic three dimensional structures were termed as Supramolecular compounds. The structure of 18C6 has 18 Oxygen and 5 Carbon atoms while the cryptand 2,2,2 is having bridge structure with N-atom. They were designed as Cryptand 2,2,2 with bridge through Nitrogen atom. Contrary to these compounds, Calix[n]arene were aromatic compounds with six aromatic rings (n=6). In quantitative analysis, alkali and alkaline earths of S-group elements readily formed complexes with various crown ethers. Several transition elements also formed coordination complexes with these ligands. The chemically similar metals like zirconium (IV) and hafnium (IV) or niobium (V) and tantalum (V) were best separated by solvent extraction with these compounds.

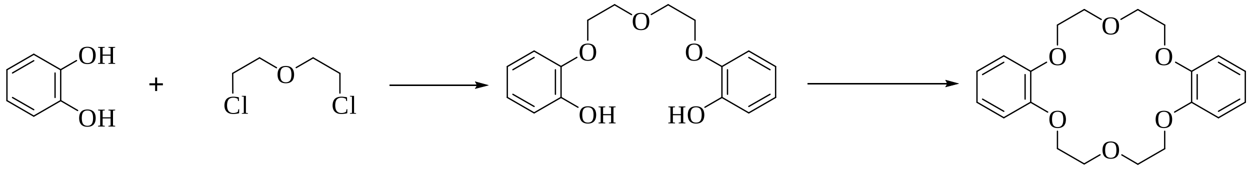
2) 2a Classification of new compounds:

These compounds are mostly used in the solvent extraction separations followed by instrumental analysis of these metals belonging to transition, inner transition or the main group elements. We have four groups of complexing ligands known as Podants, Cordants, cryptands and superamolecules. Thus 18C6 is cordant, cryptand 2,2,2 is member of cryptand group while Calix[n]arene or Calix[n]resorcinarene are supra-molecules having three dimensional structure. We have series of crown ethers such as 12C4, 15C5, 18C6 and 24C8, DB18C6, DB24C8 while the series of cryptands include cryptand 1, 1, 1 cryptand 2, 2, 1 cryptand 2, 2, 2 and cryptand 2, 2, 2B. Those are closed ring bridge spherands useful for extractive separations.

However, compounds with cyclic structures with n = 4, 5, 6 belong to the series of the calix(n)arene or analogous compounds. They are giant molecules with symmetrical structures which are most useful for solvent extraction and separation of s- and d- block metals.

The metal complexes have typical host guest structures. In these complexes, the metal is guest while the complexing ligand is host e.g. $[K^+$DB18C6]. The host is DB18C6 while K+ is guest. Large number of monographs and review articles[16-35] have appeared in literature in the last few deceedes.

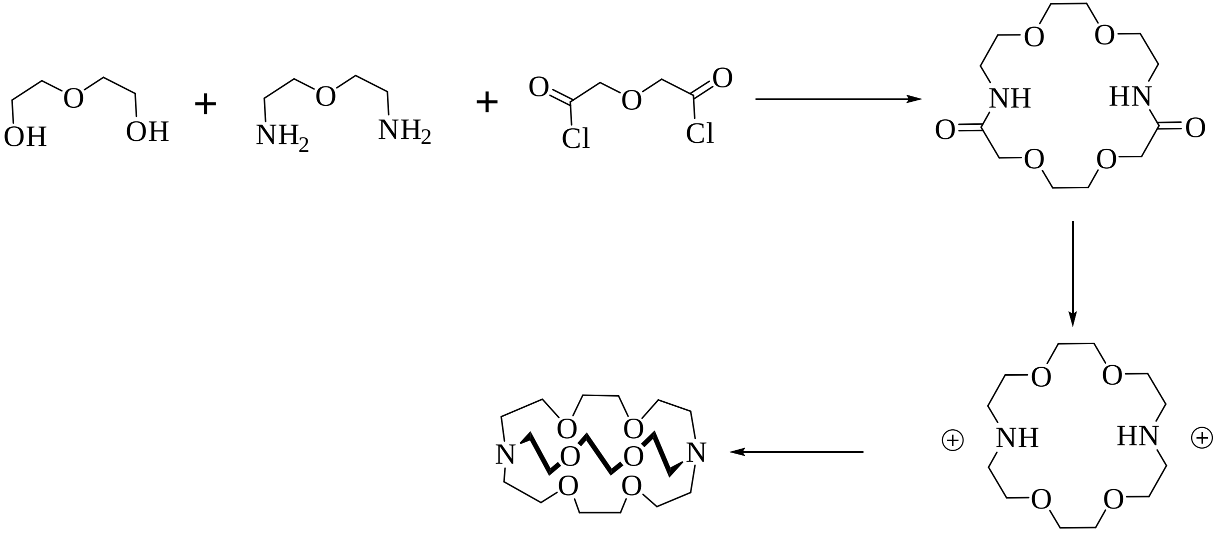
2b Consider the synthesis of a typical crown ether



The original synthesis[36-38] included reactions shown above. Thus, hydroxyl benzene was created with base to produce an intermediate compound called dianion which on interaction with 2,2 dichlorother undergoes Williamson reaction[36] in the presence of Sodium hydroxide in butanol results in a new product (III) DB18C6 with 40% yield. The process involves Template effective[37]. The Oligomerisation was performed with increase in base strength. The presence of metal enhances the yield. The hydrogenation of DB18C6 gives us lipophilic DC18C6, another new compound belonging to crown ether series. The size of the ring in the presence of heteroatom and nature of side arms are vital[36-37] for good synthesis. Catchol and dichloroether proved useful in the synthesis. Photoresponsive crown ether[38-39] and were obtained by specific method.[40-41]

2c Synthesis of cryptands:

Cryptand 2,2,2 (Viii) was synthesized by Lehn & Dietric[40-43] as follows:

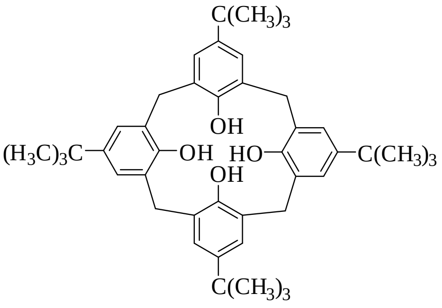


Too many steps are involved in the synthesis to reduce diamide and promote cyclocondensation. The high dilution was conductive for production of product. It prevented polymerisation. Other similar compounds such as aza- and thia- crown ethers were obtained by similar kind of synthesis. Similarly the chiral crown ethers diester were obtained.

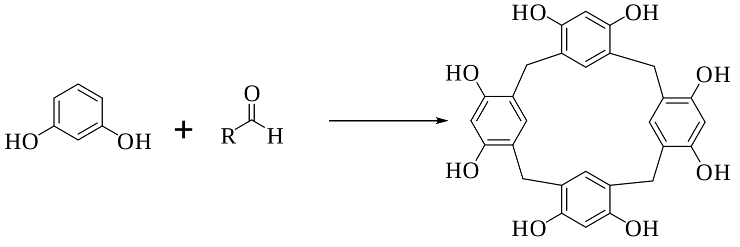
2(d) Synthesis of Calix[n]arenes and Calix[n]resorcinarene:

These compounds were synthesized by base or acid catalyzed reaction respectively[44] Acyclic tetramer is formed from aldehyde (Xi) and phenol (X) to give Calix[4]arene[45] (XII).

The subsequent compound containing CH3 is replaced by R=C (CH3) CH2CH2 Or –C (CH2CH3) or C(CH3)CH2C(CH3) C6H5 by the known reaction. It is termed as phenolformaldehyde reaction which includes cyclisation. Calix[n]arene has deep cavity with the shape of a cone in the center. It can accommodate metal ion to form complex. The overall process involves the induced oligerisation of phenol and formaldehyde to form Calix(n) arene[45]. The formation of linear product followed by cyclisation is critical. While, Calix[n]resorcinarene is obtained by acid catalysed reaction between resorcinol and acetaldehyde to form tetrameric complex in acid media in the presence of ethanol at 80°C.[46-49] We should avoid formation of the linear oligomer. A Friedel Craft reaction[50-51] with chloromethylmesitylene gives good yield. Finally the synthesis of chiral Calix[n]arene involves acid catalyzed reaction. These compounds have lower and upper rims, which occupy functional groups.[49-50] They readily form complexes with transition metals. In calyx[n]arene upper rim, p-position is occupied by tetrabutyl, p-tetraoctyl- or pentyl groups of the phenolic ring. The process is facilitated by the reversed Friedel Craft reaction. It is dealkyation process. The supramolecular species undergoes electrophilic substitution wherein the nitration reaction facilitates such substitution leading to the formation of water soluble compound. The introduction of aryl group in the para-position furnishes many of its derivatives. This led to recent discovery of compound called 'Rotaxane' having wheel structure. The chromogenic Supramolecules are also synthesized. The cyclodextrin and crown ethers have served as an example of the host guest chemistry. The metal ion is guest while Calix(n)arene is host moiety. This led to discovery of Buckiminsler Fullerane with C-60 atoms.



IX



X

XI

XII

3 Coordination Chemistry of the complexes

The complexation reaction proceeds through two entities viz. metal ion and ligand to form the complex. Such complexes contain either ionic or covalent compound and may have close ring structure. They are usually called chelate. Such chelates are stable while most of the complexes with supramolecules are cyclic closed ring structures. Such structures have a cavity or hole in the center of ligand. As told earlier when the hole size of the ligand and atomic (ionic) size of metal ion matches it leads to the formation of the stable complex. This combination leads to formation of planner (with crown) or a three dimensional (Cryptand, Calixerene) structures. In the supramolecules, the metal ion is entrapped inside cone shaped cavity of supramolecule.

The ion dipolar interaction facilitates complex formation. Such complexes are soluble in non-polar solvents. The kind of bond involved is electrostatic with ion dipolar interaction within cation and negatively charged oxygen donor atom from the crown / crypland compound. Such complexes are characterized by UV- visible, IR, NMR and MS techniques. The size of the cation, the number of oxygen atoms, the stereochemistry as well as the basicity of the donor (oxygen)atom, steric hindrance and charge determine their stability. Such stabilization of the complex is called as "the macrocyclic effect".

The stability is related to the value of stability constant of the complex. It was noted that stabilization and steric congestion are enthalpic in nature in these host guest complexes. Thus, during complexation reaction, there is competition between solvation of the solvent and the coordination of complex crown ether which leads to weak solvation. Therefore a solvent with high dielectric constant is preferred to enhance the process of solvation.

As regard complexes of metals with cryptands with two Nitrogen atoms in the bridge structure, it leads to formation of stable complex with negative reduction potential[53-55] with a caged structure. The S-block metal complexes have absorption maxima in near IR region of 645 to 745 nm. The coordination complex with Calix(n) arene has two rims which facilitate complextion with remote possibility of changing the cavity size of ligand facilitating further interaction. Such complexes have good flexibility to adjust to the shape of the metal cation with controlled solubility during extraction. They have capacity to incorporate various functional groups on each rim to provide better complexation with selectivity. The naked Calixarene has no group in upper ring while chloromethyl group in upper rim was useful for the preparation of the synthetic precursor. In calixarene, the lower rim accommodated groups such as ester, ethers, carboxylate while attachment of polymeric groups support the process of immobilization in solvent extraction separations. Also membrane transport by calixarene is quite rapid and significant. The cone shaped calix[n]arene is good for extraction in comparison with partial cone as 1-3 alternate or 1-2 alternate or 1-3 alternate Calix[4]arene. Thus the donor effect of phenyl ring is of vital importance in improving selectivity as well as stability[57] of complexes while lower rim substitution increases stability.[58] The chromogenic calixarene are used as ion sensors[59] with absorption maxima at 550 nm. Those of the calixarene which have end cap are called as Calixspherands[60]. The inner transition metals form good complexes with calixarenes with phenolic group in lower rim with favoured deprotonation while upper rim substituted calixarene are good for transition metal complexation[61,62]. In spectral characterization of Calixarene complexes,[13] CNMR has distinguished between liner and cyclic aligomers of the p-tertiary calix(4) arene but NMR was of no use for the characterization of the calixarenes (n=3,5,7). Mass spectrometry is used to determine mass of the calixarene and calixresorcinarene wherein signal at e/m 1872 is seen indicating the presence of the Octomer in the structure. By and large, their steriochemistry is most fascinating. The spectral characterization however is more reliable for the detection of cyclisation during their synthesis. The X-ray technique and thermal methods are useful to study host guest chemistry. The donor atoms such as Oxygen and Nitrogen are used to stabilize the crown or cryptand complexes specially for study of the solid complexes. They exhibit structures of different orientation as per cavity of the crown and ionic diameter of the cation.[63] The structure in solution shows absence of lattice force but presence of polorisation of each anion which is influenced in these structures. [64-66]

4. All about Solvent Extraction Separations:

Let us consider the theoretical aspect of Solvent extraction procedures – the technique is simple, rapid and useful for the purpose of separations using just a separating funnel, with subsequent analysis of metal ions using spectral, radiochemical or electroanalytical instruments methods. The process of extraction is classified into four categories depending upon the nature of the extracted species. The four categories are Chelate extraction, solvation, ion pair formation and synergic extraction. The extraction of uranium(VI) with hydroxyquinolone is Chelate extraction while the extraction of iron with dimethylether is an example of ion-association or the solvation phenomena. The extraction of zinc (II) with Trioctylamine (70A) in acid media is an example of extraction by ion pair extraction while one involving two extractions like with Thenoyltrifluoroaccelone (TTA) and Tributyl phosphate (TBP) is called as the synergic extraction. We consider now extraction equilibrium. After metal extraction, the metal ion is stripped back to the aqueous phase. Thus during extraction procedure various diluents which are non-polar or polar are used as stripping agents. The extraction equilibrium is represented by following expression.[67]

$$D = \frac{[MRX_n]}{[M^{n+}]} = \frac{[MRX_n]_0}{[M^{n+}]\,[MR^{n+}]} = K_{Dx}[X^{n-}]\,[R]_0$$

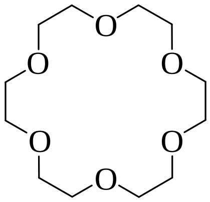
In above expression 'D' is overall distribution ratio, K_{Dx} is extraction coefficient of metal complex, M^{n+} is metal ion with valency as n, X is anion and R is a reagent namely crown, cryptand or Calix[n]arene with subscript o-indicating organic phase. We now consider the effect of these variables used during extraction on D, K_{Dx}, K_{DR} (reagent coefficient). In the total extraction (D), all constants have highest magnitude e.g. K_{DX}. Large value indicates good complexation reactions, K_{DR} shows good solution of complexing ligand while K_{ex} shows good extractability and formation of the metal complex.

Thus more the solubility of reagent viz. crown ether or cryptand, better is the extent of extraction. It is reflected in the value of K_{DX} of the complex. Further substitution of crown ether ring with dibenzo- or dicyclo-) (DB or DC) group enhances the extent of extraction. It also depends upon the nature of diluent. The solubility of ligand in aqueous phase (K_{DX}) is of great significance. Consequently greater the value of K_{DX} larger is value of K_p with high magnitude for D. More the hydrophobic complex, better is the extent of extraction but the co-extraction of water due to hydration of cation should be minimum in the organic phase in order to stop or arrest competition between complex and the molecule of water in the coordination sphere[67]. The role of counter anions used to make the complex electrically neutral during extraction is also important. One uses counter instances such as picrate, dipicrylamine, dinitrophenol. Those having large molecular volumn[68] give maximum extraction. The hydrophobicity of the counter anion also increases the extractability of the complex as planner organic counter anion gives best results. Finally role of diluent is also of equal importance. High value for dielectric constant of the solvent favours dispersion of solvated species due to high hydration energy. Further the dispersion of the extracted species promotes more extractable complex species[69-70].

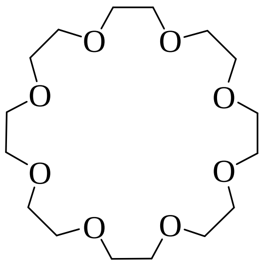
Other factors influencing extraction includes nature of crown ether, selectivity of extractant and temperature. A low reagent concentration is always preferred for better extraction. The chemical structure should also be favourable as the absence of electron withdrawing group on reagent stabilizes the complex.[71-74] The salting – out agent enhances extraction as it facilitates removal of water molecule from the coordination sphere of the metal ion. Entropy controls extraction.[75-76] Finally the nature of the stripping agents is important. Mostly one uses mineral acids or salts for stripping to dissociate metal complex in organic phase. This is actually the undoing process of complex formation and hence extraction.

5 (a) Solvent extraction Separations with Crown ethers:

Many crown ethers like 15C5 (XVI), 18C6(XVI), 24C8(XIV) and their derivatives as DB18C6 (XI), DCI18C6 (XVI) and DB24C8 were used for the separation of several elements. The unique discovery of extractive separation of alkali and alkaline forms was a significant contribution to analytical separations.



XIII



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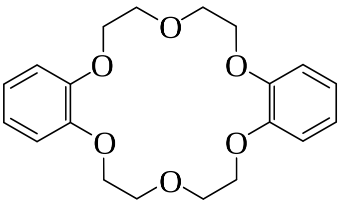
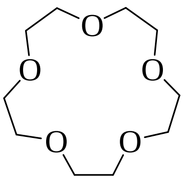


XV

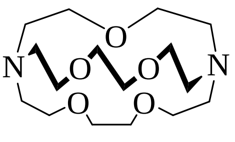


XVI

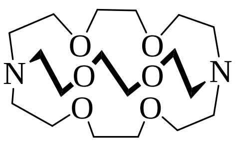
Several main group and transition elements were extracted with crown ethers. The inner transition metals such as europium, uranium were thoroughly investigated. Also, gallium, indium, thalium were studied. Similarly iron zinc and copper group elements were extracted. At pH 2-4, alkali metals were extracted while other metals were extracted at pH 4-7 in Chloroform dichloromethane. 24C7 was used for the extraction of caesium with counter anions as picric acid, xylenol yellow, bis-C_2 ethylhexyl) (phosphoric acid) and hexyl phosphoric acid. TTA was used with crown ethers[77, 78] for Synergic extractions. Uranium (VI) was extracted[79] with methylene blue as the counter anion. Americium[80] was similarly extracted. For Group I and II metals, extraction was rapid with these compounds. The best results were obtained in the separation of mono and bivalent Thallium[81].

5(b) Solvent extraction Separations with Cryptands:

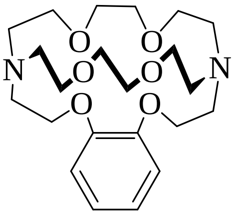
Most commonly used cryptands are cryptand 2, 21 (XVII), 22, 2 (XVIII) and 2,2,2B(XIX).



XVII



XVIII



XIX

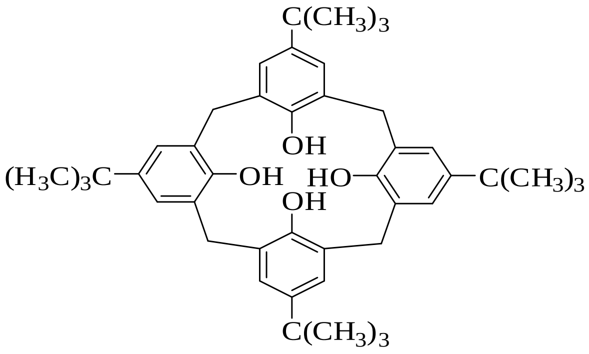
These are ligands with three dimensional structures. They are useful for extraction of heavy metals specially for metal pollutants from the environment.

Selective methods[82-88] were developed for the solvent extraction separation of lead[82] thallium[83] nickel[84] manganese[85] cadmium[86] Copper[87] and mercury[88]. Similarly uranium[89] Zirconium[90] and Calcium[91] were extracted with cryptands. Cryptand 2,2,2B as extractant, is ideal for the separation of lead with picrate as counter ion with toluene or ethylene chloride as the diluents. There is no stress between two nitrogen atoms as hole size gets expanded to trap the metal ions with strong bond formation between nitrogen and oxygen in the ring. The colored counteranions such as eosin and erythrosine facilitate simultaneous extraction and direct spectro-photometric determination of metalions. Calcium[92] is separated at pH ~7.2 – 8.6 with cryptand 2,2,2, in cyclohexane[93] with PMBP as counter anion. Trioctyl phosphine oxide (TOPO) is used as the synergic extractant[93] in such process.

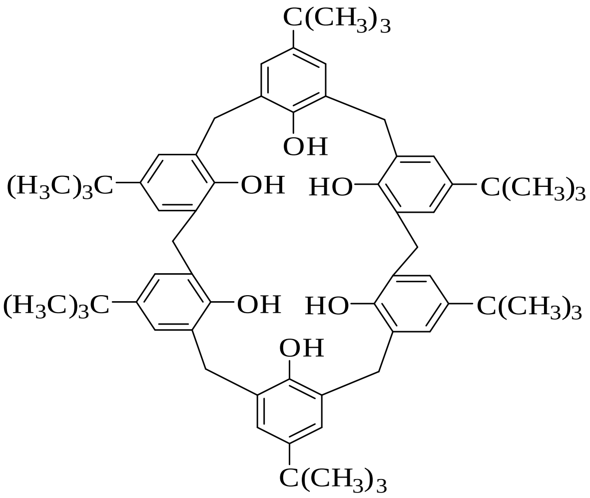
5c Solvent extraction separations with Calix[n]arenes:

These are phenolformaldehyde condensation aligomers obtained in base catalyzed reaction to form calix[n]arenc(XX) while acid catalysed reaction of resorcinol with acetaldehyde gives Calix[n] resorcinarene(XXI)

Calix[n]arenes are phenolformaldehyde oligomers (XX). The important members have n=4, or 6, 8 where n – represents aromatic rings. They have different configurations such as cone, partial cone, 1-2, cone 1-3 in the stereochemistry of the ligands. They are easily synthesized and can accommodate various functional groups.



XX



XXI

On the upper rim, they have several applications while the other Calix[n]resorcinarene (XXI) has limited applications inspite of the fact that their synthesis is quite simple. Calix[n]arene forms inclusion complexes with four configurations. Ziegler[84] and GuHz[95] provided a very systematic synthesis of these ligands. They are all water soluble while the sulphate form is most useful for extraction of metals[96] Conc 1-3 is best[97] for complexation when $R=(S0_3)^-$, Na^+, $R'=CH_3$. Uranium (VI) readily forms complex with Calix[6]arene[98]. The template effect facilitates synthesis with tertiary butyl as the substitute in upper rim position - metacyclophone[99]. They do not have optical isomers and are metacyclophones. Many metals like Fe^{3+},. Pd^{2+}, Pt^{2+}, Ag^+, Hg^{2+} and Pb^{2+} are extracted by Calixarene[100] and its Carboxylate derivatives[101-107]. Calix[6]arene is preferred for extraction when several aromatic hydrocarbons are used as the diluents. The inner transition metals are easily extractable. In fact, Calix[n]arenes have several advantages over crownether or cryptands. The lower rim is substituted by nitrate or suphate ions. A large number of papers[108-111] have been published in the last two decades on Calix[n]arene. Unfortunately Calix[6]resorcinarene finds limited applications may be due to presence of several OH^- group in the structure. This is not very conductive for solvent extraction purposes[108-111].

6. Simultaneous extraction and spectrophotometric analysis:

In conventional solvent extraction, the metal ion is stripped back into aqueous phase for subsequent quantitative analysis. In a few instances, the chromogenic ligand is complexed with metal ion in the organic phase during extraction. One can also use the colored counteranions such as eosin, erythrosine, Tropolien, Rhodamine and picric acid to facilitate formation of colored complexes followed by spectral analysis. In few instances, the stripping of the complex is followed by spectral method or suitable instrumental techniques.

6(a) Crownether and photometry:

We have two stages, where in first stage, the complex with crown ether is extracted followed by interaction with chromogenic ligand to form a colored complex which is analysed photometrically at a suitable wave length.

Many crownethers like 1SC5, B15C5, 18C6, DB18C6, DC18C6 and DB24C8 are used with suitable chromogenic ligands to form colored complexes which are analysed using spectrophotometric methods. Other counteranions like dipicrylamine[112] Zincon[113], Xylenol

(C114) Orange[116], 1-2 pyridylazonapthol PAN[115] and Metanillyellow[116] were also used. The S- block metals were extracted at pH 3-7 while inner transition metals were extracted at pH > 9. Copper is extractable at pH13[117] while p-block metals were extracted at pH 3-8[119]. Cobalt[120] was also quantitively extracted. In a few instances, external Chromogenic reagents were used[121-125]. Novel methods were developed for the separation of chemically similar metals like Zirconium and hafnium[126]. The isolation of niobium[126-127] was carried out successfully. Dithizone[128] PAR (4-2 Pyridy) azo resorcinol) was used for the synergic extraction of uranium[129]. Many counter anion and chromogenis reagents were identical[130]. The typical example is Vanadium (V) separation. Extensive work was carried out on Potassium[131-136] with DB18C6 with Tropolien as counter anion. DC24C8 with propylene as the diluent was used for calcium extraction[137] while Cerium (IV) was extracted with 15C5[138] Uranium (VI) was extracted with DC18C6 from 6-8 M hydrochloric acid with Arsenium-III as the counteranion and it was determined photometrically at 665 nm[139] Gallium[140] was quantitatively extracted with 18C6 with PAR as the counteranion. Metanil yellow with 18C6 extracted lead[141] while Bismuth was extracted with 18C6 in dichloroether with potassium iodide as counteranion. Iodide was used as counteranion and was analysed at 520nm[142] photometrically.

6(b) Extractive photometry with Cryptand:

These are three dimensional complexes with bonding through nitrogen atoms. Most of the extractions were carried at pH 6.0 – 9.0 with chloroform as the diluent Eosine and Erythrosin were used as the counteranion cryptands Commonly used cryptands were – 2,2,2, 2,2,1, 211 and 2,2,2B. Similarly zirconium and uranium were extracted with cryptand (143-144) cryptand – 2,2,2 with best results[145].

Uranium (VI) was quantitively extracted at pH 6.0 while lead was isolated with cryptand 2,2,2B in toluene with fluoroscene as the counter anion. It was measured photometrically at 540nm.

6(C) Extractive Photometry with Calix(n) arenes:

Calixarene showed remarkable effect of substitution in the upper and lower rims with appropriate functional groups. Tertiary butyl or octyl proved very good in the upper rim while sulphate was best in the lower rim of Calix[6]arene. The hydroxyl anion is used to replace keto- group but acetyl group favoured several separations when n=6 in Calix[n]arene. Lanthanides[145-147] were extracted with Calix[4]arene diamide with sulphate in one rim. Cerium was extracted with p-tertbutyl Calix[6]arene[148]. Thorium was extracted with Thoron at pH 7.5 with hexaacetone derivative. Several metal ions were similarly extracted. Buckminster C60 fullerene was extracted with Calix[8]arene[148-153] with great success.

7(a) Atomic absorption spectroscopy:

The s-block and large s-block metals were extracted with DC18C6, DB18C6 while d-block transition metals were extracted with 18C6, 24C8 at pH 5.0-7.0 with a variety of counteranions such as HDEHP(Bis 2-ethylhexyl) phosphoric acid Extraction was possible with TTA, TOP0 cryptands[154-155]. Several metals like copper, thallium, lead, manganese and cadmium were extracted with cryptand 2,2,2 with eosin at pH 5.5 – 6.8. While caesium[156] was extracted with DB24C8[157] when cryptand – 2,2,2B proved to be best extractant while for lead eosin[155] served as counteranion. Calix[4]arene extracted silver but strontium 2+[157], copper[158, 159-169] calcium[157] were extracted with crownether. In all these cases, metal ions were stripped back into aqueous phase and determined by AAS at suitable wavelengths.

7(b) Plasma Emission spectroscopy:[159]

1CP-AES proved to be most effective and useful method for quantitative analysis of metals after extraction and stripping of the complex[160] Flame photometry was rarely used. Rubidium+[161] was analyzed by flame emission spectroscopy. Potassium +[168] was extracted with cryptand of 222 and then analyzed by 1CP-AES with nitrobenzene as the diluent[162] while Thorium (IV) was extracted with Calix[4]arene[163] and analyzed by Plasma Emission Spectroscopy[164].

7c Fluorimetric analysis:

This is one of the powerful methods specially for supramolecules as complexing ligands. Among the counteranions used in extraction were eosine. erythrosine, Rhodamine and picric acid. These ligands serve not only as the counter anions facilitating extraction but also permit simultaneous spectrophotometric determination, acting as chromogenic ligands Thallium (III) silver were extracted with cryptand – 2,2,2[165-166] with these counter anions. Similarly Europium, Lithium were analysed by molecular luminescence method. These metals[166-170] were extracted by DB18C6 with eosine at pH 12-0[170]. Many more metals are extractable with cryptands and crownethers.

Conclusions

Let us compare crownethers, cryptands or Calix[n]arene as extractant with existing well established organic extractions. Most of the complexes formed by oxine, oximes, napthols, etc. were plannar. Their extraction was time consuming, needed critical control of reagent concentration, acidic pH and had to apply classical methods for quantitative analysis. While the proposed extractions were used at microgram, counteractions with powerful techniques for the quantitative analysis such as AAS, ICP-AES, fluoroscence and Polarographic methods. Several of counter anions were used which acted as trychromogenic ligands. Further the three dimensional cone structure of Calixarenes facilitated selective stripping and metals ions with varying ionic size. Large size metals were first selectively stripped followed by the final stripping of metals with small ionic size at the end of experiment.

In spite of advancements in chromatographic and electrophoric methods of separations, solvent extraction remains a powerful tool in separation chemistry. It started with large scale counter current technique at pilot scale followed by batch extraction on laboratory scale. This in turn was followed by development of the method of the extraction chromatography handling microgram concentration of metals. The recent invention is the use of liquid membrane technique to isolate the nanogram concentration of metals. This mode of liquid liquid extraction will continue to be used on account of its simplicity with wide scope of discovery of novel compounds like crownether, cryptands and calyx[n]arenes in chemistry.

References

1. Khopkar, S.M., 2002, Analytical Chemistry of Macrocyclic and Supramolecular compounds 2nd Edition Springe-Veilag-Norosa.
2. Khopkar, S.M., 2019, Basic Concepts of Analytical Chemistry 4h Edition, New Age International Publishers.
3. Khopkar, S.M. and Gandhi M. N., 1994, Crownethers and Cryptands in Solvent Extraction, Unpublished Monograph loc Cit.
4. Vogtle, E. and Weber W., 1985, Host Guest complex Chemistry Macrocycles Springs – Verlog Berlin.
5. Gokel, G.W., Korzeniowski S.H., 1982, Macrocyclic Polyethers Chemistry Springer Verlag.
6. Melson, C.A., (Ed), 1980, Coordination Chemistry of Macrocyclic Compounds Planum Press NY.
7. Izatt, R.M. and Christen J.J. (Ed), 1978, Sythetic Multidentete Macro Cyclic compounds Academic Press.
8. Inouse, Y. and Gokal G.K., (Ed), 1990, Cahon binding by Macrocycles Marcel Dekker NY.
9. Gokel, G.K., 1991, Crown ethers and Cryptands – Royal Chemical Soc.
10. Hiraoka, M., 1982, Crown ethers and analoges compounds, Elesvier Publishing.

11. David Gutsche C., 2004, Calixarene New Edition Royal Chemiacl Society London.
12. Weber, E., 1987, Crown compounds – Properties and Practice, E. Merck Schucbert Monograph.
13. Coope, S.R., 1992, Crown compounds (+) towards future applications VCH Verlag.
14. Khopkar, S.M. and Gandhi, M.N., 1996, *J. Sc. & Ind. Res.*, **55**, 139.
15. De, K., Khopkar, S.M. and Chelmers R.A., 1970, Solven extraction of Metals Ven Nostrand – Reinhold Ltd.
16. Khopkar, S.M., 2019, Solvent extraction separation with liquid ion exchangers 2nd Edition New Age International Publishers.
17. Yoshio N. and Naguchi H., 1982, *Anal. Letters*, **15**, 115.
18. Takegi M., Nekamura H., Sanui, Y. and Ueno K., 1981, *Anal. Chem, Acta.*, **126**, 185.
19. Shinkai S., 1990, *Biorgchem Frontiers*, **1**, 161.
20. Rasso L. and Voltle R., 1982, *Topics in Current Chemistry*, **113**, 1.
21. Pedersen, C.J., 1972, *Org. Synthesis*, **52**, 66.
22. Pederson, C.J., 1988, *J. Inclusion Phenomena*, **6**, 337.
23. Blasius, E. and Janzen K.P., 1981, *Topics in Current Chem.*, **98**, 163.
24. Pedersen, C.J., 1971, *Aldrchimics*, **4,** 1.
25. Bradshaw, J.S. and Hui J.K., 1974, *J. Het. Chem.*, **11**, 649.
26. Chritstensen, J.J., Earoug, D.J. and Izatt R.M., 1974, *Chem. Rev.*, **74**, 351.
27. Cram, D.J. and Cram J.M., 1974, *Science*, **183**, 803.
28. Kapoor, P.N. and Merhotra R.C., 1974, *Coord. Chem. Rev.*, **74**, 1.
29. Lehn, J.M., 1971, *Pure and Applied Chem.*, **49 & 57**.
30. Lehn, J.M., 1978, *Acct. Chem. Res.*, **11**, 49.
31. Poonia, N.S. and Bajaj A.V., 1978, *Chem. Reviews*, **79**, 389.
32. Lehn, J.M., 1981, *La Recharche*, **127**, 12, 13.
33. Kolthnoff, M., 1979, *Anot. Chem.*, **51**, IR.
34. Irving, M.H., 1978, *Pure and App. Chem.*, **50**, 1129.
35. Sekine, T., Y., 1979, *Hasegawa Kageku no Raykil*, **33**, 464.
36. Takagi, M., Nakamura H., Sanui Y. and Ueno K., 1981, *Anal. Chim. Acta.*, **126**, 185.
37. Krakowiak, F.K.E. and Bradshaw J.S., 1992, *Israel J. Chem.*, **32**.
38. Pedersen, C.J., 1967, *J. Am. Chem. Soc.*, **89**, 7017.
39. Eliel, E.L.F., 1962, Sterochem of Carbon Compound McGrow Hill.
40. Gokel, G.W., Dishang, D. and Diamond, C.J., 1998, *J. Chem. Soc.*, **10**, 53.
41. Shinkai, S., Miyazcki K. and Menebe O., 1987, *J. Chem. Soc.*, **449.**
42. Dietrich, B., 1984, Inclusion Compouns Academic Press., Vol.**2337.**
43. Dietrich, B., Lehn J.M. and Savage J.P., 1969, *Tetrahedron Letters*, 2885.
44. David Gutsche C., 1999, Calixerene First Edition Royal Chem. Society

45. Asfari, Z. and Vienn J., 1988, *Tetrahedron Letters*, **29**, 2659

46. Hodberg, G.S., 1977, Ph.D Thesis, Royal Inst. Tech. Stockholm.

47. Hogberg, G.S., 1980, *J. Org. Chem.*, **45**, 4498.

48. Cram, D.J., Karbach, S., Kim, H.E., Knober, C.B. and Maresick E., *J. Am. Chem. Soc*., **10**, 2229.

49. Hunder, R.F. and Tarner C., 1957, *Chem. & Ind*., **72**.

50. Shinkai, S., Mori, S., Tsubeki T. and Marisbe O., 1984, *Tetrahedron Letters*, **25**, 5315.

51. Shinkai S., Araki K., Tsubaki T., Arimova T. and Monnabe O., 1987, *J. Chem. Soc*., 2297.

52. Takeshita M. and Shinkar S., 1995, *Bull Chem. Soc., Japan*, **68**, 1088.

53. Kappenstein, G., 1974, *Bull Soc. Chem. France*, 89.

54. Truter, M.R. and Pedersen C.J., 1974, *Endeavour*, **32**, 142.

55. Tracter, M.R., 1973, *Structure and Bonding*, **6**, p71, Springer Valag.

56. Arenci, G., Cali G. R., Lombardo, G., Rizzcrelli F., Scotta, D., Urigcro R. and Casnati A., 1992, *Sup. Chem*., 119.

57. Ikeda A. and Shinkai S., 1992, *Tetrahedon Lett.*, **33**, 7385.

58. Armoudveu F., Bariet, G., Owns, S.V.M.H. and Mokerrey M.H., 1993, *Inorg. Chem*., **32,** 2644.

59. Shimizu H., Iwanata K., Fujimato K. and Shinkai S., 1991, *Chem. Lett*., 2147.

60. Deng K., Sakaki K., Nokoshim S., Shinkai, 1992, *Chem. Letters*., **1287**.

61. Gutche D. and Nem K.C., 1988, *J. Chem. Soc*., **110**, 6153.

62. Floridri D. and Jaeby A. Chiest, 1989, Villa, C. Gusteni – *Anal Chem*., **28**, 137.

63. Givaud Z., Seherrens M., Lever O., Lapervole B. and Das, C., *J. Chem. Soc*., **2**, 981.

64. Desantisgiancario, F., Luigi, L., Maurizio, P. and Piersanotro, 1992, *Coord. Chem. Rev*., **120**, 237.

65. Round Hill D.M., 1995, *Prog. Inorg. Chem*., **43,** 533.

66. Wiese, C., Dieleman, C.B. and Malt D., 1997, *Coord. Chem. Rev*., **165**, 93.

67. Michell, W. and Shanki D.L., 1995, *Anal. Chem*., **47**, 647.

68. Takeda Y. and Takahashi F., 1960, *Bull Chem. Soc., Japan*, **53**, 1167.

69. Eisenman S. Ciani, and Zebo G.S., 1969, *J. Memb. Bio*., **1**, 294.

70. Gandhi, M.N., 1992, Ph. D. Thesis, I.I.T. Mumbai.

71. Rebek, R.V. and Wallley, 1980, *J. Hetchem*., **7**, 749.

72. Kolthoff Z.M., 1981, *J. Chem*., **59**, 1548.

73. McDowell, W.J. and Shouri R.R., 1979, *Procd. Int. Conf. Solv. Extn*., **21**, 93.

74. Gloe, 1979, *Z. Chem*., **19**, 382.

75. Sadakane I. and Iwan Chido K. Toe, 1975, *Bull Chem. Soc., Japan*, **48**, 60.

76. Marcus, Y. and Asher L.F., 1978, *J. Phy. Chem*., **82**, 1246.

77. Ensar D.O. and Reynolds P.S., 1988, *J. Less Comm. Metals*., **149**, 287.

78. Imura H. Mito, 1995, *J. Radio & Nucl. Chem.*, **189**, 229.
79. Tang C.M. Wai, 1986, *Anal. Chem.*, **50**, 3233.
80. Nair, G.M. and Prabhu, D.R., 1988, *J. Radianal & Nucl. Chem.*, **121**, 83.
81. Sekine T., Wekaboyshi H. and Hasegcwg Y., 1978, *Bull. Chem. Soc., Japan*, **51**, 645.
82. Gandhi, M.N. and Khopkar S.M., 1991, *Ind. J. Chem.*, **30A**, 706.
83. Gandhi, N. and Khopkar S.M., 1992, *Anal. Chem. Acta.*, **270**, 87.
84. Gandhi, N. and Khopkar, S.M., 1992, *Chem. Analy. (Warsaw)*, **37**, 437.
85. Gandhi, M.N. and Khopkar, S.M., 1992, *Analyt. Sci.*, **8**, 65.
86. Gandhi, M.N. and Khopkar, S.M., 1992, *Micro Chim. Acta.*, **111**, 93.
87. Gandhi, M.N. and Khopkar, S.M., 1993, *Chem. & Enr. Res.*, **1**, 389.
88. Gandhi, M.N. and Khopkar, S.M., 1994, *J. Sc. & Ind. Res.*, **53**, 630.
89. Mathew, V.J. and Khopkar S.M., 1995, *J. Radioanal and Nucl. Chem.*, **201**, 281.
90. Mathew, V.J. and Khopkar S.M., 1997, *Chem. Analy. (Warsaw)*, **42**, 651.
91. Ajgaonkar, H.S. and Khopkar, S.M., 1999, *Chem. Analy. (Warsaw)*, **44**, 61.
92. Saski, T., Gmetani, U.S., Matsui M. and Sarabou, T., 1994, *Chem. Letters*, 1193.
93. Dimetriemka, S.G., Pasekova, G., Sirusaveva D., Far Monorskii Zoltoy, A. and Mikhurost, V., 1990, *Vest Mesk Unikhim*, **31,** 378.
94. Mohite, B.S. and Khopkar, S.M., 1987, *Anal. Chem.*, **59**, 1200.
95. Gutsche, C.D., Iqbal, M. and Kwart, D.S., 1988, *J. Org. Chem.*, **51**, 72.
96. Weng, H., Yogi, K. and Smid, J., 1974, *J. Mem. Bio.*, **18**, 379.
97. Shinkai, S., 1980, *Pure & Appl. Chem.*, **58**, 1523.
98. Izalt, S.R. and Christensen, J. J., 1983, *J. Am. Chem. Soc.*, **105**, 1782.
99. Takeshita, M. and Shinkai, S., 1995, *Foull Chem. Soc. Japan.*, **68**, 1088.
100. Hilgenfeld, R. and Saenger, N., 1982, Host Guest Complex Springe Verlog.
101. Gupta & Khopkar, S.M., 1995, *Talanta*, **42**, 1493.
102. Mathew, V.J. and Khopkar, S.M., 1997, *Talanta*, **44**, 1699.
103. Khandwe, R.A. and Khopkar, S.M., 1998, *Talanta*, **48**, 321.
104. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 1999, *J. Radio Analytical Chem.*, **241**, 179.
105. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 1998, *Cand. J. Anal. Sci.*, **43**, 143.
106. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 1999, *Anal. Sci.*, **15**, 1.
107. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 2000, *Ind. J. Chem. Tech.*, **7**, 7.
108. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 1999, *Ind. J. Chem.*, **38A**, 1079.
109. Malkhede, D., Dhadke, P.M. and Khopkar, S.M., 1995, *Madhya Bharat, J.* **3510**, 3913.
110. Malkhede, D. and Khopkar, S.M., 2002, *J. Ind. Council Chemists*, **20**, 1.

111. Malkhede, D.M. and Khopkar, S.M., 2003, *Ind. J. Chem.*, **42**, 2945.

112. Molmsted, M., Siegl, G., Hope, H. and Power, P.P., 1985, *J. Am. Chem. Soc.*, **107**, 8087.

113. Motomiza, S., Onido M., Oshima, M. and Twenchido, J., 1985, *Analyst*, **113**, 742.

114. Namdeo, R.P. and Khopkar, S.M., 1995, *Ind. J. Chem.*, **34A**, 840.

115. Deorkar, V. and Khopkar, S.M., 1991, *Analyst.*, **116**, 961.

116. Nezareka, A.Y. and Leoneko, Y.I., 1985, *Vist. Kiev Ser. Uni. Khim.*, 66.

117. Eiko N., Khimiya, O. and Nemiki, H., 1982, *Japan Analyst*, **31**, 602.

118. Nazerenko, Y. and Leonanko, y. i., 1985, *Visa Kie Uni. Khim.*, 66.

119. Deorkar, N.V. and Khopkar, S.M., *Bull Chem. Soc., Japan.*, **64** (19).

120. Nabgawa, K.S., Okade, Y., Inouc A., 1988, Jai T. Hckushi – *Anal. Chem.*, **60**, 2527.

121. Manchond, V.K. and Chang, G.A., 1986, *Anal. Chem.*, **58**, 2269.

122. Schlen, I., Salhin, A. and Saad, B., 1986, *Analyst.*, **120**, 2861.

123. Agarwal, Y.K., 1993, Proc. Int. Conf. Solvent Extn. Conference, York.

124. Yoshio, M., Ugamura, H., Naguchi, N. and Nagemalsu, 1989, *Anal. Letter.*, **11**, 281.

125. Deorkar, V. and Khopkar, S.M., 1989, *J. Anal. & Nucl. Chem.*, **134**, 433.

126. Deorkar, V. and Khopkar, S.M., 1991, *Anal. Chim. Acta.*, **245**, 27.

127. Deorkar, N.Y., 1991, Ph.D. Thesis, IIT, Mumbai.

128. Poddubriykh, L.P., Dimtrienko, S.G., Kuzimm, M.M. and Zolotoy, Y.A., 1987, *Zevd. Lab.*, 53.

129. Mohite, S., Patil, J.M. and Zombre, D.N., 1993, *J. Radioanal & Nucl. Chem.*, **170**, 125.

130. Inonchido, T., Sadkane, A. and Tobi, K., 1978, *Bull Chem. Soc., Japan.*, **50**, 629.

131. Mohite, S. and Patil, J.M., 1991, *J. Radioanal. & Nucl. Chem.*, **150**, 207.

132. Deokar, N.V., 1991, Ph.D Thesis IIT, Mumbai.

133. Rane, S.S. and Khopkar, S.M., 1990, *Ind. J. Chem. Tech.*, **3**, 363.

134. Abe, S., Fuji, K. and Sone, T., 1994, *Anal. Chem. Acta.*, **293,** 325.

135. Yu, A. and Nazarenko, 1983, *Uki Khim. Zh.*, **49**, 279.

136. Znong, F. and Liao, T., 1984, *Fenxi Huaxue*, **12**, 153.

137. Motomiza, S., Oshima, M., Yenedo, N. and Wwan Chindo, T., 1990, *Anal. Soc.*, **6**, 215.

138. Deorkar, N.V. and Khopkar, S.M., 1989, *Analyst*, **114**, 105.

139. Mohite, B.S., 1990, Ph.D Thesis IIT, Mumbai.

140. Vibhute, C. and Khopkar, S.M., 1989, *J. Ind. Chem. Soc.*, **66**, 720.

141. Nazorenko, A.Y. and Lpaneko, X.I., 1985, *Vesi Kier Uni Khim.*, 66.

142. Nakashima, K., Machinzuki, N.M., Nakalyis S., Akiyama, S., Kannode, J. and Sum, S.M., 1983, Chem.

143. Ya, A., Nazevenko, T.A. Tiltorenko, 1983, *Ukr Khim Zu.*, **49**, 650.

144. Ageeva, V.V., A. Yu., Nazarenko, 1987, *Vesi Khim Uni Sar Khim*.

145. Wezepaniak B., Juskowiak, 1987, *Chem. Analyst*, **82**, 787.

146. Espersen, F. and Jensen, A., 1979, *Anal. Chem. Acta*., **108**, 241.

147. Mathew, V.K. and Khopkar, S.M., 1994, *J. Radioanal and Nucl. Chem. Letters*., **132**, 315.

148. Beer, D., Drew, M.G.B., Grieve, A. and Ogdan, M., 1995, *J. Chem. Soc*., 3455.

149. Yoshida N. Xemamoto R. Sagara Kueno. D. Ishis Shinke, 1991, *Chem. Soc. Letters*, **12**, 2015.

150. Khandwe, R.M. and Khopkar, S.M., 1998, *Talanta*, **46**, 515.

151. Vaidya, S.B., Zok, J., Bastions, G.J. and Parteretal, M.D., 1995, *Anal. Chem*., **67**, 4101.

152. Mathew, V.K. and Khopkar, S.M., 1997, *Talanta*, **45**, 1699.

153. Malkhede and Khopkar, S.M., 2000, *Ind. J. Chem. Tech*., **9**, 7.

154. Vibhute, G. and Khopkar, S.M., 1991, *J. Radioanal. & Nucl. Chem*., **152**, 487.

155. Rudierse, B., Massodi, A. and Browness, J.P., 1993, *Talanta*, **40**, 805.

156. Rusdiavso, B., Massoudi, A. and Branett, J.P., 1993, *Talanta*, **40**, 805.

157. Hasegawa, T., Suzuki K. and Sekine, T., 1991, *Chem. Letters*, **8**, 1075.

158. Saito, S., Murkami, A., Muromatsu F. Sekio, 1990, *Anal. Chim. Acta*, **237,** 245.

159. Billah, M. and Honjo, T., 1997, *Z. Anal. Chem*., **357**, 61.

160. Mohite, B.S., Zambre, D.N. and Mahadik, B.E., 1994, *Anal. Chem*., **66**, 4097.

161. Mohite, B.S. and Khopkar, S.M., 1985, *Talenta*, **37**, 565.

162. H. Gerow J.H. Smith J., D.M. Wijun, 1981, *Sep. Sci. & Tech*., **16**, 519.

163. Yakshin, V.V., Fedorova, A.T. and Laskorin, B.N., 1995, *Zh. Anal. Khim*, **40**, 45.

164. Agarwal, Y.K. and Senyal, M., 1995, *Analyst*, **120**, 2759.

165. Sanz – Medel, A., Glanco, G.D. and Garcia, A.V.R., 1981, *Talanta*, **8**, 425.

166. Blanco, G.O., Andies Gracia, E., Flunte, E.A. and Arribas, S.J., 1984, *Talanta*, **31**, 515.

167. Andres, G.E., Fueptie, A.E. and Blenco, G.D., 1992, *Anal. Letters*, **25**, 339.

168. Kimura, Ketani, S.I. and Shono, T., 1987, *Anal. Chem. Acta*., **203**, 85.

169. James, J.D. and Shinkai, S., 1995, *J. Chem. Soc*., 1483.

170. Sanz Medel, A., Blanco, G.D., Fuinte, F. and Arribas, J., 1984, *Talanta*, **31**, 213.

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Analysis of Physiochemical and Microbial Parameters to Evaluate the Drinking Water Quality in Five Different Villages in the Vicinity of Saphale, District Palghar, Maharashtra State, India

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Abstract

The drinking water quality was investigated in five villages in the vicinity of Saphale, District Palghar, Maharashtra State, India. The objective was to investigate the source of safe and clean drinking water. The water samples from five villages viz. Sartodi, Karwale, Kandarvan, Ghatim and Navghar were analysed. These villages are adopted under "Unnat Bharat Abhiyan" a programme of Higher Education, Ministry of Human Resource Development, Government of India. Ten water samples were collected and studied for physiochemical and microbial parameters to evaluate the drinking water quality The water samples were collected in the months of October and December.. Parameters such as pH, conductivity, Total dissolved solids (TDS), salinity, Total Hardness, Dissolved Oxygen, and microbial activity were evaluated. The values obtained were compared with the standard values set by the World Health Organisation (WHO) and local standards like National Drinking Water Quality Standards (NDWQS). The values of most of the parameters were outside the safe limits set by WHO and NDWQS. Out of the eight locations, water from only one location was found to be safe for drinking purpose. It is also important to study the other parameters like Biological and Chemical Oxygen Demand and heavy metal content of the water samples.

Keywords: *Unnat Bharat Abhiyaan, Drinking quality, Most Probable Number, Salinity, Hardness, Dissolved Oxygen*

Introduction

Clean water can be considered as a fundamental right of human beings. Around 750 million people do not have access to clean and safe water and around 2.5 billion people do not have proper sanitation. As a result, around 6-8 million people die each year because of water borne diseases.[1] In India, the Ministry of Drinking Water and Sanitation is primarily responsible for policy, planning, funding and coordination of the programmes for safe drinking water and sanitation facilities. Accordingly, the Government is focussing on Swachh Bharat Mission (SBM-G) and National Rural Drinking Water programme (NRDWP). In recent years, the priorities of government has shifted to SBM-G than NRDWP; the share of allocation of budget for NRDWP has dropped (from

87% in 2009 to 31% in 2018-19). During the same period, the share of allocation of SBM-G has increased from 13% to 69%.[2] In the current research, we have focused on drinking water facility of five villages which have been adopted by us since 2019. All the five villages have shown improvement in sanitary facilities. The source of the drinking water is in the form of wells and hand pumps i.e. mainly groundwater. The village Sartodi and Karwale have Karwale dam on their east side. The survey conducted for 790 houses indicated greater demand of safe and clean drinking water. The ground water always gets polluted due to contamination from agricultural chemicals like fertilizers and pesticides, faecal matter, house hold waste etc. In India 70% water resources are contaminated with biological, organic and inorganic pollutants.[3]

A number of scientific protocols are available in literature survey to assess the contaminants in water. [4-5]. These procedure include all the physical, chemical and biological parameters that need to be studied to assess the quality of water for different purposes. These parameters affect the drinking water quality if their values are above the safe limits given by regulatory bodies like WHO and BIS.[6]

E. coli is widely distributed in the intestine of humans and warm-blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora that maintain the physiology of the healthy host. *E. coli* is a member of the family Enterobacteriaceae, which includes many genera, including known pathogens such as *Salmonella*, *Shigella*, and *Yersinia*. In 1892, Shardinger proposed the use of *E. coli* as an indicator of faecal contamination. This was based on the premise that *E. coli* is abundant in human and animal faeces and not usually found in other niches. Furthermore, since *E. coli* could be easily detected by its ability to ferment glucose (later changed to lactose), it was easier to isolate than the known gastrointestinal pathogens. Hence, the presence of *E. coli* in food or water became accepted as indicative of recent faecal contamination and the possible presence of frank pathogens[14].

MPN (Most probable number) is a part of the harmonized compendial chapter on bacterial enumeration (USP, Chapter on "Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests," United States Pharmacopeia 32, vol. 1, pp 71-75, 2009a) and has been part of the "Microbial Limits Test" chapter in the United States Pharmacopeia (USP) since the inception of the chapter in USP XVIII[15]. Serial dilution tests measure the concentration of a target microbe in a sample with an estimate called the most probable number (MPN). The MPN is particularly useful for low concentrations of organisms (<100/g), especially in water. The basic assumptions of the MPN method are that bacteria follow Poisson statistics, and that a single viable cell will result in turbidity of the test media under the given conditions[16]. Coliforms ferment lactose with the production of acid and gas. An inverted Durham's tube is used to indicate the presence of gas formation. The medium used is lauryl tryptose broth with lauryl sulfate to suppress the growth of other organisms that may be present in water and thus making the medium more selective for coliform organisms. A change in color of the medium to yellow and gas bubbles in Durham's tube indicate the presence of coliforms.

Table 1: Physico-chemical parameters of water

Parameter	Max. allowable limit	Ill effects
TDS	2000mg/L	Undesirable taste
pH	6.5-8.5	Affects mucous membrane, bitter taste and affect aquatic life
Hardness	600mg/L	Leads to skin irritation and bad quality of food
Chloride ion	1000mg/L	High blood pressure,

Material and Methods

Study Area: The five villages identified for the study are from Palghar district, Post Umbarpada, on the east side of Saphale railway station of Western Railway line of Indian Railways. Geographic location is close to 19^0N, 72^0E. The south side of the villages Navghar and Ghatim is surrounded by Vaitarna Creek whereas on the east side of these villages is Karwale dam. The main source of drinking water is hand pumps and open wells as per the survey conducted under Unnat Bharat Abhiyaan

Table 2: Sources of water in villages

Sartodi		Karwale		Kandarvan		Ghatim		Navghar	
HP	OW	HP	OW	HP	OW	HP	OW	HP	OW
42.1	24.6	17.2	58.2	56.3	4.0	48.7	20.9	77.0	15.6

* HP- Hand Pump (values in percentages)
* OW- open well (values in percentages)

Selection of Sampling Points: The villages selected do not have any industry and the land is used for agriculture or is forested. The sanitary facilities have reached to almost 90% families with soak pit type of waste disposal. The percentages of land under irrigation and non-irrigation activities is given in Table 3.

Table 3: Percentage of land under irrigation and non-irrigation activities

Sartodi		Karwale		Kandarvan		Ghatim		Navghar	
IR	NIR	IR	NIR	IR	NIR	IR	NIR	IR	NIR
56.3	43.7	9.5	90.5	66.7	33.3	88.5	11.5	71.6	28.4

* IR- Land under irrigation (values in percentages)
* NIR- Land under Non Irrigation (values in percentages)

The samples were collected from 10 locations (Table 4).

Table 4: Water Samples collected from villages

Sample No	Sample Name	Sample Code
Sample 1	Sartodi Well	SAR-W
Sample 2	Karwale Grampanchayat Hand pump	KAR-H
Sample 3	Bogadipada Well	KAR-B-W
Sample 4	Patilpada Hand pump	KAR-P-H
Sample 5	Kandarvan Hand pump	KAN-H
Sample 6	Ghatim Well	GHT-W
Sample 7	Ghatim Hand pump	GHT=H
Sample 8	Navghar Hand pump 1	NAV-H1
Sample 9	Navghar Hand pump 2	NAV-H2
Sample 10	Navghar Well	NAV-W

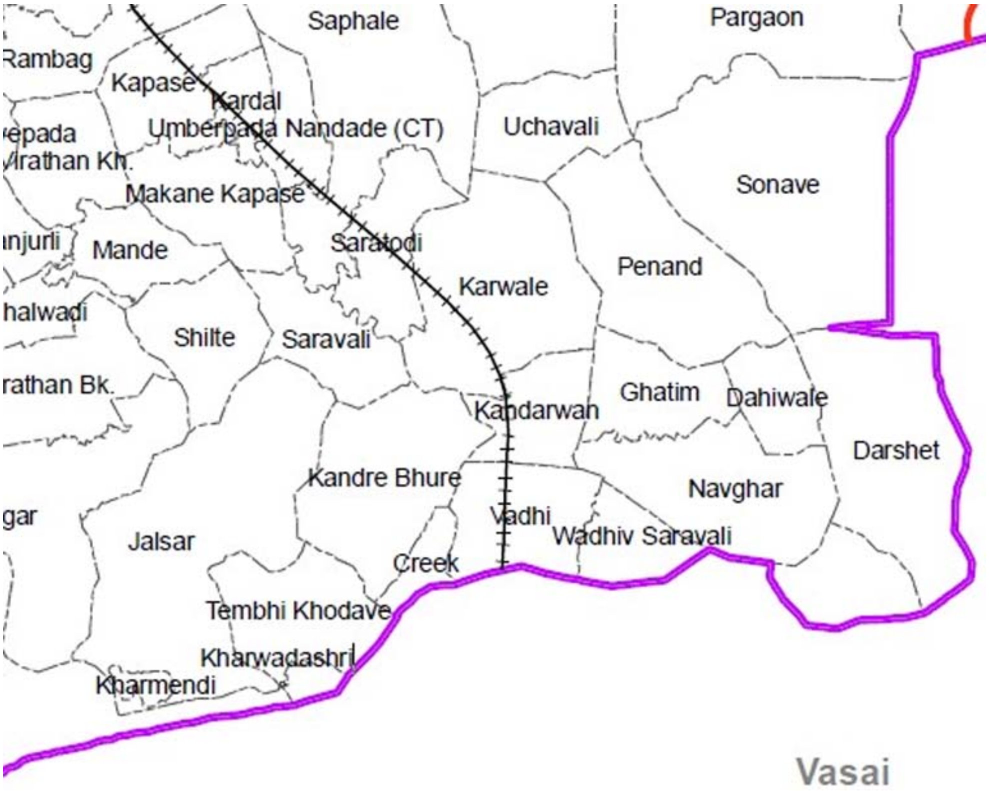

Fig. 1: Map of the area where the water samples were collected

Collection of samples

Samples were collected from ten locations in 5 villages. The samples were collected in the months of October and December 2019. The water samples were collected in sterile plastic or glass bottles. The samples were analysed within 48 hours of the collection. The samples were analysed for chemical parameters such as pH, Conductance, TDS, Hardness, Dissolved Oxygen, Salinity and microbial test.

Analytical Instrumentation

pH and Conductivity were determined as per the standard protocol and methods. pH of the water samples was measured by using a pH meter. The pH meter was standardised by using standard buffer solutions of pH 4, and 9.2. A combined glass-calomel electrode was used to measure pH.

The conductivity of the water samples was measured using conductivity meter. The probe was calibrated using standard solution of known conductivity. The conductivity of each water sample was measured carefully taking care of cross contamination.

Laboratory Analysis

Water samples were analysed to determine Total Dissolved Solids (TDS), Total Hardness, Dissolved oxygen and Salinity of the water using standard methods.

The microbial test for detection of faecal coliforms [intestinal organisms] present in drinking water was carried out by the most probable number method. If coliforms are present in drinking water, it indicates faecal contamination and the water is not potable.

MPN Methods

Bacteriological analysis was carried out for indicator organisms i.e. total and faecal coliform (*E.coli*) by the most probable number (MPN) method:

1. Fifteen tubes containing lauryl tryptose broth were arranged in three rows (5 tubes each).
2. First row contained 10 mL double strength lauryl tryptose broth which was inoculated with 10 mL of water sample.
3. Second row contained 10 mL single strength lauryl tryptose broth which was inoculated with 1 mL water sample.
4. Third row contained 10 mL single strength lauryl tryptose broth which was inoculated with 0.1 ml water sample.
5. The tubes were incubated in an incubator at 44°C for 24 hrs.
6. After incubation, the number of tubes in which lactose fermentation occurred with acid and gas production were counted.
7. Finally, by referring to the probability table (Mcrady table- (FDA Bacterial Analytical manual) the MPN of coliform in 100 mL water sample was estimated.

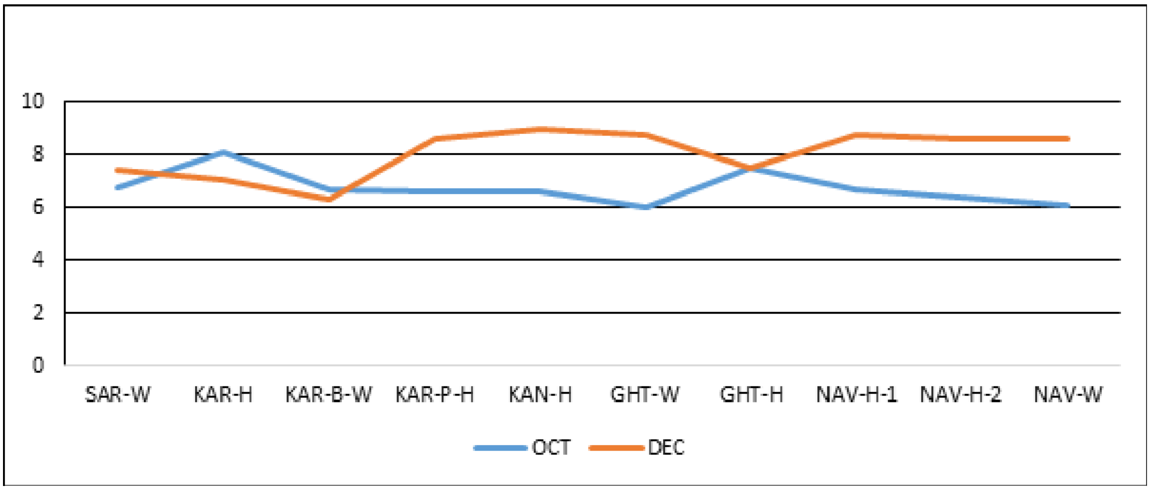

Figure 2: Seasonal pH variation of water

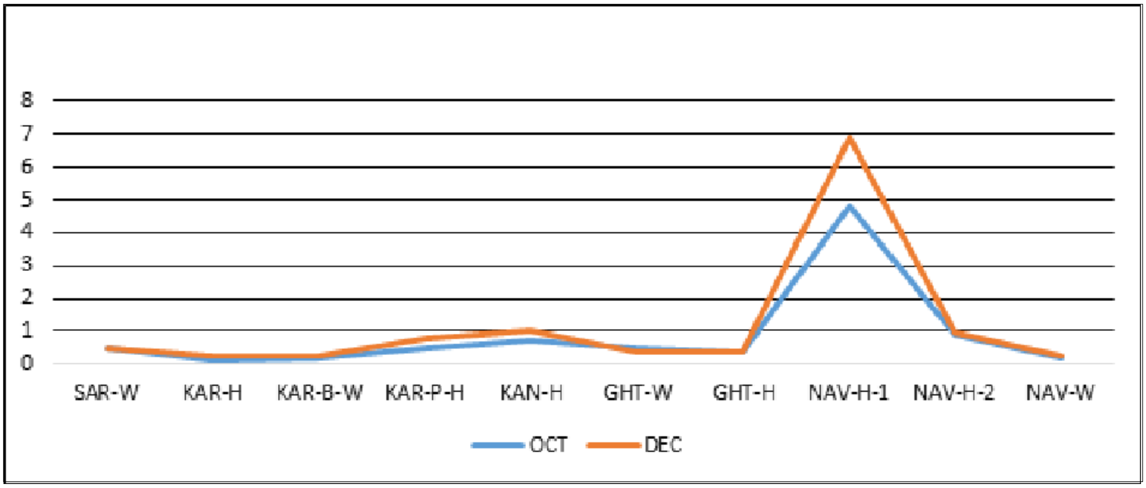

Fig. 3 : Seasonal variation of conductance of water

Table 5: Range of TDS and Total Hardness

Total dissolved solid (TDS)		**Total Hardness:**	
Value (mg/ L)	Quality	Value (mg/ L)	Quality
Less than 300	Excellent	0-43	Soft
300-600	Good	43-150	Slightly Hard
600-900	Fair	150-300	Moderately Hard
900-1200	Poor taste	300-450	Hard
		More than 450	Very Hard

Table 6: TDS values of water samples

TDS in mg/ L	Oct	40	30	130	570	32	28	12500	1100	52	43
	Dec	60	45	240	1620	40	40	33720	1700	80	60
Sample Code		SAR-W	KAR-H	KAR-B-W	KAR-P-H	KAN-H	GHTI-W	GHT-H	NAV-H1	NAV-H2	NAV-W

Table 7: Hardness values of water samples

Hardness in ppm	Oct	160	48	80	176	176	272	224	704	256	64
	Dec	288	112	112	232	512	240	288	1184	560	208
Sample Code		SAR-W	KAR-H	KAR-B-W	KAR-P-H	KAN-H	GHTI-W	GHT-H	NAV-H1	NAV-H2	NAV W

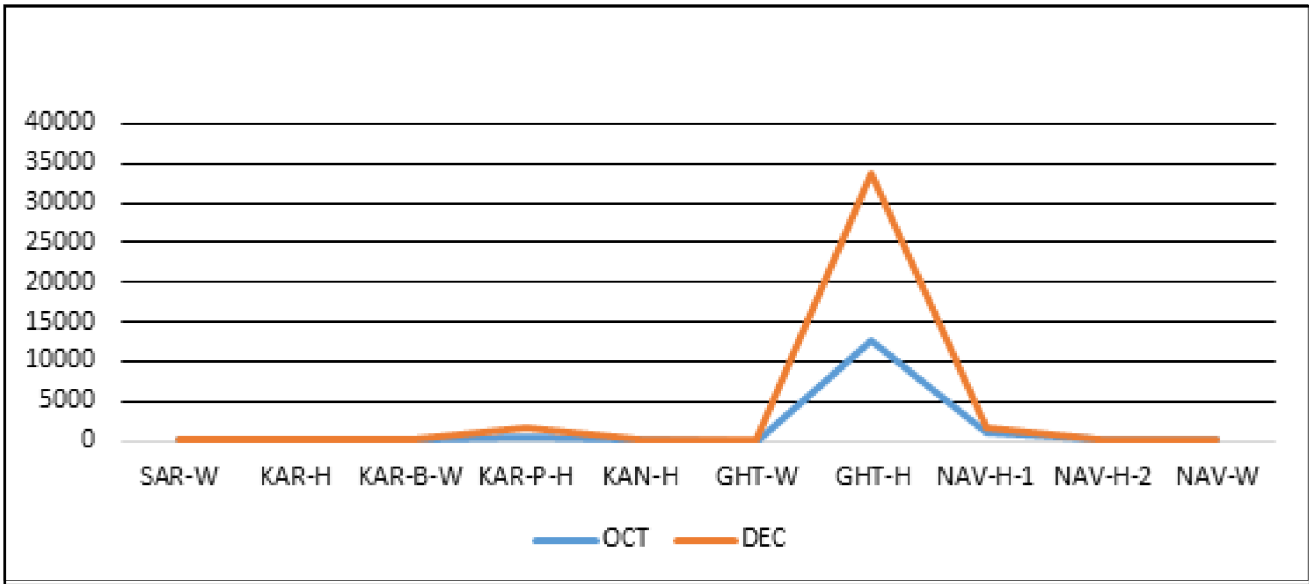

Fig. 4 Seasonal TDS values of water

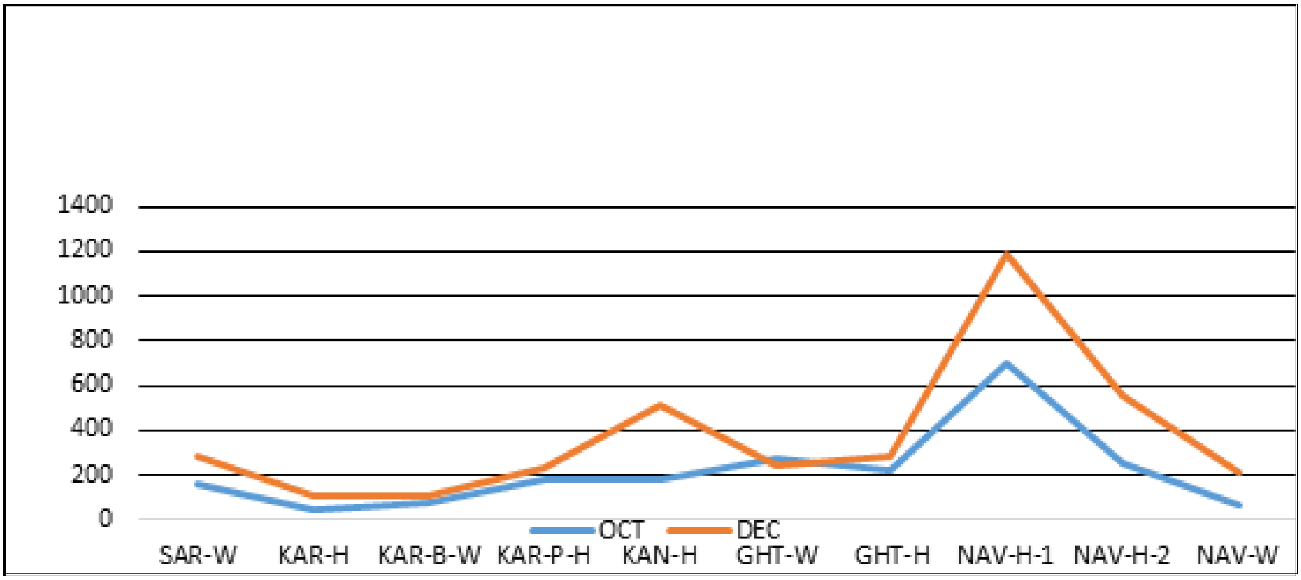

Fig. 5 Seasonal Hardness values of water

Table 8: Ranges of Dissolved Oxygen and Salinity

Dissolved Oxygen(D.O)		Salinity	
Value (mg/ L)	Quality	Value (mg/ L)	Quality
9.5-12	Excellent	Upto 2000	Acceptable
6.5-9.5	Good	Above 2000	Not accabale
4.0-6.5	poor		
0.0-4.0	Very bad		

Table 9: Seasonal variation of Dissolved oxygen values of water

Dissolved oxygen in ppm	Oct	3.23	1.9	6.14	4.2	1.9	2.9	3.23	7.43	2.9	5.5
	Dec	3.10	1.8	6.0	4.05	1.9	2.6	3.06	7.29	2.62	5.34
Sample Code		SAR-W	KAR-H	KAR-B-W	KAR-P-H	KAN-H	GHTI-W	GHT-H	NAV-H1	NAV-H2	NAV-W

Table 10: Seasonal variation of Salinity of water

Salinity in mg/L	Oct	140.4	112.32	168.7	84.2	112.3	168.5	199.6	1881.3	365	140.4
	Dec	140.4	210.2	365	168.6	702	325.6	533.5	2892	842	842
Sample Code		SAR-W	KAR-H	KAR-B-W	KAR-P-H	KAN-H	GHTI-W	GHT-H	NAV-H1	NAV-H2	NAV-W

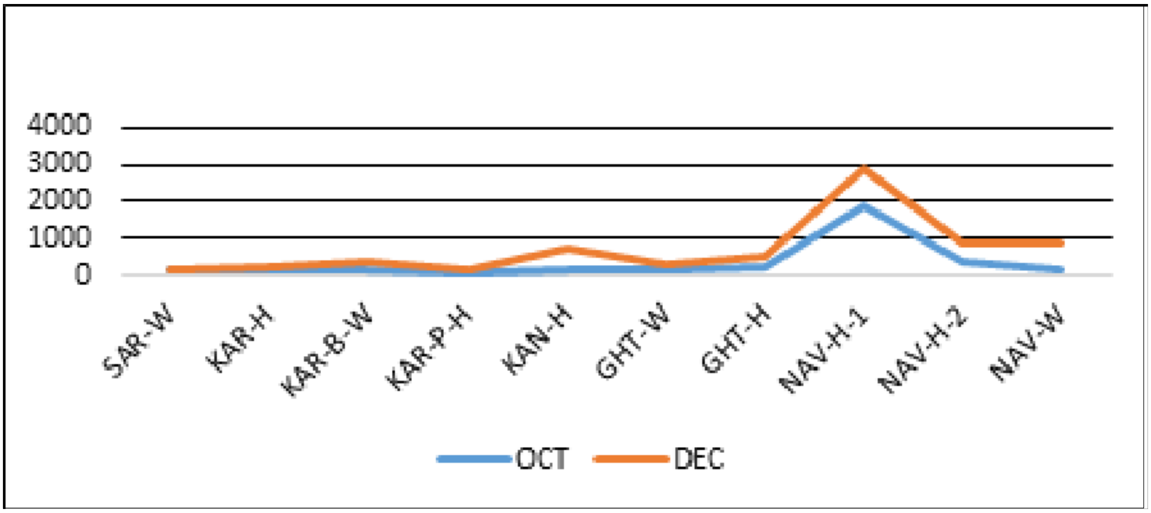

Fig. 6 Seasonal variation of Salinity of water

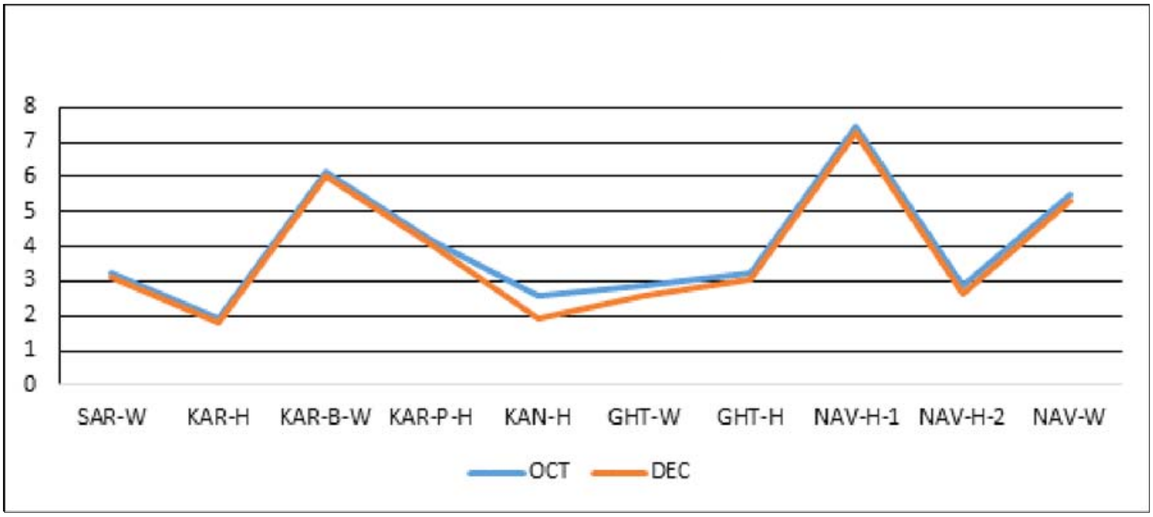

Fig. 7 Seasonal variation of Dissolved Oxygen of water

Results and Discussion

The results of the analysis of water samples from ten different resources of five villages adopted under the Ministry of Higher and Technical Education "Unnat Bharat Abhiyaan" are shown in Table 11. The water samples from the ten resources were collected in the months of October and December 2019 for the proposed study. As per the results of MPN analysis all the water resources indicate non potable nature of water due to high microbial load as shown in Figure 8. The MPN values are high for October 2019 due to heavy rainfall (extended monsoon) which is the major reason of contamination. Though the extent of contamination was low, as determined by low MPN values in December 2019, yet the samples were not safe for drinking purpose.

Table 11: Physiochemical parameter of water

Parameter	pH		Conductance [×10^{-3}]		TDS		Hardness [ppm]		Dissolved Oxygen [DO]		Salinity [mg/1lit]		Microbial test	
Sample code	**OCT**	**DEC**	**OCT**	**DEC**	**OCT**	**DEC**	**OCT**	**DEC**	**OCT**	**DEC**	**OCT**	**DEC**	**OCT**	**DEC**
SAR-W	6.74	7.44	0.513	0.49	40	60	160	288	3.23	3.10	140.4	140.4	80	2
KAR-H	8.13	7.1	0.107	0.22	30	45	48	112	1.9	1.8	112.32	210.2	130	14
KAR-B-W	6.7	6.29	0.214	0.22	130	240	80	112	6.14	6.0	168.7	365	30	22
KAR-P-H	6.6	8.65	0.495	0.81	570	1620	176	232	4.2	4.05	84.2	168.6	>1600	130
KAN-H	6.66	9.01	0.723	1.04	32	40	176	512	2.6	1.9	112.32	702	190	17
GHT-W	6.03	8.74	0.495	0.37	28	40	272	240	2.9	2.6	168.5	325.6	70	4
GHT-H	7.5	7.52	0.397	0.38	12500	33,720	224	288	3.23	3.06	199.6	533.5	170	140
NAV-H-1	6.72	8.81	4.8	6.9	1100	1700	704	1184	7.43	7.29	1881.3	2892	500	2
NAV-H-2	6.41	8.65	0.915	0.96	52	80	256	560	2.9	2.62	365	842.4	23	2
NAV-W	6.13	8.65	0.179	0.24	43	60	64	208	5.5	5.34	140.4	842.4	>1600	2

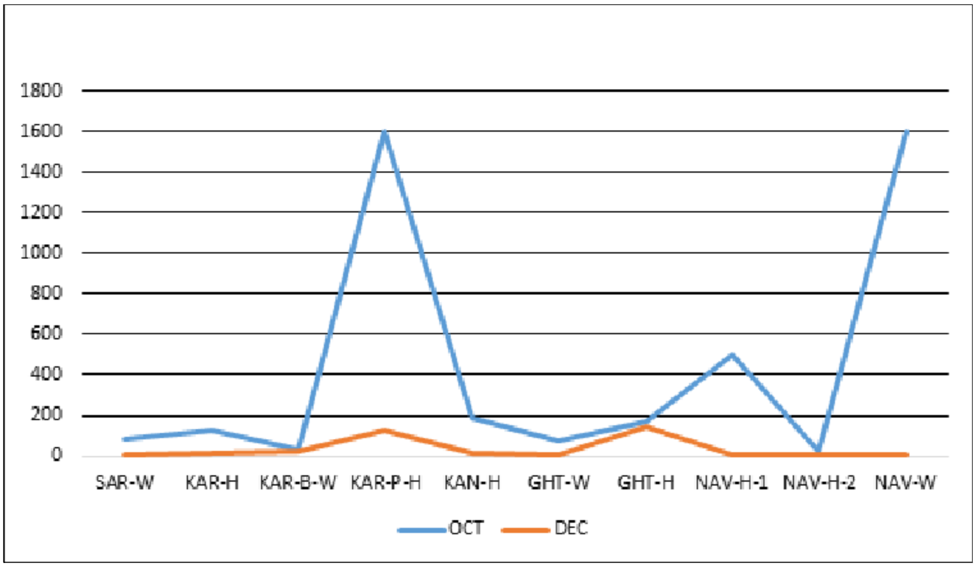

Fig. 8 Seasonal variation of MPN

Conclusions

The physicochemical parameters of water quality have been determined for water samples from five villages near Saphale, Maharashtra State, India. Microbial risk is associated with ingestion of water that is contaminated with human or animal (including bird) faeces. Faeces can be a source of pathogenic bacteria, viruses, protozoa and helminths and is a source of great concern.

References

1. Rahmanian, N., Siti Hajar Bt Ali and Homayoonfard, M., 2015, *Journal of Chemistry*, Article Id 716125.
2. prsindia.org/ theprsblog/status-drinking water and sanitation-rural-india
3. Chandrashekar, S.V.A. and Kodarkar, M.S., 1996, *J. Aqua. Biol.*, **9 (1 and 2)**, 30-33
4. Dissmeyer, G. E., 2000, Drinking water from Forests and Grasslands, South Research Station, USDA Forest Service, Ashville, NC, USA.
5. L.M.L. Nollet, 2000, Handbook of Water Analysis, Marcel Dekker, New York, NY, USA.
6. India water portal.org/articles/ Indian-standards-drinking-water-bis-specifications-10500-2012-second -revision. posted Thursday 2020-02-20
7. who.int/water_sanitation_ health/dwq/Chemicals/ tds.pdf
8. Chemistry.bd.psu.edu/jircitano/water05.pdf
9. Ber. Deutsch chem. Gos.21, 2843
10. Environmental Chemistry of Bostan Harbor_January-IAP-2006/labs/dissolved_oxygen_pdf.
11. nodc.noaa.gov/ archive/arc0001/9900162/2.2/data o-data/igofscd /files/protocol/ cap 5 and 6.html.
12. APHA,2005, Standard methods for examination of water and waste water, 21st Edition, American public Health association/ American water works association/ water environment federation, Washington DC.
13. (WHO Guidelines for drinking-water quality [electronic resource]: incorporating 1st and 2nd addenda, Vol.1, Recommendations. – 3rd ed. Volume 1 https://www.who.int/water_sanitation_health/dwq/ fulltext.pdf)
14. FDA Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. Chapter 4.
15. USP, “Microbial Limit Tests,” USP XVIII, p. 846, 1970.
16. Sutton, S., 2010, *Journal of Validation Technology*, 35-38
17. American Public Health Association (APHA), 1998 and Water Pollution Control Federation, Standard methods for the examination of water and waste water 20th ed, Washington D.C.
18. *IOSR Journal of Environmental Science, Toxicology And Food Technology (IOSR-JESTFT)* e-ISSN: 2319-2402,p- ISSN: 2319-2399. **4(3)**, 17-22: https:/ /www.iosrjournals.org/iosr-jestft/papers/vol4-issue3/ C0431722.pdf?id=3757)
19. WHO Guidelines for drinking-water quality [electronic resource]: incorporating 1st and 2nd addenda, Vol.1, Recommendations. – 3rd ed. Volume 1 https://www.who.int/water_sanitation_health/dwq/ fulltext.pdf)

Conference Alerts

1) European Symposium on Organic Chemistry (ESOC 20210
July 5-6, 2021, Online Symposium
Website: http://www.esoc2021.org

2) 56th International Conference on Medicinal Chemistry (RICT 2021)
July 7-9, 2021, Online Conference, France
Website: http://www.rict2021.org/?

3) The 5th International Conference on Materials Chemistry and Nano materials (ICMSN20210
July 13-15, 2021, London, UK
Website: http://www.icmsn.org

4) 6th International Conference on Green Energy Techniques (ICGET2021)
July 14-16, 2021, Frankfurt, Germany
Website: http://www.icget.org

5) The 7th International Conference on Chemical Materials and Process (ICCMP2021)
July 18-20, 2021, Paris, France
Website: http://www.iccmp.org

6) The 12th International Conference on Chemistry and Chemical Engineering (ICCCE2021)
July 18-20, 2021, Paris, France
Website: http:www.iccce.org

7) 5th International Conference on Green Chemistry and Sustainable Engineering (GREEN 21)
July 21-23, 2021, Rome, Italy
Website: https://greenchem-20.com/

8) 13th International Conference on Chemical, Biological and Environmental Engineering (ICBEE 2021)
August 6-8, 2021, Hangzhou, China
Website: http://www.icbee.org

9) The 6th International Conference on Advanced Functional Materials (ICAFM2021)
August 25-27, 2021, Online Conference, Tokyo, Japan
Website :http://www.icafm.org

10) International Symposium on Medicinal Chemistry (EFMC-ISMC 2021)
August 29-September 2, 2021, Online Conference, Switzerland
Website: http://www.efmc-ismc.org?

11) Chemistry World Conference
September 6-8, 2021, Rome, Italy
Website: //chemistryworldconference.com/

12) Global Summit and Expo on Materials Science and Nanoscience
September 6-8, 2021, Lisbon, Portugal
Website: https://www.thescientistt.com/materials-science-nanoscience/

13) 4th World Chemistry Conference and Exhibition
October 4-6, 2021, London, UK
Website: https://worldchemistry.org/

14) 8th ICSTR Dubai International Conference on Science and Technology Research
October 25-26, 2021, Dubai, UAE
Website: https:straevents.org/conference/dubai-icstr-25-26-oct-2021

15) 32nd Dubai International Conference on Chemical, Biological and Environmental Engineering (DCBEE-21)
Online Conference, November 15-16,2021,Dubai
Website: http://drabi.org/conference/329

16) 33rd Johannesburg International Conference on Chemical, Biological and Environmental Engineering (JCBEE-21)
Online Conference, November 17-19, 2021, Johannesburg, South Africa
Website: https://www.iicbe.org/2021/18/18/127

17) 27th ISBC International Conference on Futuristic Directions in Chemistry and Biology (ISCBC2021)
November 29-December 1, 2021, Thapar Institute of Engineering and Technology, Patiala, India
Contact: Prof Amjad Ali, Convenor, Email: iscbc.tiet@gmail.com

18) 5th International Conference on Nanomaterials and Biomaterials (ICNB2021)
Online Conference, December 20-22, 2021, Tokyo, Japan
Website: https://www.icnb.org

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