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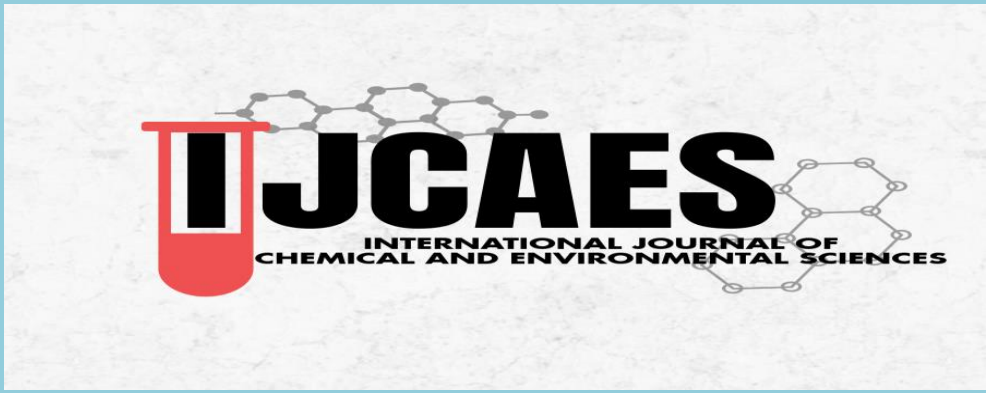

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From Managing Editor's Desk

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Study of Acetolactate Synthase and its Mechanism of Inhibition by Sulfonylurea Active Ingredients: Amidosulfuron, Nicosulfuron, Cyclosulfuron – *In-silico Approach*

Received for publication, May 12, 2020, and in revised form, May 25, 2020

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Abstract

Acetolactate synthase (ALS) or Acetohydroxyacid synthase (AHAS) catalyzes the first step in the synthesis of the branched-chain amino acids i.e., valine (2-amino-3-methylbutanoic acid), leucine (2-amino-4-methylpentanoic acid), and isoleucine (2-amino-3-methylpentanoic acid), in plants, bacteria, algae and fungi but not in humans. AHAS is the main target enzyme for sulfonylurea active ingredients; Amidosulfuron, Nicosulfuron and Cyclosulfuron those assist in lowering branched-chain amino acid synthesis through inhibition to form the complex of Lactyl-ThDP(TDL) to ALS with great practical importance. Amino acid composition, evolutionary and sequence analysis of the ALS protein from *Arabidopsis thaliana* and its homologous were systematically studied. Composition analysis reveals that ALS is a soluble protein. Moreover, the phylogenetic tree showed different clusters based on the source organism and multiple sequence alignment depicts conservative nature in amino acid residues. Furthermore, molecular docking has been conducted to study the interactions between ALS of *Arabidopsis thaliana* and TDL in presence/absence of the active ingredients of sulfonylurea herbicide groups. Molecular docking studies confirm active ingredients are effective to inhibit the binding of TDL to ALS. Our obtained results can be very useful to study specific protein interactions along with developing new herbicides using computational methods.

Key Words: Acetolactate synthase (ALS), Branched-chain amino acid synthesis (BCAA), Lactyl-ThDP (TDL), Herbicide, Molecular docking.

Introduction

Herbicides are chemicals which control or kill undesirable plants commonly known as weed killers. Potent low dose herbicides are known to target Acetohydroxyacid synthase (AHAS) or Acetolactate synthase (ALS), which makes this enzyme, as an important subject of research to weed scientists. Studies particularly relating to changes in amino acids Leucine, Valine, Isoleucine in plants that confer herbicide resistance, have remained the matter of interest for intensive studies.[1] The biosynthesis of neutral, nonpolar, hydrophobic branched-chain amino acids (BCAA) i.e., Leucine, Valine, Isoleucine, having an aliphatic side-chain with a branch (a central carbon atom bound to three or more carbon atoms) in plants, occurs through a series of parallel reactions, mediated by AHAS (E.C

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4.1.3.18) or acetolactate synthase (ALS).[2][3]

Weed Science Society of America (WSSA) and Herbicide Resistance Action Committee (HRAC) divided herbicides according to their mode of action into 27 groups (https://www.intechopen.com/books/herbicides-physiology-of-action-and-safety/modes-of-action-of-different-classes-of-herbicides) like lipid synthesis inhibitor, branched or aromatic amino acid synthesis inhibitor, carotenoid synthesis inhibitor (refer **supplementary Table 1.**) etc.[4][5][6] Based on their mechanism of action herbicide possess an active chemical ingredient which is active in plants, affecting the plant tissue and inhibit many metabolic activities and ultimately leads to plants death.[7]

AHAS from *Arabidopsis thaliana* crystallize as a tetramer whereas AHAS from *Saccharomyces cerevisiae* acts as a dimer.[8] AHAS composed of two monomers and each monomer contains three domains α (85–269), β (281–458), γ (463–639). α domains and γ domains form subunit interface, β domains are faraway from them and play a minor role in stabilizing the dimer interface.[9] There is a C-terminal tail also called capping region (650–687) which is observable when herbicide bound to AHAS and the polypeptide segment is then called mobile loop (580–595). Two types of subunits present in AHAS, one for catalysis, the catalytic subunit that comprises the cofactor thiamine diphosphate (ThDP; also referred to as Thiamine phosphate, TPP) and the regulatory subunit significantly trigger the action of the catalytic subunit even with zero AHAS activity but can confers susceptivity to feedback inhibition by one or more of the branched-chain amino acids synthesis.[10]

Process of condensation of pyruvate to acetolactate, AHAS enzyme requires three co-factors such as Thiamine diphosphate (ThDP), divalent cation i.e., Mg2+, Flavine Adenosine Diphosphate (FAD) in complex with catalytic subunit of *Saccharomyces cerevisiae* as reported by Pang et al., in 2002.[8][9][11][12] This reaction is emphatically mediated by co-factor ThDP with the help of 2-ketobutyrate enzyme and produce 2-aceto-2-hydroxybutyrate.[2][13]

In the first step Thiamine diphosphate (ThDP) is protonated in C2 position and then ionizes to a reactive yield. Then this nucleophilic yield attracts a pyruvate to give Lactyl-ThDP (L-ThDP). At third step ThDP is decarboxylated in presence of pyruvate to give resonating Hydroxyethyl-ThDP/emine intermediates. Emine of Hydroxyethyl–ThDP undergoes separation of charge to give α-carbanion that reacts with another pyruvate or 2-keto acid and to generate product complex. Finally, product is released and ThDP regenerated.[14][15]

Sulfonylurea herbicides were discovered by Levitt and his colleagues, the most active herbicide for inhibition of AHAS enzyme.[16] Sulfonylurea forms complex with Acetolactate synthase (ALS) of *Arabidopsis thaliana* and inhibits binding of pyruvate to ALS by the way of competitive or uncompetitive inhibition.[17][18] It contains an aromatic ring attached to the sulphur atom by the sulfonyl urea bridge and a heterocyclic ring attached to the nitrogen atom. Aromatic ring is ortho substituted where the heterocyclic ring is meta substituted. Earlier studies have been carried out on the expression, purification, crystallization, structural

modelling of AHAS and describe how AHAS bound with some sulfonylurea herbicide but the mechanism of inhibition is not clear till date.[9][19][20][21]
In this paper, molecular docking has been conducted to study the interaction between AHAS of *Arabidopsis thaliana* and Amidosulfuron, Nicosulfuron and Cyclosulfamuron; selective active ingredients of sulfonylurea herbicide group to know their inhibition mechanism. Amino acid composition, evolutionary and conserved domain analysis of AHAS had also been evaluated. Our analysis will be beneficial for further development of new and effective herbicides in future.

2. Materials and method

2.1 Retrieval of the sequences

Initially we have collected amino acid sequence of ALS of *Arabidopsis thaliana* genome from Plant ensembl (http://plants.ensembl.org/index.html). Then we have searched for the homologous sequences using blastp (Basic Local Alignment Search Tool – protein to protein) 2.2.32 (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and ALS protein sequences of *Arabidopsis thaliana* as query.[22][23][24] On the basis of query coverage 70% and e-value 0.0 we have selected most related 53 sequences from different organisms (refer in **supplementary Table 2.**). FASTA format of those homologous ALS protein sequences were retrieved from NCBI (National Center for Biotechnology Information).

2.2 Analysis of the protein primary and secondary structure and prediction of the conserved domains

We know that Domains are responsible for function and interaction of particular protein and these are the conserved part of protein sequence. To identify domains present in these selected protein sequences, we have analysed the sequences by using conserved domain search tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).[25] The secondary structural characteristics/content was predicted through Self-Optimized Prediction Method with Alignment (SOPMA) tool. It is available free from http://npsaprabi.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_sopma.html.[26]

2.3 Multiple Sequence Alignment and Phylogenetic Analysis

To contemplate the concise sequence alignment of the selected homologous ALS protein sequences from different organisms along with *Arabidopsis thaliana*, multiple sequence alignment was executed using Clustal Omega tool (www.ebi.ac.uk/tools/msa/clustalw2) with default parameter values and jalview display option was used to visualize along with sequence logo option for the same. A 1000-time boot strapped phylogenetic tree was generated following neighbour joining method based on the multiple sequence alignment of their protein sequences and displayed using Molecular Evolutionary Genetic Analysis (MEGA V6.06) program, which will depicts the evolutionary relationships among those selected organisms on the basis of the mutation acquired by AHAS.[22][27]

2.4 Physicochemical property

Physiochemical properties of selected proteins including molecular weight, theoretical pI, negative charge, positive charge, instability index, GRAVY (grand average hydropathy) etc. was calculated by using ProtParam tool available on Expert Protein Analysis System (ExPASy) proteomics server (http://web.expasy.org/protparam/).[25][28]

2.5 Statistical analysis

Individual amino acid frequencies of the selected ALS protein sequences were calculated using in-house PERL script. Some statistical analysis such as average, standard deviation and Z value of amino acids frequencies and groups on the basis of amino acids physicochemical properties for those ALS proteins were measured in MS Excel 2007.[29][30]

$$Z=(X_c-X_g)/\sqrt{((S_c^2+S_g^2)/(N_c+N_g)}$$

Here 'X_i' and 'S_i' represents the average and standard deviation of individual amino acids of AHAS from the selected organisms where 'i' represent c and g to denote Brassicales and Rosales order respectively and 'N' represents the sample size.[31]

2.6 Identification of interacting proteins of ALS

We have searched within STRING online resources at http://string-db.org/ to know the interacting proteins of ALS in *Arabidopsis thaliana.*[32] Ketoacid reductoisomerase, Dihydroxyacid dehydratase, 2-isopropylmalate synthase and 3-isopropylmalate dehydrogenase are procured as frequently interacting proteins of ALS in *Arabidopsis thaliana*.

2.7 Retrieval of AHAS (Arabidopsis thaliana) homologous sequences and their three dimensional structure

Again we have used blastp and protein sequence of ALS from *Arabidopsis thaliana* against PDB (Protein Data Bank) (http://www.rcsb.org) to search for the related sequences whose 3D structure is also available. From blastp result, protein sequences were collected based on query coverage 82% and e-value 8e-47 as threshold. ALS sequences in FASTA format from 6 related organisms including *Arabidopsis thaliana(ath), Cadida albicans SC 5314(cal), Saccharomyces cerevisiae(sce), Pseudomonas protegens(pfl), Basillus subtilis PY79(bsp)* and *Klebsiella pneumoniae(kpn*) were retrieved from NCBI and boot strapped (1000 times) phylogeny analysis of these six sequences was performed through MEGA V6.06 to know their closeness.[27] The PDB id's of the selected sequences are as follows 5WJ1(ath), 6DEK(cal), 1JSC(sce), 5AHK(pfl), 4RJJ(bsp) and 1OZF(kpn). Further more, structure alignment was also performed to see structural similarity between the closely related sequences using PyMOL (version 2.1.0).[33][34]

2.8 Molecular Docking study

Molecular docking is the computational study that involves interaction of two or more molecules (e.g., drug and protein or enzyme) with ligand (small molecules) and predicts the ligand binding site as well as ligand affinity of the targeted molecule (protein).[35]
For docking, at first 3D structure of active ingredients of sulphonylurea chemical family herbicides: Amidosulfuron (CID: 91777), Nicosulfuron (CID: 73281), Cyclosulfamuron (CID: 6451137) and Lacty-lThDP(L-ThDP/TDL were collected from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and reserved the 3D structure as SDF file. That SDF file was converted to PDB file by using Auto dock and UCSF Chimera (an extensible software platform).[36] PDB file of ALS protein of *Arabidopsis thaliana* (PDB ID 5K6Q) was retrieved from RCSB PDB protein data bank (https://www.rcsb.org/) following molecular docking by using Autodock vina tool and Autodock (version 4.2.6) tool, forming a grid above the selected protein structure. The selected ligands were bind to targeted protein and this compound was then saved as PDB file. The best interaction between the ALS protein and active ingredients was displayed through LIGPLOT+ v.2.1 tool.[37] We have used 5K6Q in place of 5WJ1 as the later one is in a complex with other small molecule whereas the previous one is only the structure of *Arabidopsis thaliana*, acetohydroxyacid synthase catalytic subunit and their sequences are identical to each other along with their structural similarity judgment through PyMOL.

3. Results and Discussions

3.1 Conserved Domain Analysis

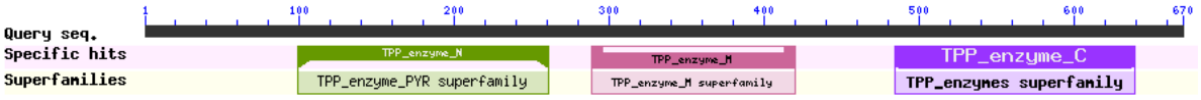

Fig1. Conserved domain structure of ALS protein from *Arabidopsis thaliana* obtained from NCBI Conserved Domain Search – NIH.

A number of enzymes require thiamine pyrophosphate (TPP) (vitamin B1) as a cofactor. It has been shown that some of these enzymes are structurally related. Some of them are TPP_enzyme_N, TPP_enzyme_M and TPP_enzyme_C. The N-terminal TPP binding domain of TPP enzymes represents the alpha(α) subunit, or the N-terminal region. The M-terminal TPP binding domain of TPP enzymes represents the gamma(γ) subunit, or the M-terminal region. Nearly every member of the C-terminal TPP binding domain of TPP enzymes is the beta(β) subunit, or else the C-terminal region. Aligning query sequence of TPP_enzyme_N, TPP_enzyme_M and TPP_enzyme_C are approximately ranges from 100-250, 290-420, 485-640 respectively. Conserved domain structure of ALS protein of every considerable organisms (fig.1) are quite similar to each other (for complete graphical representation refer **supplementary** (fig.1).

3.2 Multiple Sequence Alignment (MSA)

Multiple sequence alignment (MSA) methods refer to a series of algorithmic solution for aligning more than two biological sequences (DNA, RNA or Protein) at a time considering evolutionary events (mutations, insertions, deletions etc.).

Multiple sequence alignment was performed to study amino acid conservation at different site of selected AHAS sequences. There are three conserved domains present in AHAS protein sequence of *Arabidopsis thaliana* N-terminal domain(α), M-terminal domain(γ) and C-terminal domain(β) and sequences from selected organisms i.e., TPP_enzyme_N, TPP_enzyme_M, TPP_enzyme_C. Sequence alignments have revealed conservation of interacting amino acids. Conserved domain of amino acids are present from position within alignment 140 to 302 (fig 2a.), 334 to 466 (fig 2b.) and 530 to 686 (fig2c). In the jalview representation corresponding height of the different amino acid residues in sequence logo further the colour conservation at the different sites, unveil a few variations in the ALS encoding gene, suggesting the level of conservation of amino acids.

Fully conserved amino acids (site number in sequence alignment) are as follows: G(140), D(142), L(144), A(147), E(149), L(148), A(150), G(152), V(153), V(156), F(157), A(158), Y(159), P(160), G(161), G(162), E(166), I(167), H(168), Q(169), A(170), L(171), T(172), R(173), G(200), G(203), L(217), M(241), G(243), A(253), A(255), E(264), I(272), R(287), H(300), H(301), H(302), L(334), Q(336), R(339), I(440), I(442), G(448), A(468), K(449), K(451), K(462), A(464), G(530), G(532), H(534), Q(535), M(536), W(537), A(539), Q(540), G(555), G(557), L(602), P(603), L(614), Y(625), H(631), S(635), L(683) respectively. In contrast to the fully conserved amino acid sites, the non- or semi-conserved sites equip with more beneficial information for evolutionary study. The amino acid pattern present in the non/semi conserve sites yield quite useful facts on the possible places of changes or mutations occurred in these sequences during evolution, bring out differences in the grouping of the identified proteins in different clades, as evidenced in the phylogenetic analyses (fig. 3.).

Less conserved site in sequence alignment indicates nearly similar pattern within organisms in orders.[9] From this multiple sequence alignment and jalview representation along with sequence logo, we are able to understand about the effect of mutation acquired in the ALS encoding gene and reflection on phylogeny. However many of the mutations are silent mutation, *Tarenaya hassleriana, Arabis alpina. Trema orientale, Parasponia andersonii* belongs to Brassicales and Rosales respectively and most non conserved site within ALS domains of these organisms are mutated and their effect of mutations are notable on phylogeny for these organisms (e.g., pointed as no.; i, ii, v, vi, viii) besides a few with hardly any impact over phylogenetic tree (e.g., pointed as no.; iii, iv, vii, ix, x).

A number of mutations have been mentioned below supporting the analysis carried out with jalview (For detailed record refer **supplementary Table 3. A., 3. B., 3. C.**).

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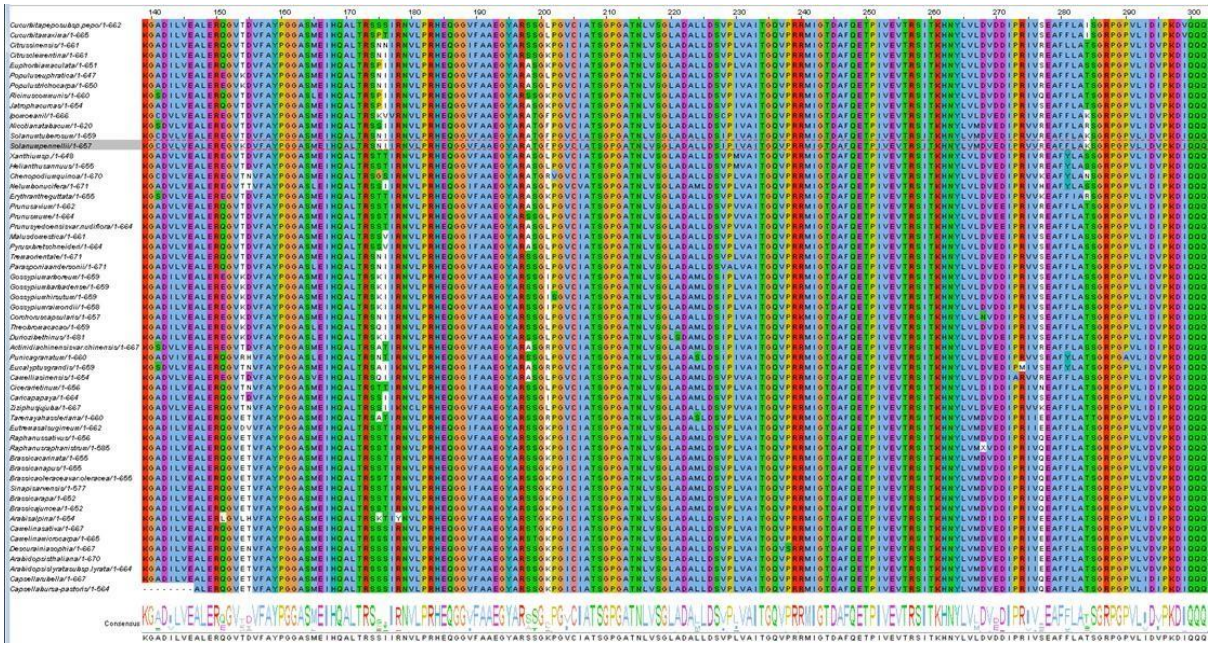

Fig 2A**.** JalView representation with sequence logo of multiple sequence alignment using Clustal Omega of the ALS sequences from selected organisms for the domain TPP_Enzyme_N

From the above jalview representation (Fig2A**.**) we have observed many non-conserved amino acids present in this conserved domain. Depending upon that we have detected a number of mutable sites as follows with positive impression over the phylogenetic tree besides some with no impact:

i. At site 151 in aligned condition is occupied by amino acids Glutamine, Glutamic acid and Leucine. Brassicales, Rosales, Myrtales, Cucurbitales are possess Glutamine. *Populas euphratica, Populus tricocarpa* belongs to Malpighiales order, Solanales, Asterales, Proteales, Laminales, Mavales, Ericales possess amino acid Glutamic acid and only *Arabis alpina* belongs to Brassicales which possess Lysine at this site. Glutamic acid and glutamine both are polar and hydrophilic whereas Lysine is nonpolar, hydrophobic and Glutamine and Lysine both are neutral whereas Glutamic acid is acidic. Therefore, at this site mutation has occurred.

Effect of mutation observed in Brassicales (*Arabis alpina*), Malpighiales (*Populas euphratica, Populas triocarpa*) on phylogenetic tree.

ii. Likewise, site 178 is almost occupied by amino acid Arginine with some differences for *Arabis alpina, Ziziphus jujube, Eucalyptus grandis* which belongs to Brassicales, Rosales, Myrtales respectively. *Arabis alpina* (Brassicales) possess amino acid Tyrosine while *Ziziphus jujuba* and *Eucalyptus grandis* possess Lysine. Arginine and Lysine are basic whereas Tyrosine is neutral. Lysine and Tyrosine are hydrophobic in nature but Arginine is hydrophilic. Therefore, mutation occurred in this site. Effect of mutation on phylogenetic tree observed in *Ziziphus jujuba* (Rosales) and *Arabis alpina* (Brassicales).

iii. Site 221 is occupied by Alanine and only *Durio zibenthus* belongs to malvales and possess Serine amino acid at this site. Alanine and Serine both are neutral but Alanine is nonpolar, hydrophobic whereas Serine is polar, hydrophilic in nature. Even while mutation occurs at this site but no phylogenetic impact was observed.

iv. Similarly, site 274 is almost occupied by Arginine. *Eucalyptus grandis* (Myrtales) possess Methionine. Arginine is basic, polar and hydrophilic whereas Methionine is neutral, nonpolar hydrophobic hence mutation occurs yet no phylogeny impact observed.

Just as in conserved domain TPP_Enzyme_N, kind of similarity noticed for the mutable sites from the above representation (Fig 2B) too.

v. Site 344 is almost occupied by amino acid Serine. *Xanthium sp., Helianthus annus* (Asterales), *Chenopodium quinoa* (Caryophyllales), *Actinidia chinensis ver chinensis* (Ericales), *Capsellarubella* (Brassicales), *Capsellabursa-pastoris* (Brassicales) occupied by amino acid Alanine.

Serine and Alanine both are neutral other than Serine is polar, hydrophilic but Alanine is nonpolar and hydrophobic. Considering this, mutation occurs at this site and phylogenetic impact is observed on Brassicales order.

vi. Site 346 is hold up with amino acid Lysine, Arginine, Methionine. *Punica granatum (Myrtales), Eutrema salsugineum, Raphanus sativas, Raphanus raphanistrum, Brassica rapa,*

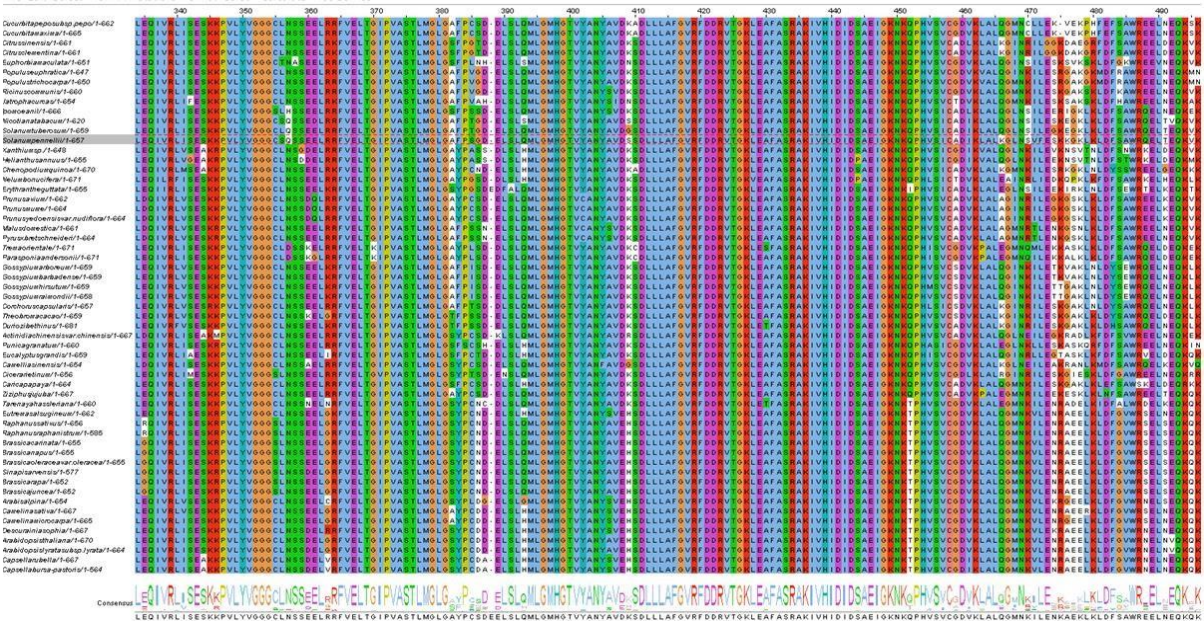

Fig2b**.** JalView representation with sequence logo of Multiple Sequence Alignment using Clustal Omega of the ALS sequences from selected organisms for the domain TPP_Enzyme_M.

Brassica juncea, Brassica carinata, Sinapis arvensis, Arabis alpine (Brassicales), Mulus domestica, Pyrus X bretsctneideri (Rosales) filled by amino acid Arginine. *Actinidia chinensis ver. chinensis* belongs to Ericales order and occupied by amino acid Methionine. Lysine and Arginine both are basic, polar and hydrophilic bow out Methionine as neutral, nonpolar and hydrophobic. Hence mutation occurs at this site and *Mulus domestica and Pyrus X bretschneideri* cut off from other plants which belongs to Rosales.

vii. In contrast, at site 405 it is almost occupied by amino acid Tyrosine. *Camelina sinesis* belongs to Ericales and filled up with Phenylalanine. Tyrosine is neutral, polar, hydrophilic in other hand Phenylalanine is neutral, nonpolar, hydrophobic. Although, mutation occurred at this site but no phylogeny impact is notable.

By the same token in the above case (fig2c.) of conserved domain TPP_Enzyme_C some inequalities are noted in the event of mutation at different mutable sites.

viii. Site 570 of alignment is almost occupied by Alanine and Serine. Only Brassicales order (except *Tarenaya orientale, Arabis alpina*) possess with amino acid Serine. Where Alanine is neutral, nonpolar, hydrophobic in nature, Serine is neutral, polar and hydrophilic. That is why, mutation occurs with evident of phylogenic impact in Brassicales order.

ix. Site 572 is occupied by amino acid Alanine. Only Solanales order possess with amino acid Glycine. Alanine and Glycine both are neutral, nonpolar but alanine is hydrophobic and glycine is hydrophilic. Therefore, mutation occurs at this site with no positive phylogeny impact.

Fig 2c. JalView representation with sequence logo of Multiple Sequence Alignment using Clustal Omega of the ALS sequences from selected organisms for the domain TPP_Enzyme_C.

x. Site 678 is almost reserved with amino acid Proline. Cucurbitales order possess with amino acid Glutamate. Proline is neutral, nonpolar, hydrophobic but Glutamine is acidic, polar and hydrophilic. Hence, mutation occurs at this site but no phylogeny impact is observed.

These above mentioned outcomes support the evolutionary relationship of ALS protein from selected organisms as obtained in phylogenetic tree.

3.3 Phylogenetic analysis

Phylogeny is the evolutionary development of an organ or of a kind of organism. Perusal of Biological evolutionary relationship between two or more organisms shared common ancestors in the past referred to as phylogenetic analysis.

Phylogenetic tree (Fig3) on the basis of AHAS sequences across the selected organisms were categorized into fourteen different clades which supports taxonomical order such as Brassicales, Rosales, malvales, malpighiales, Solanales, Asterales, Laminales, Ericales, Caryophyllales, Cucurbitales, Myrtales, Fabales, Sapindales according to their phylogenetic tree. The twelve different colors in taxonomical order indicate members of the corresponding clades.

Cucurbetales and Fabales are close to each other and Cucurbetales are also related to Malvales. Brassicales are close to Fabales whereas *Carica papaya* situated in Brassicales close to Malvales. *Helianthus annus* belongs to Asterales and *Xanthiam sp.* is closely related to Asterales. Therefore, sequence of ALS from *Xanthiam sp.* is similar to *Helianthus annus*. Solanales, Caryophyllales and Laminales orders are closely associated to each other. Rosales are situated close to Proteales and Solanales. *Ricinus communis, Jatropha curcas, Populus euphratica, Populus trichocarpa* are belong to Malpighiales but *Ricinus communis* and *Jatropha curcas* are more close to *Euphorbia maculate* than, *Populus euphratica, Populus trichocarpa with* Sapindales.

This phylogenetic tree indicates that Brassicales order is the most common ancestor among the selected organisms.

3.4 Analysis of physicochemical properties

Next we have analyzed the physicochemical properties of ALS protein from *Arabidopsis thaliana* and other selected organisms to determine the molecular characteristics. The ALS protein comprised of an average of 600-670 amino acid residues with an average molecular weight of ~65-72 k Da. From physicochemical analysis, obtained grand average hydropathy (GRAVY) score is negative. Higher (positive) GRAVY score indicates that the protein is membrane bound protein where as negative GRAVY score suggests that the protein is soluble in nature. Hence, ALS from *Arabidopsis thaliana* and selected organisms is soluble in nature.[8]

Protein structure and function is contributed by its compositional/constituent amino acids.[20][28] Each of the 20 most common amino acids, with their specific chemical characteristics, contributes their unique role to determine a protein structure as well as its function. Significant positive Z-values were obtained for amino acids glutamate, methionine, glycine and isoleucine, indicates that these amino acids are present with higher frequencies within ALS from Brassicales, whereas, significant negative Z-values for amino acids serine, alanine and proline indicates the presence to a greater extent of these amino acids within ALS from Rosales (**Table$_1$.**). Non-polar aliphatic side groups like methionine, glycine and isoleucine are hydrophobic ("water fearing") in nature. In aqueous solutions, the protein which are made of these amino acids, fold into their characteristic three-dimensional shape, to protect these hydrophobic side chains in the protein interior.

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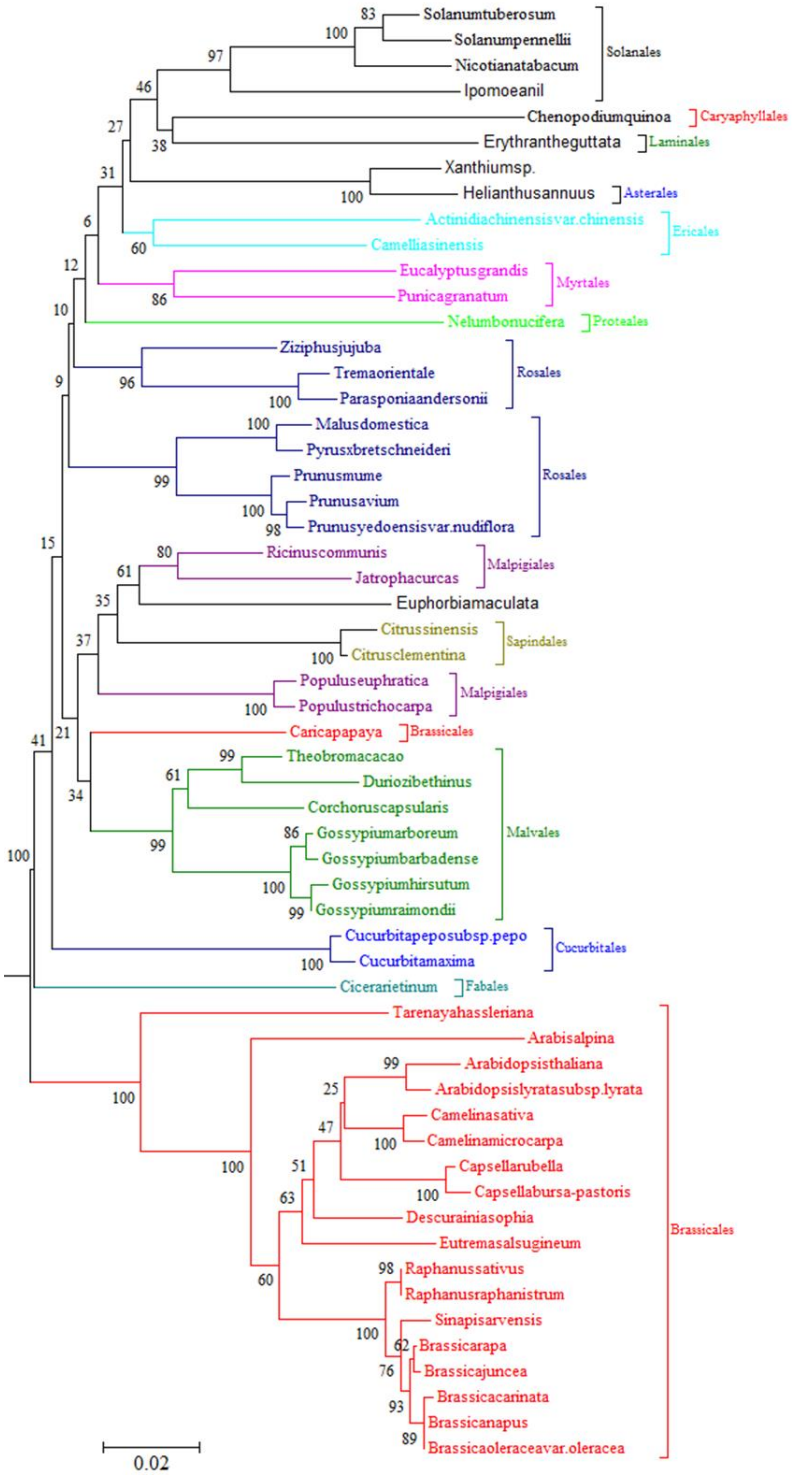

Fig. 3. Phylogenetic relationship of ALS of different organisms on the basis of AHAS sequences using the Neighbour Joining (NJ) method. Amino acid sequences were aligned with Clustal Omega tool (www.ebi.ac.uk/tools/msa/clustalw2) and the phylogenetic tree was constructed using MEGA V6.06 software (http://www.megasoftware.net/). The branching order is validated by 1000 steps of bootstrap replicates. The bootstrap values are shown at the nodes. The number in each node indicates the confidence value of that branch after bootstrapping the phylogenetic tree.

3.5 Statistical analysis

Table 1. Z – scores of different amino acids and their physic-chemical groups of ALS enzyme in two different orders i.e., Brassicales and Rosales. Z- value greater than +1.96 and less than -1.96 ($p<0.05$) are significant**.**

Amino acid	**Z- score**	**Amino acid**	**Z- score**
Negative	**5.827**	**Non polar aliphatic**	-1.575
Glutamate	**6.62**	Proline	**-10.907**
Aspartate	0.353	Methionine	**6.353**
Positive	**3.554**	Glycine	**5.679**
Lysine	0.38	Alanine	**-5.047**
Arginine	0.994	Valine	-1.479
Histidine	1.603	Isoleucine	**3.547**
Aromatic	0	Leucine	-1.616
Tyrosine	-1.058	Polar Uncharged	**-2.129**
Tryptophan	1.806	Glutamate	**2.735**
Phenylalanine	0.34	Asparagine	-1.585
		Serine	**-2.403**
		Threonine	-0.025
		Cysteine	-1.516

Table 2. Secondary structure content and responsible region within corresponding organisms to provide different secondary structure, domain architecture, active sites, binding sites etc. obtained from SOPMA for the selected ALS protein sequences

Organisms	**Alpha helix**	**Extended strand**	**Beta turn**	**Random coil**
Brassicales				
Arabidopsis thaliana	29.40%	20.75%	7.31%	42.54%
Arabidopsis lyrata subsp. lyrata	29.07%	20.33%	7.23%	43.37%
Camelina sativa	29.69%	19.04%	6.75%	44.53%
Raphanus sativus	30.79%	19.21%	7.32%	42.68%

Brassica napus	29.92%	19.08%	6.87%	44.12%
Brassica carinata	29.92%	18.78%	6.87%	44.43%
Brassica oleracea var. oleracea	29.92%	19.08%	5.95%	45.04%
Brassica rapa	31.29%	18.10%	6.29%	44.33%
Brassica juncea	31.44%	18.56%	6.90%	43.10%
Capsella rubella	30.28%	19.04%	6.75%	43.93%
Arabis alpina	29.97%	20.03%	6.57%	43.43%
Raphanus raphanistrum	33.33%	18.63%	8.03%	40.00%
Tarenaya hassleriana	31.36%	17.73%	6.36%	44.55%
Sinapis arvensis	33.97%	19.93%	8.49%	37.61%
Capsella bursa-pastoris	33.69%	19.68%	8.87%	37.77%
Carica papaya	32.53%	18.83%	6.63%	42.02%
Rosales				
Prunus mume	33.58%	17.77%	7.23%	41.42%
Ziziphus jujuba	30.28%	19.49%	7.80%	42.43%
Trema orientale	29.66%	18.63%	7.15%	44.56%
Prunus avium	29.15%	18.88%	7.10%	44.86%
Prunus yedoensis var. nudiflora	33.73%	17.47%	6.93%	41.87%
Malus domestica	29.80%	18.76%	7.11%	44.33%
Pyrus x bretschneideri	30.12%	18.67%	6.78%	44.43%
Parasponia andersonii	29.21%	19.23%	7.00%	44.56%

Brassicales and Rosales are consequences similar nature in the secondary structural characteristics within ALS among the selected groups. The percentages of the alpha(α) helix and the random coils are highest in the ALS of both the groups (**Table$_2$.**). Proteins those are in absolute dearth of well-defined secondary structure, generally exhibits Random coil structures as an alternate. Adjacent residues through the peptide bonds is the atmost alliance among the amino acids those occupy Random coil structures.

3.6 Role of interacting proteins

By using STRING software, interacting partners of ALS and its co- expression genes were identified in *Arabidopsis thaliana* (fig 4**.**) Some protein including Ketoacid reductoisomerase, Dihydroxyacid dehydratase, 2-isopropylmalate synthase and 3-isopropylmalate dehydrogenase are found to be common interacting proteins of ALS in *Arabidopsis thaliana*.In the Isoleucine biosynthesis pathway initially Threonine dehydratase converts Threonine to 2-ketobutyrate and in the 2nd step Acetohydroxyacid synthase converts 2-ketobutyrate to 2-acetohydroxybutyrate in case of Isoleucine and for Valine and Leucine it kick off the

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biosynthesis by forming Acetolactate from Pyruvate. Ketoacid reductoisomerase then produces 2,3-Dihydroxy-3-methylvalerate in the 3rd step for Isoleucine and Dihydroxy isovalerate in the 2nd step for Valine and Leucine biosynthesis. In the 4th step of Isoleucine, Dihydroxyacid dehydratase forms 2-Oxo-3-methylvalerate and 2-Oxoisovalerate in the 3rd step of Valine and Leucine. Aminotransferase produce Isoleucine and Valine in the final step of Isoleucine and Valine biosynthesis respectively. Now Isopropylmalate synthase forms 3-Carboxy-3-hydroxyisocaproate for synthesis of Leucine in the 5th step. Then 3-carboxy-2-hydroxyisocaproate and 2-Oxoisocaproate are produced by Isopropylmalate isomerase and Isopropylmalate dehydrogenase in the 6th and 7th step respectively. Finally Aminotransferase converts 2-Oxoisocaproate to Leucine as a final product.

These all proteins are involved in branched-chain amino acid synthesis pathway for biosynthesis of Valine, Isoleucine, and Leucine conserved in plants, fungi and algae. In plants, the syntheses of these amino acids arise by a series of analogous reactions, mediated by AHAS or ALS.[8][9][12] Unlike most bacterial and fungal AHAS that is sensitive only to Valine, a characteristic of plant that AHAS is sensitive to each of the three branched-chain amino acids is conferred by the regulatory subunits. Further determination of the 3-D structure of the plant AHAS regulatory subunits, and catalytic plus regulatory subunits, could substantially accommodate in assimilation the cross-talk between the subunits, and inevitably, how herbicide resistance mutations in the catalytic subunit affect the enzyme activity and its sensitivity.[38]

3.7 Sequence and Structural similarity among homologous sequences within PDB

Further more, the phylogenetic tree based on the retrieved homologous sequences whose structure is available along with the query sequence depicts their closeness. This phylogenetic tree (**FIG. 5.**) exhibits a clear outline of ALS into two clusters. Phylogenetic tree which we obtained resembles that sequence of ALS from *Arabidopsis thaliana* indistinguishable to the sequence of ALS from *Saccharomyces Cerevisiae* that belongs to Fungi.

3.8 Docking result

We have used UCSF Chimera and Autodock vina to perform the molecular docking that is the study of molecular interaction between the receptor and ligand molecule (refer **supplementary Table 4.**). Molecular docking studies through UCSF Chimera and Autodock vina offer 10 best predictions of interaction and for each and every possible interaction it will give a binding energy value which indicates about the significance of interaction. More negative binding energy indicates more favourable interaction between the receptor and ligand molecule. Then Ligplot + v.2.1 is used to obtained the interaction sites.[36]

3.8.1 Interaction between Acetolactate synthase (ALS) and Lactyl-ThDP (TDL)

Acetolactate synthase (ALS) of *Arabidopsis thaliana* form complex with Amidosulfuron,

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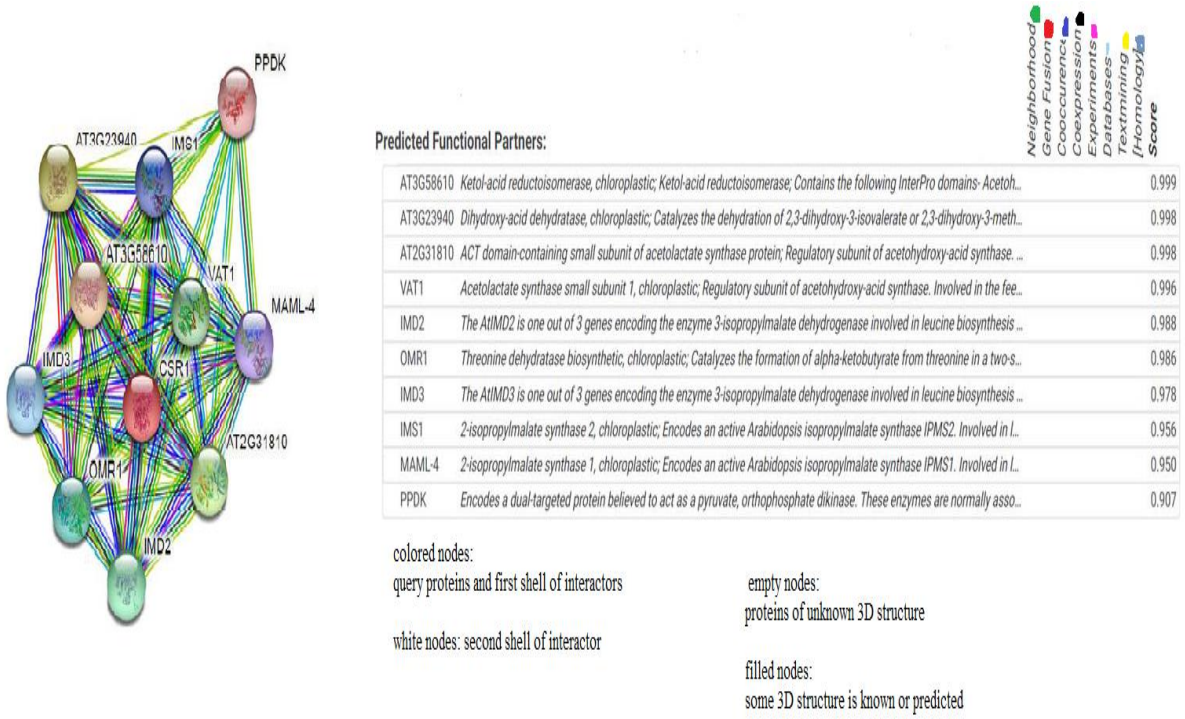

Fig 4. AHAS interacting partners as well as its co expression genes predicted by STRING software (https://string-db.org/cgi/network.pl?taskId=I7kzqYzL745x)

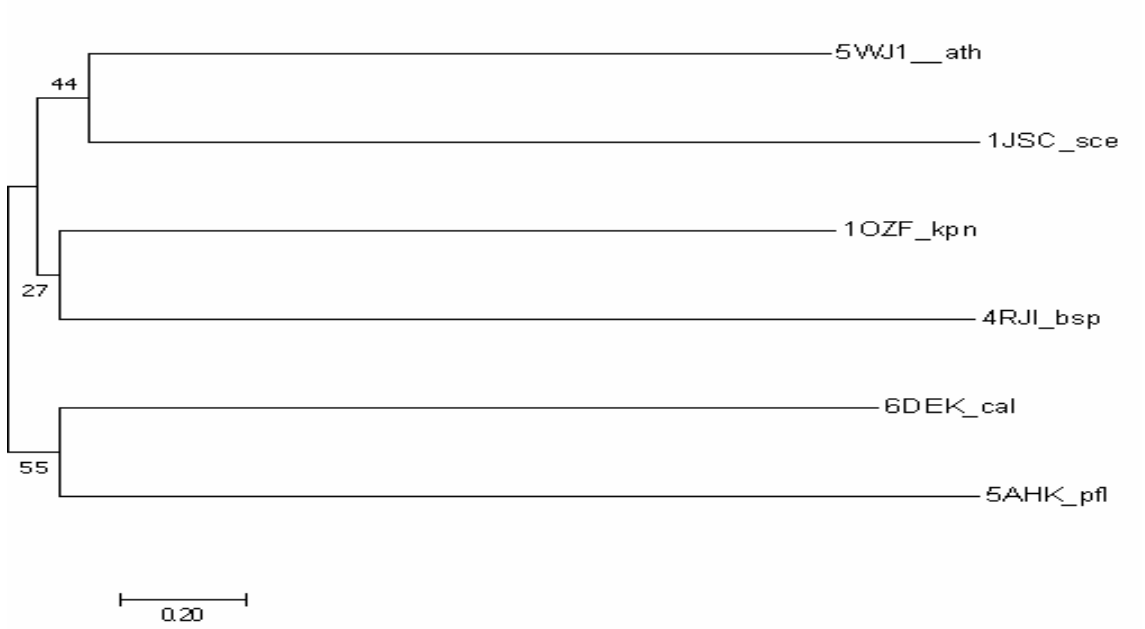

Fig. 5. 1000 times bootstrapped phylogenetic tree obtained through MEGA V 6.06 software based on the 6 homologous sequences whose structure is available in PDB.

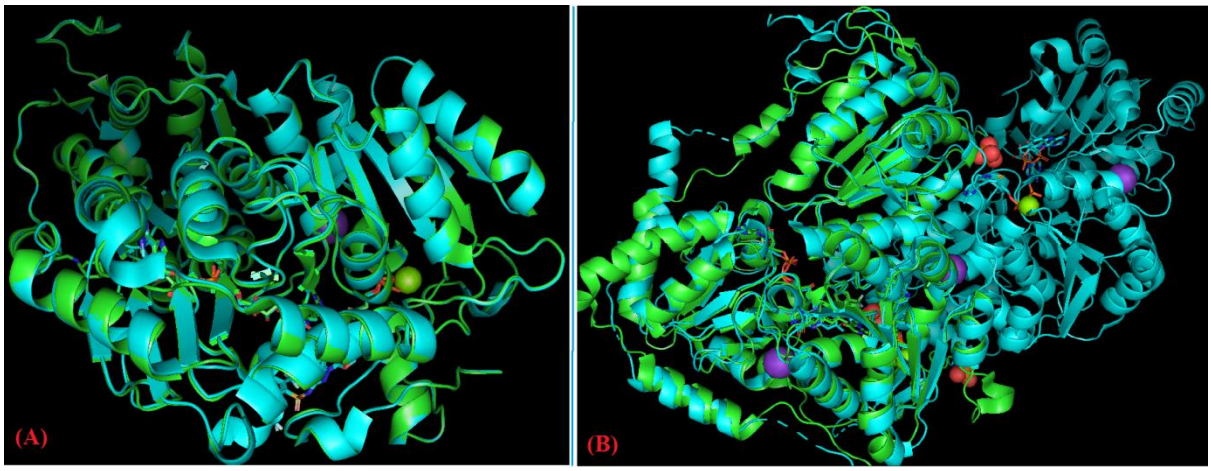

Fig. 6. Structure alignment between **A.** 5K6Q(Green) and 5WJ1(Cyans) with RMSD 0.227; and **B.** 5WJ1(Green) and 1JSC(Cyans) with RMSD 0.824.

NOTE: Moreover, RMSD (Root-Mean-Square-Deviation) values obtained from the structure alignment between 5K6Q and 5WJ1 through PyMOL is 0.227 (Fig.6a.) and between 5WJ1 and 1JSC it is 0.824 (Fig.6b.) which are very low and indicates about their structural similarities.

Nicosulfuron, Cyclosulfuron and inhibits binding of TDL (Lactyl-ThDP) to ALS. This inhibition is either competitive or uncompetitive inhibitions.[17][18]

At first we have used Acetolactate synthase as a receptor molecule and Lactyl-Thdp as a ligand. From these molecular docking studies we have obtained the interacting site molecules of ALS with TDL and binding affinity between ALS and TDL (Fig.7a.). After that we have framed an interaction between ALS and one of the considered active ingredients to form a complex of ALS and active ingredient. Next we analyzed the molecular interaction between ALS-active ingredient complex and TDL (Fig7b.). From the successive molecular docking studies we came to know how the binding affinity decreases when sulfonylurea herbicides binds to ALS and inhibit binding of TDL to ALS.

3.8.2 Interaction between ALS and Amidosulfuron a commonly used sulfonylurea herbicide

Here ALS acts as a receptor protein and Amidosulfuron (Unk1) acts as a ligand . From this molecular interaction (Fig 8a) it is clear that Amidosulfuron binds to ALS by hydrogen bond and hydrophobic bond. The responsible amino acids from ALS to form hydrogen bond are Arg246, Arg279 and for hydrophobic interactions are as follows: Gly245, Tyr276, Asp397, Leu183, Lys220 and co factor NHE 703 of chain A (Fig8b). The binding affinity for this interaction is -6.3 kcal/mol.[39]

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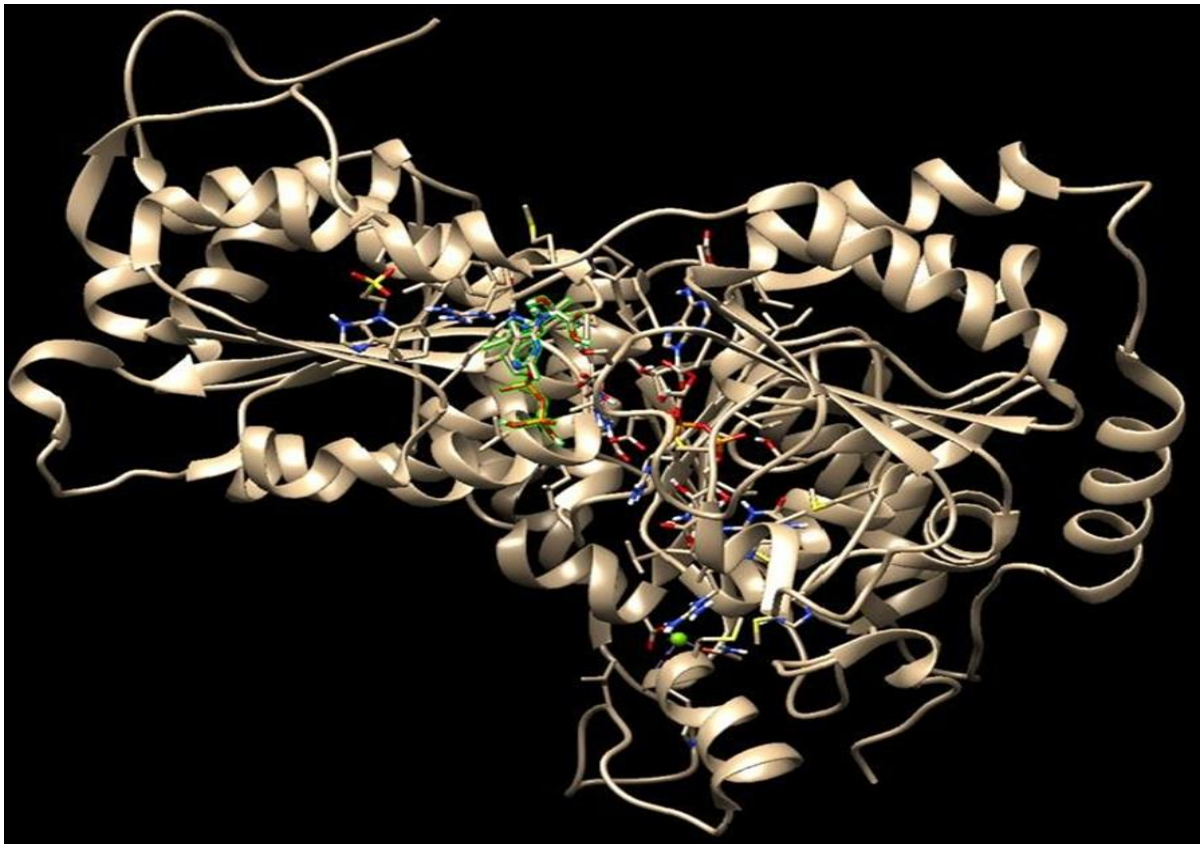
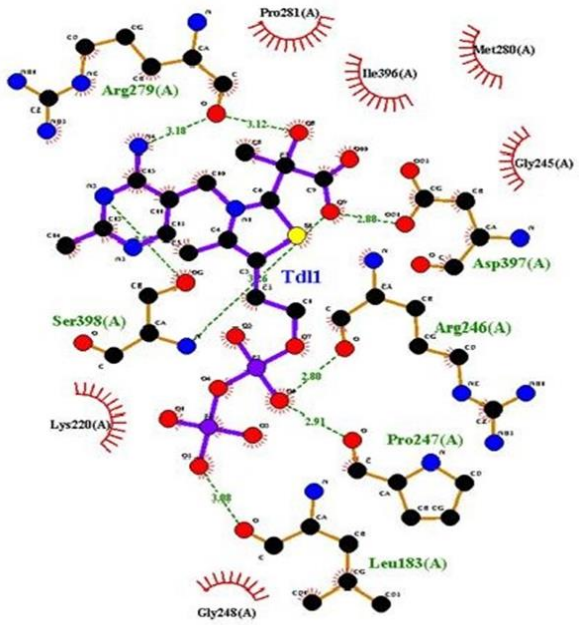


Fig.7a. Docked structure of ALS and TDL by using Autodock vina



ALS+TDL

Fig.7b**.** Interaction site of ALS and TDL by using LigPlot+ v.2.1. where green coloured dashed lines indicate hydrogen bond and red sun like symbol indicates hydrophobic bonds**.**

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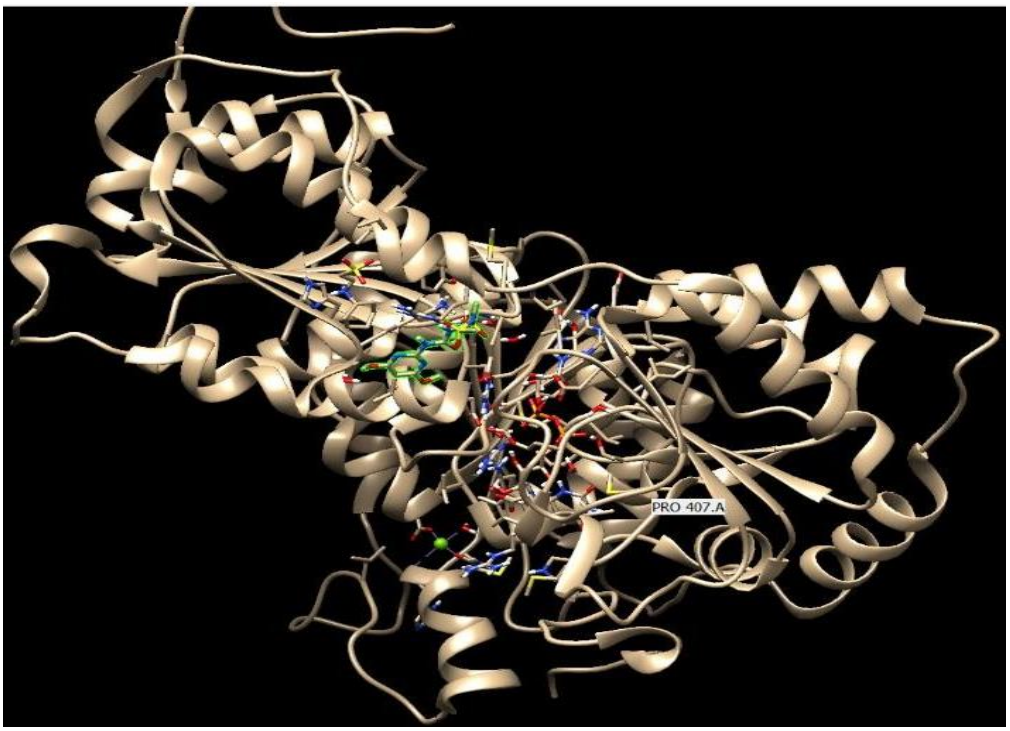

Fig 8a**.** Docked structure of Amidosulfuron and ALS using by Autodock vina

3.8.3 Interaction between TDL and ALS -Amidosulfuron complex

In this molecular docking study (Fig 9a) we can observe when TDL interacts with ALS – Amidosulfuron (ASM) complex, Arg246, Arg279 from ALS is responsible to forms hydrogen bond and Gly245, Tyr276, Asp397, Leu183, Lys220 and co-factor NHE703 forms hydrophobic bond with TDL (Fig9b). The binding energy for this interaction is -6.1 kcal/mol which is less than the binding energy between ALS and TDL i.e., -7.0 kcal/mol and more over these interaction sites are exactly same to ALS- Amidosulfuron interaction. Therefore, we have concluded that Amidosulfuron inhibits TDL by competitive inhibition.[39]

3.8.4 Interaction between ALS and Nicosulfuron (Nsf)

In this molecular docking study (Fig9A) we can observe when TDL interacts with ALS – Amidosulfuron (ASM) complex, Arg246, Arg279 from ALS is responsible to forms hydrogen bond and Gly245, Tyr276, Asp397, Leu183, Lys220 and co-factor NHE703 forms hydrophobic bond with TDL (Fig9B). The binding energy for this interaction is -6.1 kcal/mol which is less than the binding energy between ALS and TDL i.e., -7.0 kcal/mol and moreover these interaction sites are exactly same to ALS- Amidosulfuron interaction. Therefore, we have concluded that Amidosulfuron inhibits TDL by competitive inhibition.[39]

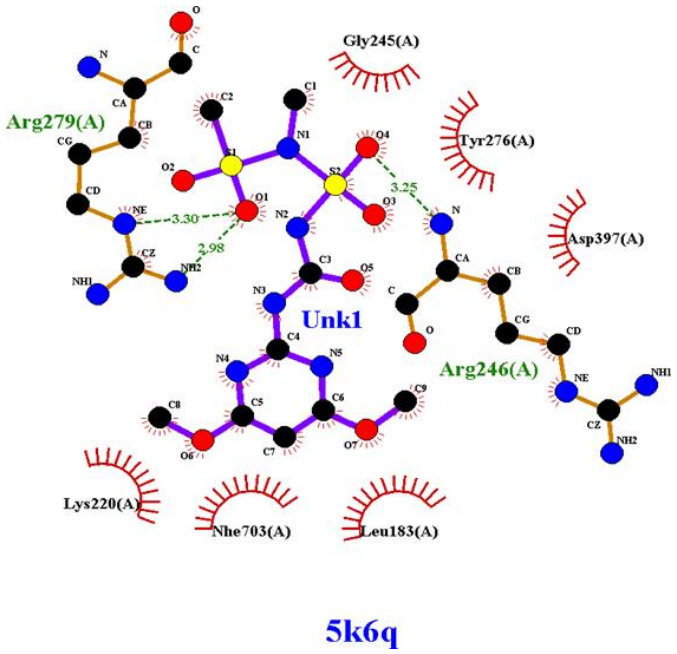

Fig.8B. Interaction site of ALS and Amidosulfuron (in picture denoted by Unk1) by using LigPlot+ v.2.1. where green colored dashed lines indicate hydrogen bond and red sun like symbol indicate hydrophobic bonds.

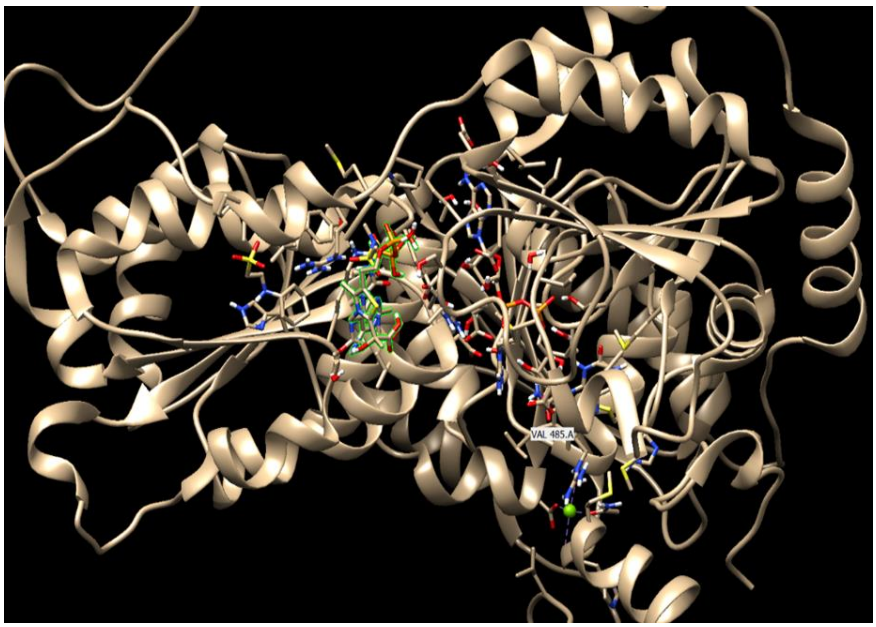

Fig 9A. docked structure of tdl binds with als -amidosulfuron complex

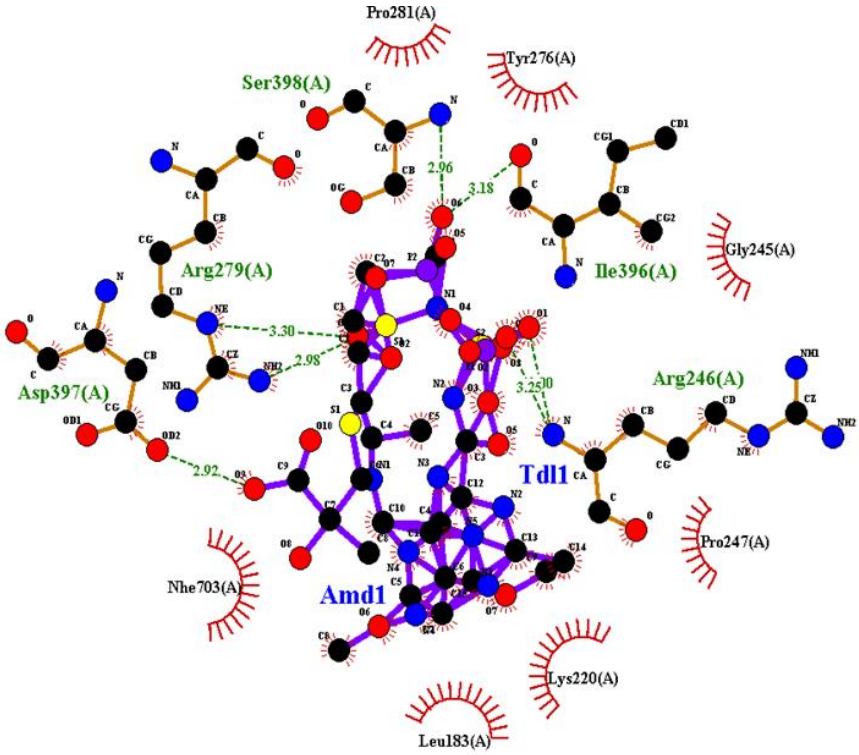

Fig 9B**.** Interaction sites of Amidosulfuron-ALS complex and TDL visualized through LigPlot+v.2.1.

3.8.4 Interaction between ALS and Nicosulfuron (Nsf)

Nicosulfuron interacts with ALS through hydrogen and hydrophobic bond formation (Fig. 10A.). Thr662, Ser454 of ALS are the participating amino acids for hydrogen bond formation and His646, Gly664, Leu684, Asp665, Glu663, Lys450, Phe452, Val355, Glu645

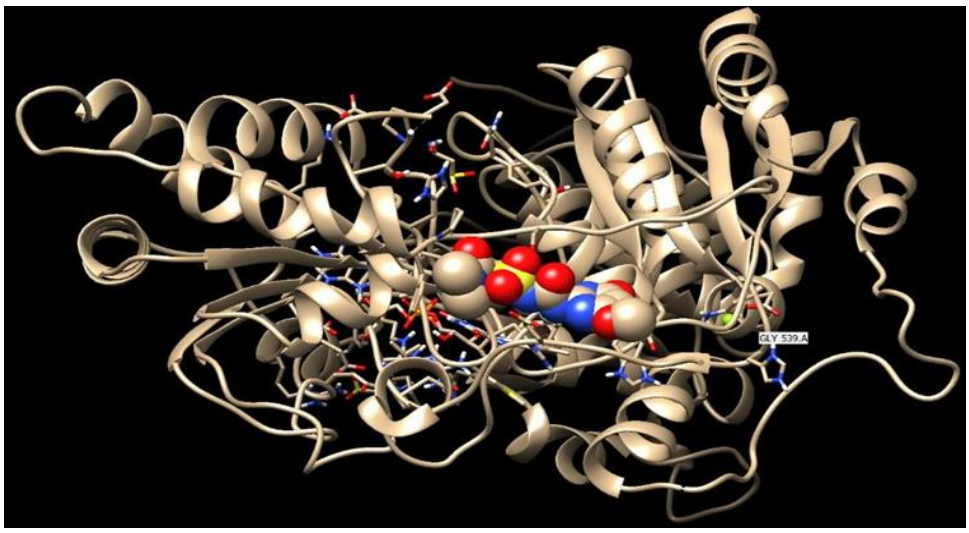

Fig 10 A**.** Docked structure of ALS and Nicosulfuron, a sulfonylurea herbicide active ingredients

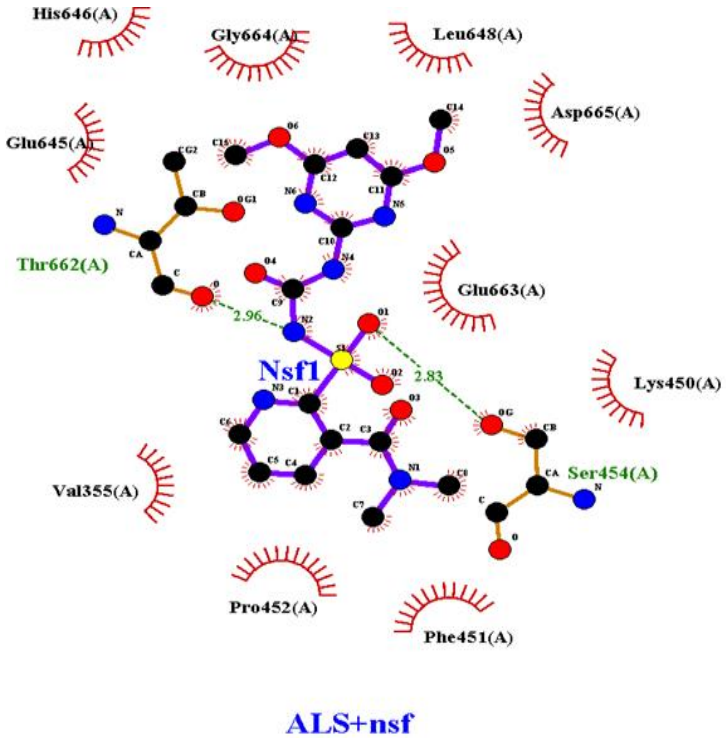

Fig 10B. Interaction site between ALS and Nicosulfuron visualized through LigPlot+ v.2.1are responsible for hydrophobic interaction with Nsf1 (Fig.10B). The binding energy for this interaction is -7.55Kcal/mole.

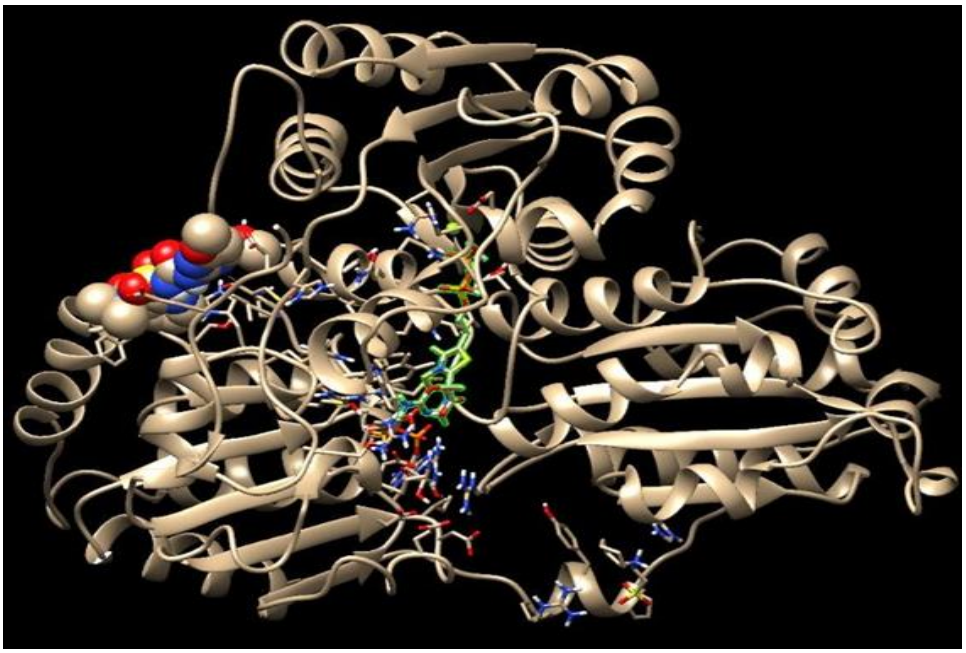

Fig. 11A. Docked structure of TDL and ALS- Nicosulfuron complex by using Autodock vina

3.8.5 Interaction between TDL and ALS -Nicosulfuron complex

TDL interacts with ALS-NSF complex (Fig 11A.) by hydrogen bond in Ser454, Thr662 and hydrophobic bond in His646, Gly664, Leu648, Asp665, Glu645, Glu663, Val355, Pro452, Lys450, Phe451 (Fig 11B**.**). Binding affinity for this interaction is -2.48Kcal/mole which is much lower than normal binding affinity -7.00 Kcal/mole of TDL- ALS. Binding sites are different from ALS-TDL binding site (Fig.7A.) and decrease the binding affinity that is why we have concluded that this inhibition is uncompetitive inhibition.

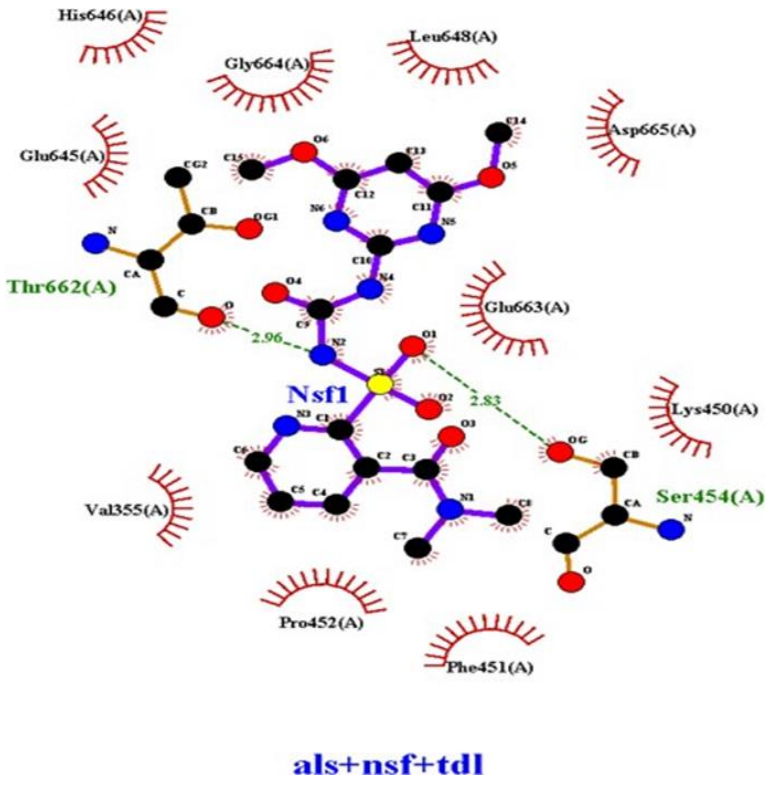

Fig11B. Interaction sites of TDL and ALS -Nicosulfuron complex obtained by Ligplot+ v.2.1

.3.8.6 Interaction between ALS- Cyclosulfamuron

Cyclosulfamuron binds to ALS (Fig12A.) by hydrogen bond and hydrophobic bond. Leu183, Thr219, Lys220, Tyr276, Met280, Gly245, Pro281 make hydrophobic bond to ALS and Arg246 and Arg279 make hydrogen bond to ALS (Fig12B.). The binding affinity for this interaction is -7.7 kcal/mol

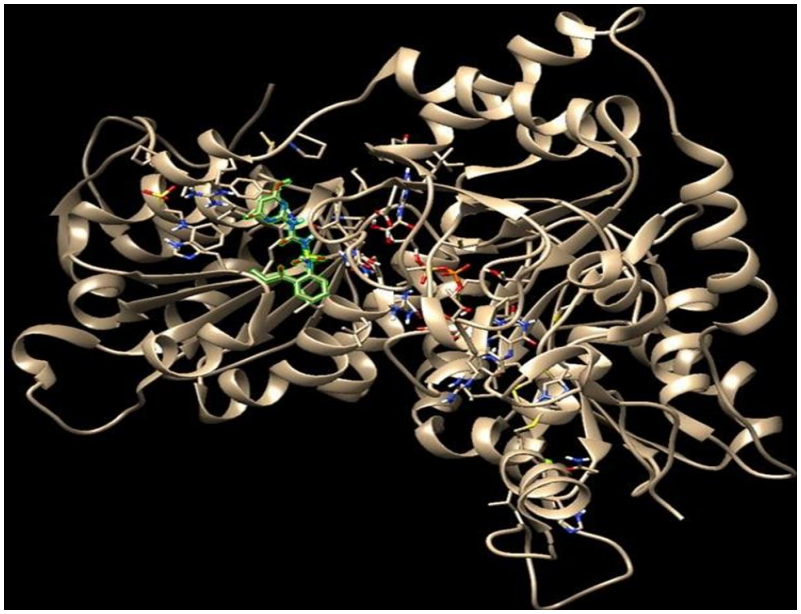

Fig. 12A**.** Docked structure of ALS and Cyclosulfamuron (CSM) interaction by using Autodock vina

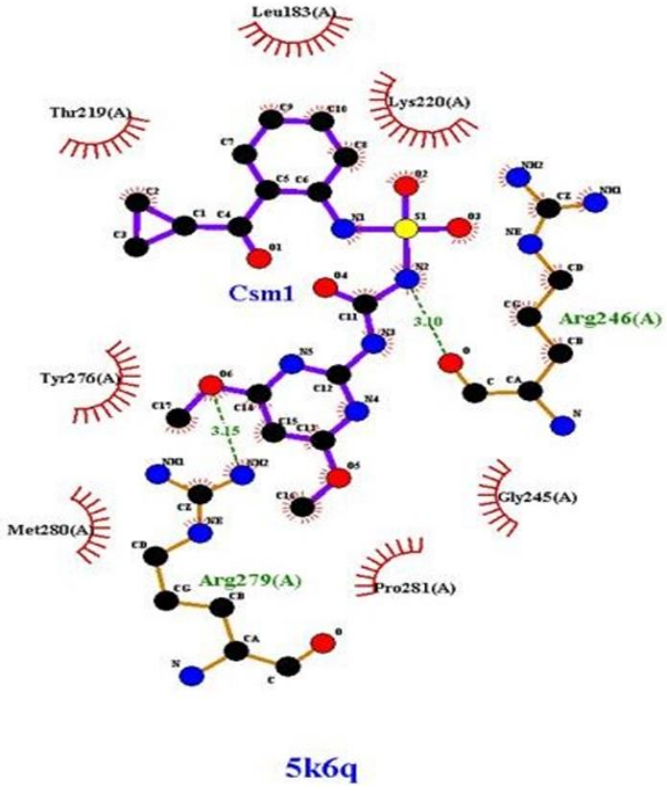

Fig. 12 B**.** Interaction sites of ALS and cyclosulfamuron

Cyclosulfamuron binds to ALS (Fig12A**.**) by hydrogen bond and hydrophobic bond. Leu183, Thr219, Lys220, Tyr276, Met280, Gly245, Pro281 make hydrophobic bond to ALS and Arg246 and Arg279 make hydrogen bond to ALS (Fig12B.). The binding affinity for this interaction is -7.7 kcal/mol.

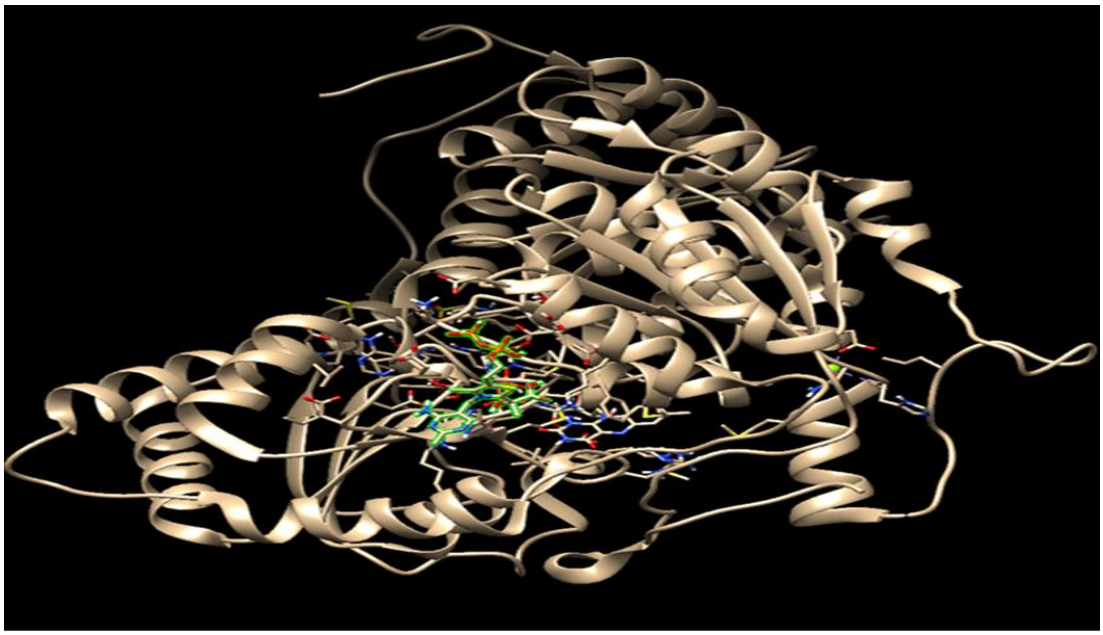

Fig. 13a**.** Docked Structure of TDL and ALS-Cyclosulfamuron complex by using Autodock vina

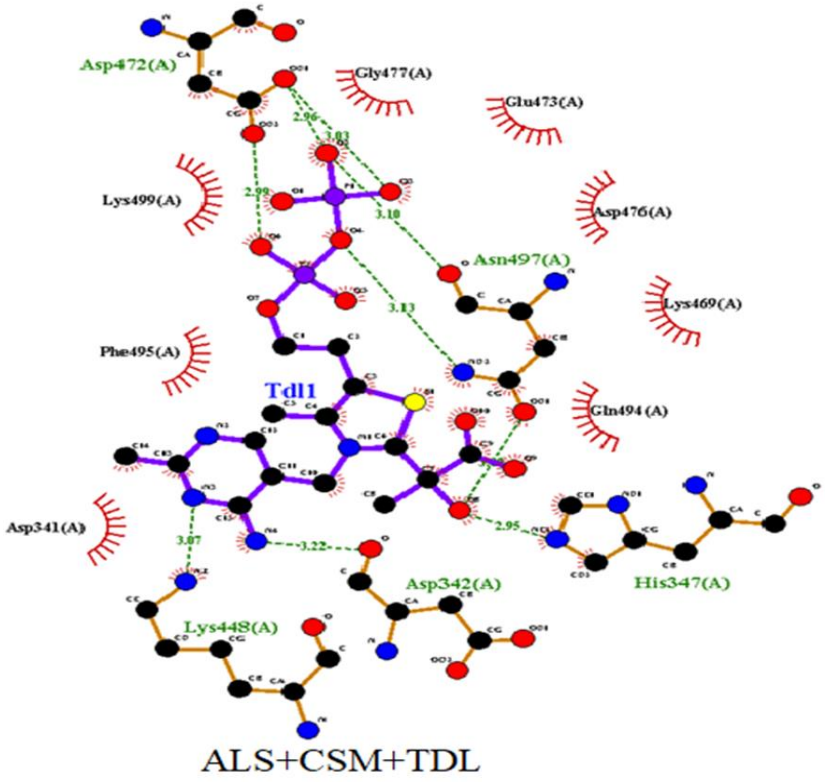

Fig.13b**.** Interaction site of TDL and ALS-Cyclosulfamuron

3.8.7 Interaction between TDL and ALS-Cyclosulfamuron complex

TDL interacts with ALS – Cyclosulfamuron complex (**FIG. 13. A.**) by hydrogen bond in Asp472, Asn497, His347, Asp342, Lys448 and by hydrophobic bond with Gly477, Glu473,

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Asp476, Lys469, Gln494, Lys499, Phe495, Asp341 (**FIG. 13. B.**) and binding affinity is -6.5 kcal/mol lower than ALS-TDL binding affinity. Interacting sites are different from the interacting sites of ALS and TDL (**FIG. 7. A.**). As for decreased binding affinity, we have concluded that this inhibition is uncompetitive inhibition.[12]

From docking result we can understand that binding of ALS to TDL is inhibited by either competitive or uncompetitive inhibition. After getting interacting site of ALS protein we have searched for the respective positional character in other organisms in jalview representation of multiple sequence alignment and from this analysis it is cleared that these interacting sites are totally occupied by same character irrespective of the organisms.Therefore, interacting site of active ingredients to ALS may be same for the other selected organisms too.

4. Conclusion

Acetolactate synthase (ALS) or Acetohydroxyacid synthase (AHAS) [E.C. 2.2.1.6] catalyses first step of branched-chain amino acid synthesis i.e., Valine, Isoleucine, Leucine and it is the prime target of the active ingredients to stop the growth of weeds. Multiple sequence alignment of these ALS protein sequences from different organisms showed conserved region with homology in amino acid residue at different site. Thereafter, phylogenetic tree constructed based on protein sequence of acetolactate synthase of *Arabidopsis thaliana* and it's homolog from different organisms revealed 14 cluster based on their taxonomical order such as Brassicales, Rosales, Malvales, Asterales, Malpighiales, Laminales, Proteales, Solanales, Cucurbitales, Myrtals, Fables, Ericales, Sapindales and Caryaphyllales. Moreover, from phylogenetic analysis we may conclude that Brassicales order is the common ancestor of the rest selected orders of organisms. The jalview representation of the multiple sequence alignment delineate that some of the sites within the conserved domain are not occupied by identical character. Further analysis resembles those apparently non-conserve sites might retain their conservation (also known as silent mutation) based on the similar physicochemical properties of the present amino acids in those sites and it has proper reflection in the phylogenetic tree with some exceptions and we can understand about mutation acquired at non-conserved site.

In this study we have also find out AHAS interacting partners as well as its coexpression genes were predicted in *Arabidopsis thaliana* from STRING resources. Some protein including ketoacid reductoisomerase, dihydroxyacid dehydratase, 2-isopropylmalate synthase and 3-isopropylmalate dehydrogenase are involved in branched-chain amino acid synthesis pathway of plants, fungi and algae are found to be common interacting proteins of ALS in *Arabidopsis thaliana*.

From compositional and physicochemical study we came to know that ALS protein comprises 600-670 amino acid residues and have an average molecular weight ~65-72 k Da and negative GRAVY score indicates it as a soluble protein.

The ALS enzyme is inhibited by Amidosulfuron, Nicosulfuron and Cyclosulfamuron are most common member of sulfonylurea herbicide family. Interaction between these active ingredients of sulfonylurea herbicide and ALS from *Arabidopsis thaliana* (PDB ID 5K6Q) shows that the formation of the complex inhibits the binding of TDL (Lactyl-ThDP) to ALS properly and results into the perturbation of the side chain biosynthesis.

From molecular docking analysis it is clear that, this inhibition is either competitive or uncompetitive inhibition. Moreover, after identifying the interacting site of protein, we have search for the respective positional character in other organism through jalview and from this study it is cleared that these interacting sites are totally occupied by same amino acids irrespective of the organisms.

With the development of bioinformatic approaches, the scopes of understanding fundamental processes have increased. Here, in this study, bioinformatic tools were used with the objective to analyse the interactions between acetolactate synthase from *Arabidopsis thaliana* and active ingredients of sulfonylurea herbicide groups. The current work done by various *in silico* tools had produced many informative results. Our obtained outcomes will be a valuable sources of enlightenment to the weed biologists and beneficial to research specific protein interactions along with developing new herbicides in near future using computational methods.

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Author contribution statement

SKM conceived and designed the experiments; SKM, MC and RD Performed the experiments; Analyzed, interpreted the data and wrote the manuscript.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Supplementary Table 1. Different groups of Herbicides, Mode of action, site of action and their chemical family.(https://www.intechopen.com/books/herbicides-physiology-of-action-and-safety/modes-of-action-of-different-classes-of-herbicides)(Schmidt, RR.1998)

Group	Mode of Action	Site of Action	Chemical Family
1	Lipid-Synthesis Inhibitors	ACCase Inhibitor	Aryloxyphenoxypropionate (FOPs), Cyclohexanedione (DIMs), Phenylpyrazolin (DENs)
2	Amino-Acid Synthesis Inhibitors	ALS Inhibitors	Imidazolinones, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinones, sulfonylureas, triazolopyrimidines
3	Root-Growth Inhibitors	Microtubule Inhibitors	Benzamide, benzoic acid (DCPA), dinitroaniline, phosphoramidate, pyridine
4	Plant-Growth Inhibitors	Site of Action Unknown	Benzoic acid, phenoxycarboxylic acid, pyridine carboxylic acid, and quinoline carboxylic acid
5	Photosynthesis Inhibitors	Photosystem II Inhibitors	Triazine, triazinone, phenylcarbamates, pyridazinones, and uracils.
6	Photosynthesis Inhibitors	Photosystem II Inhibitors	Nitriles, benzothiadiazinones, and phenylpyridazines
7	Photosynthesis Inhibitors	Photosystem II Inhibitors	Phenyl, urea, and amides
8	Shoot-Growth Inhibitors	Lipid-Synthesis Inhibitors	Phosphorodithioates and thiocarbamates
9	Amino-Acid Synthesis Inhibitors	EPSP Synthase Inhibitors	Not designated by any specific chemical family
10	Nitrogen-Metabolism Inhibitors	Glutamine-Synthesis Inhibitors	Not designated by any specific chemical family
12	Pigment-Synthesis Inhibitors	HPPD Inhibitors	Amides, anilidex, furanones, phenoxybutan-amides, pyridiazinones, and pyridines
13	Pigment-Synthesis Inhibitors	Diterpene-Synthesis Inhibitors	Isoxazolidinone
14	Cell-Membrane Disrupters	PPO Inhibitors	Diphenylether, aryl triazolinone, N-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles,

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			pyrimidindiones, and thiadiazoles.
15	Shoot-Growth Inhibitors	Very-Long-Chain Fatty Acid (VLCFA) Inhibitors	Chloroacetamide, acetamide, oxyacetamide, and tetrazolinone.
22	Cell-Membrane Disrupters	PSI Inhibitor	Bipyridilium
27	Pigment-Synthesis Inhibitors	HPPD Inhibitors	Isoxazole

Supplementary Table 2. List of selected organisms and accession number of homologous sequences with ALS protein sequences of *Arabidopsis thaliana* obtained by blastp.

Sl. No	Organisms Name	Accession Number	Sl. No	Organisms name	Accession Number
1	*Arabidopsis thaliana*	AAK68759.1	2	*Arabidopsis Lyrate subsp. lyrata*	XP_002877617.1
3.	*Camelina sativa*	XP_010503496.1	4.	*Camelina microcarpa*	AAR06607.1
5.	*Eutrema salsugineum*	XP_006404237.1	6.	*Descurainiasophia*	ACN62348.1
7.	*Brassica napus*	XP_013648961.1	8.	*Brassica carinata*	AJF23179.1
9.	*Brassica oleracea var. oleracea*	XP_013603602.1	10.	*Brassica rapa*	XP_009151430.1
11.	*Brassica juncea*	AJF23157.1	12.	*Capsella rubella*	XP_006293107.1
13.	*Carica papaya*	XP_021905626.1	14.	*Ricinus communis*	XP_002511176.1
15.	*Actinidia chinensis var. chinensis*	PSS07666.1	16.	*Cicer arietinum*	XP_004485753.1
17.	*Ziziphus*	XP_015882186.1	18.	*Tremaorientale*	PON72129.1

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	jujuba				
19	*Populus trichocarpa*	XP_002322262.1	20	*Prunus avium*	XP_021812945.1
21	*Jatropha curcas*	XP_012090695.1	22	*Prunus yedoensis var. nudiflora*	PQP93616.1
23	*Gossypiumarboreum*	XP_017649232.1	24	*Raphanus sativus*	XP_018471079.1
25	Raphanusraphanistrum	CAC86692.1	26	Theobroma cacao	EOY04840.1
27	Gossypium hirsutum	XP_016699120.1	28	*Camellia sinensis*	XP_028123767.1
29	*Citrus clementina*	XP_006436842.1	30	*Malus domestica*	RXH85640.1
31	*Gossypiumhirsutum*	XP_016699120.1	32	*Solanum tuberosum*	XP_006361740.1
33	*Cucurbita pepo subsp. pepo*	XP_023516040.1	34	*Pyrus x bretschneideri*	XP_009360722.1
35	*Eucalyptus grandis*	XP_010026500.1	36	*Gossypiumraimondii*	XP_012455043.1
37	*Euphorbia maculata*	AMB51356.1	38	*Corchorus capsularis*	OMO68011.1
39	*Ipomoea nil*	XP_019154060.1	40	*Xanthium sp*	AAA74913.1
41	*Duriozibethinus*	XP_022734870.1	42	*Chenopodium quinoa*	XP_021737451.1
43	*Punicagranatum*	OWM65493.1	44	*Cucurbita maxima*	XP_022987362.1
45	*Helianthus annuus*	XP_021984153.1	46	*Nicotiana tabacum*	XP_016478370.1
47	*Solanum pennellii*	XP_015081631.1	48	*Nelumbo nucifera*	XP_010274326.1
49	*Erythrantheguttata*	XP_012830574.1	50	*Parasponiaandersonii*	PON77293.1

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51	*Arabis alpina*	KFK34163.1	52	*Tarenayahassleriana*	XP_010550735.1
53	*Gossypium barbadense*	PPE02752.1			

Supplementary Table 3. A. Jalview analysis of conserved domain (1) TPP _Enzyme _N by using Clustal Omega to obtain amino acid conserve site.

Conserved Site	Amino acid Present	Physiochemical Property	Mutation Type	Effect on Phylogeny
141	i. Alanine	i. Neutral, Nonpolar, Hydrophobic	Mutation	NO
	ii. Serine	ii. Neutral, Polar, Hydrophilic		
143	i. Isoleucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
	ii. Valine			
144	i. Leucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	–
	ii. Isoleucine			
151	i. Glutamine	i. Neutral, Polar, Hydrophilic	Mutation	Yes
	ii. Glutamic acid	ii. Acidic, Polar, Hydrophilic		
	iii. Leucine	iii. Neutral, Nonpolar, Hydrophobic		
165	i. Methionine	All are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
	ii. Leucine			
	iii. Valine			

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177	i. Isoleucine	Both are Neutral, Polar, Hydrophobic	Silent Mutation	-
	ii. Valine			
178	i. Arginine	i. Basic, Polar, Hydrophilic	Mutation	Yes
	ii. Tyrosine	ii. Neutral, Polar, Hydrophobic		
	iii. Lysine	iii. Basic, Polar, Hydrophobic		
180	i. Valine	i. Neutral, Nonpolar, Hydrophobic	Mutation	Yes
	ii. Cysteine	ii. Neutral, Polar, Hydrophilic		
198	i. Serine	i. Neutral, Polar, Hydrophilic	Mutation	No
	ii. Alanine	ii. Neutral, Nonpolar, Hydrophobic		
199	i. Serine	Both are Neutral, Polar, Hydrophobic	Silent Mutation	-
	ii. Threonine			
202	i. Proline	i. Neutral, Nonpolar, Hydrophobic	Mutation only on *Gossypium hirsutum* (serine)	No
	ii. Valine	ii. Neutral, Nonpolar, Hydrophobic		
	iii. Serine	iii. Neutral, Polar, Hydrophilic		

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204	i. Valine ii. Isoleucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
206	i. Isoleucine ii. Valine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
221	i. Alanine ii. Serine	i. Neutral, Nonpolar, Hydrophobic ii. Neutral, Polar, Hydrophilic	Mutation	No
224	i. Leucine ii. Methionine iii. Serine	i. Neutral, Nonpolar, Hydrophobic ii. Neutral, Nonpolar, Hydrophobic iii. Neutral, Polar, Hydrophilic	Mutation	Yes
228	i. Valine ii. Isoleucine iii. Cysteine	i. Neutral, Nonpolar, Hydrophobic ii. Neutral, Nonpolar, Hydrophobic iii. Neutral, Polar, Hydrophilic	Mutation	Yes
229	i. Proline ii. Alanine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
231	i. Valine	Both are Neutral,	Silent	-

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	ii. Isoleucine	Nonpolar, Hydrophobic	Mutation	
238	i. Proline	i. Neutral, Nonpolar, Hydrophobic	Mutation	No
	ii. Serine	ii. Neutral, Polar, Hydrophilic		
267	i. Leucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
	ii. Methionine			
269	i. Valine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
	ii. Isoleucine			
274	i. Arginine	i. Basic, Polar, Hydrophilic	Mutation	No
	ii. Methionine	ii. Neutral, Nonpolar, Hydrophobic		
282	i. Lysine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
	ii. Isoleucine			
285	i. Serine	Both are Neutral, Polar, Hydrophilic	Silent Mutation	-
	ii. Threonine			
290	i. Proline	Both are neutral, polar, hydrophilic	Silent Mutation	-
	ii. Alanine			

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Supplementary Table 3. B.Jalview analysis of conserved domain (2) TPP _Enzyme _M by using Clustal Omega to obtain amino acid conserve site.

Conserved Site	Amino acid Present	Physiochemical Property	Mutation Type	Effect on Phylogeny
338	i. Valine ii. Isoleucine iii. Leucine	All are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
340	i. Leucine ii. Phenylalanine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
344	i. Serine ii. Alanine	i. Neutral, Polar, Hydrophilic ii. Neutral, Nonpolar, Hydrophobic	Mutation	Yes
345	i. Lysine ii. Arginine	Both are Basic, Polar, Hydrophilic	Silent Mutation	-
346	i. Lysine ii. Arginine iii. Methionine	i. Basic, Polar, Hydrophilic ii. Basic, Polar, Hydrophilic iii. Neutral, Nonpolar, Hydrophobic	Mutation	Yes
355	i. Cystine ii. Serine	Both are Neutral, Polar, Hydrophilic	Silent Mutation	-
370	i. Glycine ii. Leucine	i. Basic, Nonpolar, Hydrophilic ii. Neutral, Nonpolar, Hydrophobic	Mutation	Yes
405	i. Tyrosine ii. Phenylalanine	i. Neutral, Polar, Hydrophilic ii. Neutral, Nonpolar, Hydrophobic	Mutation	No
407	i. Valine	Both are Neutral,	Silent	-

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	ii. Isoleucine	Nonpolar, Hydrophobic	Mutation	

Supplementary Table 3. C.Jalview analysis of conserved domain (3) TPP _Enzyme _C by using Clustal Omega to obtain amino acid conserve site.

Conserved Site	Amino acid Present	Physiochemical Property	Mutation Type	Effect on Phylogeny
567	i. Isoleucine ii. Methionine ii. Valine	All are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
570	i. Alanine ii. Serine	i. Neutral, Nonpolar, Hydrophobic ii. Neutral, Polar, Hydrophilic	Mutation	Yes
572	i. Alanine ii. Glycine	i. Neutral, Nonpolar, Hydrophobic ii. Neutral, Nonpolar, Hydrophilic	Silent Mutation	-
578	i. Valine ii. Isoleucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
591	i. Valine ii. Leucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
608	i. Leucine ii. Isoleucine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
648	i. Leucine ii. Valine	Both are Neutral, Nonpolar, Hydrophobic	Silent Mutation	-
660	i. Arginine ii. Glutamine	Both are Neutral, Polar, Hydrophilic	Silent Mutation	-

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678	i. Proline	i. Neutral, Nonpolar, Hydrophobic	Mutation	No
	ii. Glutamate	ii. Acidic, Polar, Hydrophilic		

Supplementary Table 4. Interacting amino acids of the receptor molecules responsible for hydrogen and hydrophobic bond and respective binding affinities (kcal/mol) between receptor and ligand.

Receptor	Ligand	Hydrogen bond	Hydrophobic bond	Binding Affinity
Acetolactate Synthase (ALS)	Lactyl -ThDP (TDL)	R279, R246,D397,P247,L183,S398	P281,Ile396,M280,G245, K220,G248	-7.0
ALS	Amidosulfuron	R279,R246	G245,D397,Y276,K220, L183	-6.3
ALS-Amidosulfuron	TDL	R279,S398,D397,R246,I396	P281,Y276,G245,P247, K220, L183	-6.1
ALS	Nicosulfuron	S454,T662	H646,G664,L648,D665,E663,K450, F451	-7.55
ALS-Nicosulfuron	TDL	T662,S454	H646,G664,L648,D665,E663,K450,F451,P452 V355,E645	-2.8
ALS	Cyclosulfamuron	R246,R279	L183,K220,G245,P281,M280,Y276,T219	-7.7
ALS-Cyclosulfamuron	TDL	H347,N497,D472,D342,K448	G477,E473,D476,K469,Q494,K499,F495,D341	-6.5

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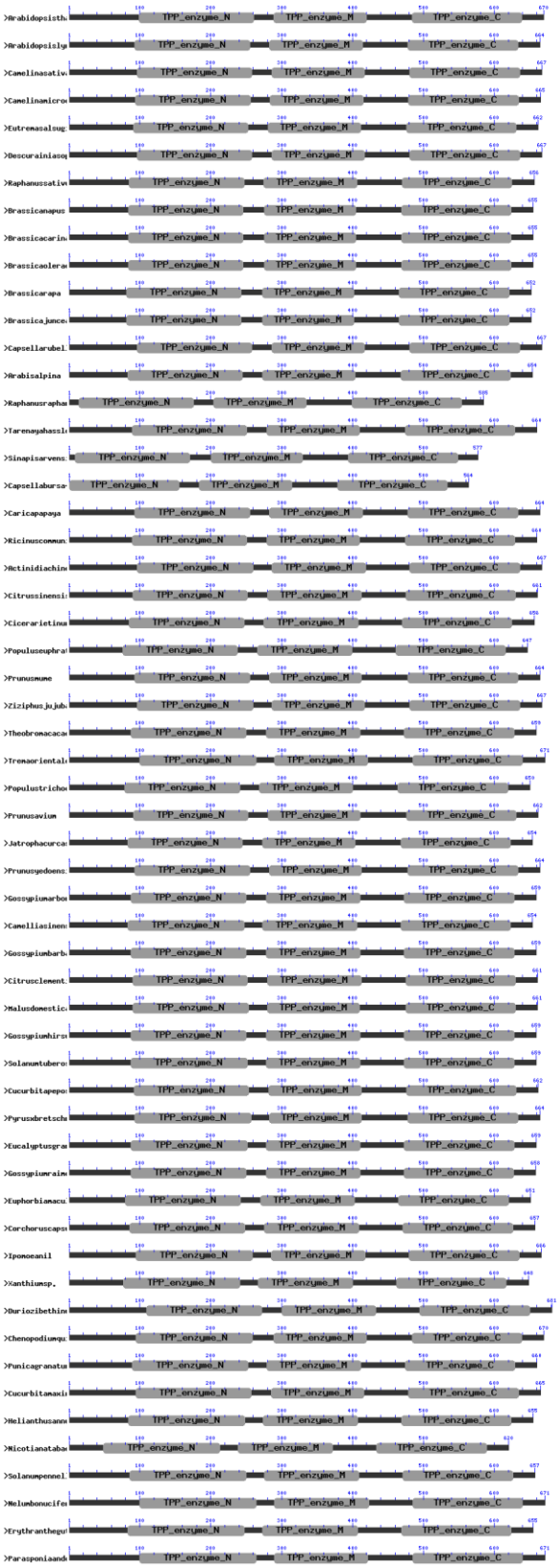

SupplementaryFIG.1. Conserved domain structure obtained from NCBI Conserved Domain Search – NIH for all selected sequences homologous to ALS protein from *Arabidopsis thaliana*.

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Study of Air pollution in Kolkata with the respect to the monthly spike in the concentration of pollutants for the year 2015

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Abstract

Air pollution in Kolkata is not a result of only the human error and negligence but it is alsoaffected by the meteorological factors like wind . In this rostrum we have simply discussed about the spike in the pollutant level in certain days of the year and have tried to explain the reason with the utilization of Hysplit Backtrajectory and Dispersion model.

Keywords: Pollution, Particulate matter, Dispersion Model

Introduction

In neoteric schedule, lack of stringent government policies and mass clustering of vehicular transports and industrial belt have spiked the concentration of pollutants in major metropolises in Eastern India [1]. Kolkata being both the financial and administrative pivot in the state of West Bengal has in contemporary period saw itself rising gradually up in the ladder for claiming the crown of worst polluted city in India. [2]The spike of pollutants in this city is attributed mainly because of the meteorological phenomenon that transpires through it and altering the chemical composition of its sky [3] While the cardinal static facets of the pollutants are located in the district of Howrah, the dynamic sources are dispersed throughout the city centre and surrounding localities. [3] Wind direction including speed contribute a major change on direction of pollutant outflow and it is observed that their direction is causing a gradual change of pollutant concentration across west to east. Bay of Bengal is a critical factor which plays a strong notion on altering the physical nature of the pollutants through the action of moisture which alters the physical nature of the pollutants through coagulation and precipitation [3]. The blunt of the spike in pollutant concentration is seen during winter season when the concentration spared to an approximate 170 $\mu g/m^3$ for PM_{10}, 293 $\mu g/m^3$ for $PM_{2.5}$, 188 $\mu g/m^3$ for NO_2 and 19 $\mu g/m^3$ for SO_2 which is not statistically significant from the mean thus signifying a homogenous distribution of pollutant concentration across the month.

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However it's been widely seen that the pollutants also act on each other altering the concentration levels. $PM_{2.5}$ undergoing coagulation produces PM_{10} thus increases its concentration while suffers a decrease of its own. While relationship also exists between gaseous and particulate pollutants, it does not have been seen widely. In recent times, further wet laboratory experiments are underway to detect those linkages.

Study Area

One of the bustling cities in the Indo-Gangetic terrain and the administrative hub of Easter India , Kolkata presents us a perfect opportunity for this unique study . The span from December to February encompasses Winter season followed by March to May as Pre-Monsoon season, June to August as Monsoon season and September to November as Post Monsoon season. The study area is under experience of heavy downpour during North –East Monsoon.

Methodology

The collection of the data was done mainly with the utilization of finalised data published at governmental sources and later modified with the help of statistical equations .The pollution levels for Kolkata were extracted from the website of West Bengal Pollution Control Boardhttp://www.wbpcb.gov.in/ .[4]

Software Analysis were done principally from NOAA (National Oceanic and Atmospheric Administration) analyser Hysplit Back trajectory Modelling employing Langarian dispersion modelling network for the detection of pollutant load arriving from various places towards Kolkata .(Fig 1) [6]The colour trend lines demotes different concentrations with blue and green been unpolluted air and red being the polluted one.

According to Fig 2, it is seen that on the month of January 2015 , the dry air is arriving from the Himalayas but laden with a big amount of pollutants mainly from the districts of Malda and Dinajpurs and dumping them over 24 Parganas and Kolkata before relieving itself to the Bay of Bengal. On 18th April 2015, the pollutants mainly arrived through winds which itself found its way right from the dry Deccan Plateau. These factors assisted in a spike in pollutant concentration. Analyzing the wind trajectories of the months of July and October, it is found that they are all following the same tract from the central plateau instead of the Bay of Bengal thus preventing any moisture from diluting the pollutant load particularly particulate matter. Thus these days showed a spike in the concentration despite the overall months showing a near homogenous value especially for the months of Monsoon.

Results: Data tabulation and hysplit analysis has revealed the true trajectory of the pollutant laden air across the southern Bengal. It varies with the winds in different season particularly the monsoon winds when they arrive from the southern part of the country

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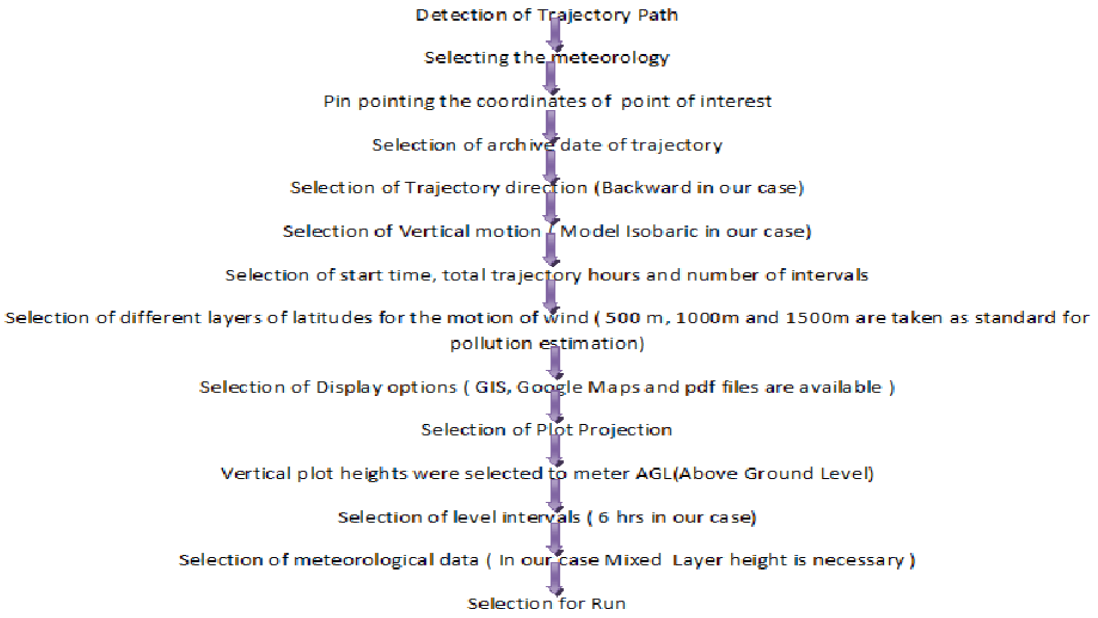

Fig 1: Steps of executing the hysplit back trajectory model

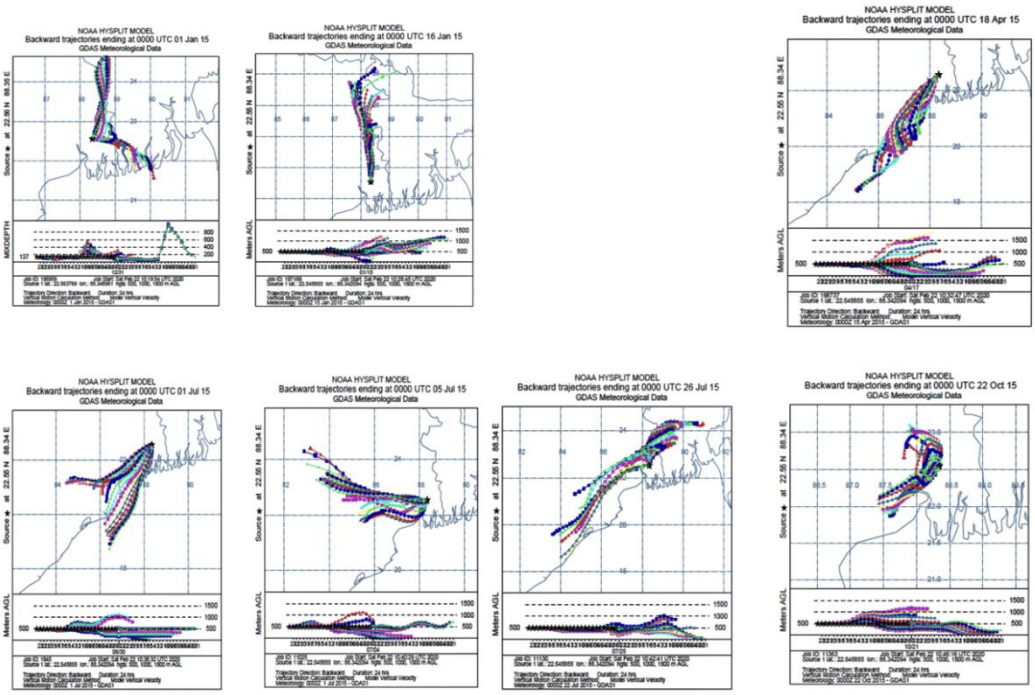

Fig 2: Figures showing the direction of pollutant laden wind from different portion of India especially during days of random spikes

In the Fig 3, we can see different pollutant concentration , deposition and plume dispersion graphs of particulate matter for the month of January .Looking closely ,it is seen that the pollutants have dispersed from eastern portion of the area of study to the

western zone before forming a hook with a northward inclination . The dilution of the pollutant increased from 40 km to 20 km within few hours . In the deposition model it is detected that the pollutants are causing widespread deposition on the western flank of the state.

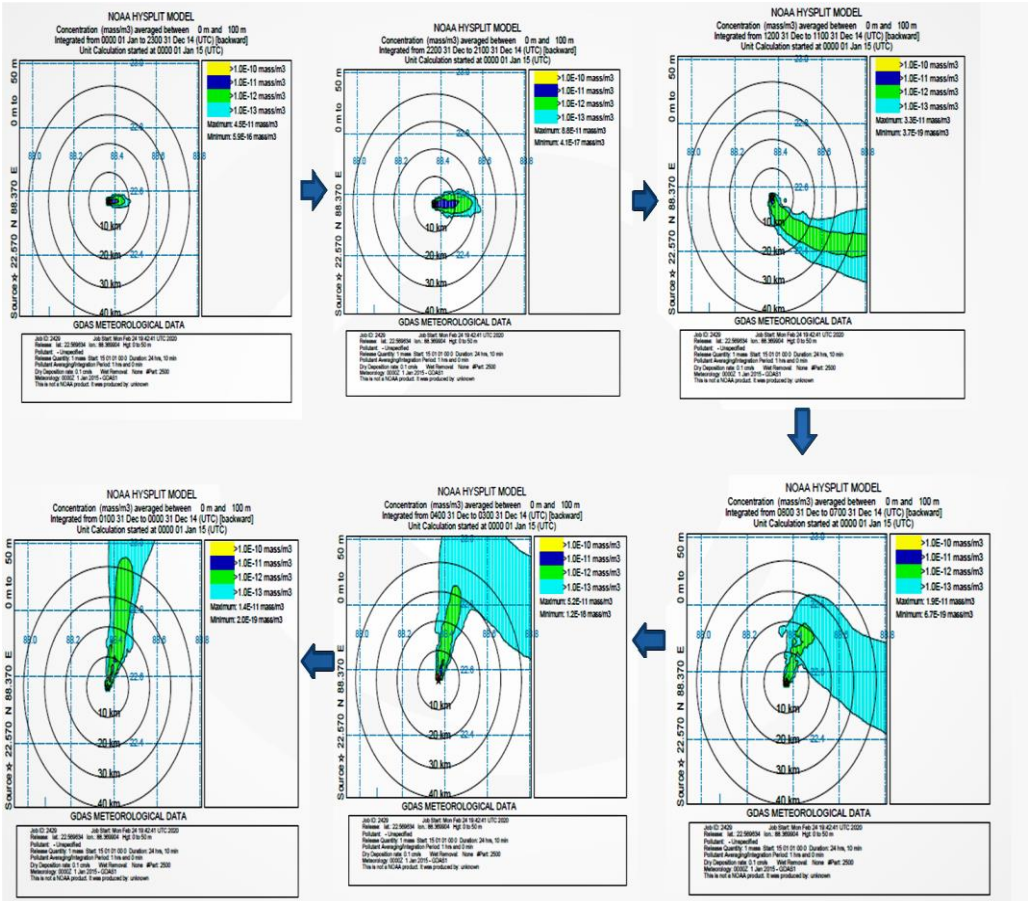

Fig 3: Showing the Dispersion projection of Particulate matters for the month of January

The distribution of pollutant concentration for four different pollutants , which are Particulate matter 2.5, Particulate matter 10 , Sulphur Dioxide and Nitrogen Dioxide varies across the months. The maximum concentrations were attained at the winter months with a near homogenous nature. Moderate concentrations were seen on the months of March till October with a heterogeneous distribution of pollutants.

The spike level are variable at different dates across the year of 2015 with the month of December attaining the highest one followed by January and November and more closely by February and October .(Fig 4)

Statistical analysis of the pollutants has revealed very contradictory consequences which showed that the meteorological conditions are inversely proportional to the pollutant concentrations. Rise in temperature will cause a decrease in pollutant concentration owing to the utilization it caused by increasing vertical dilution and

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mixing height. Increase in meteorological parameters denotes a decrease in pollutant concentration through coagulation and precipitation. Increase of wind will always wash out the pollutants.(Fig 5)

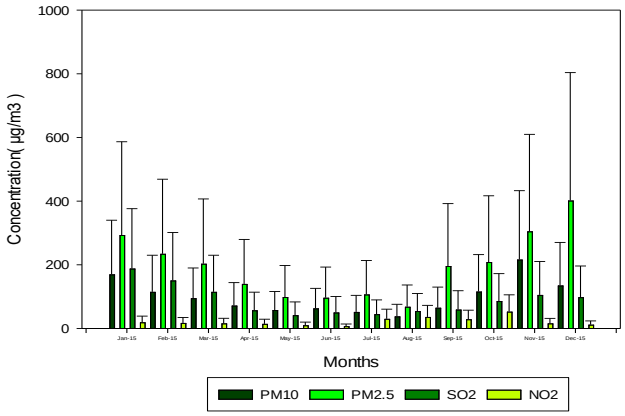

Fig 4: Showing the spikes in pollutant concentrations

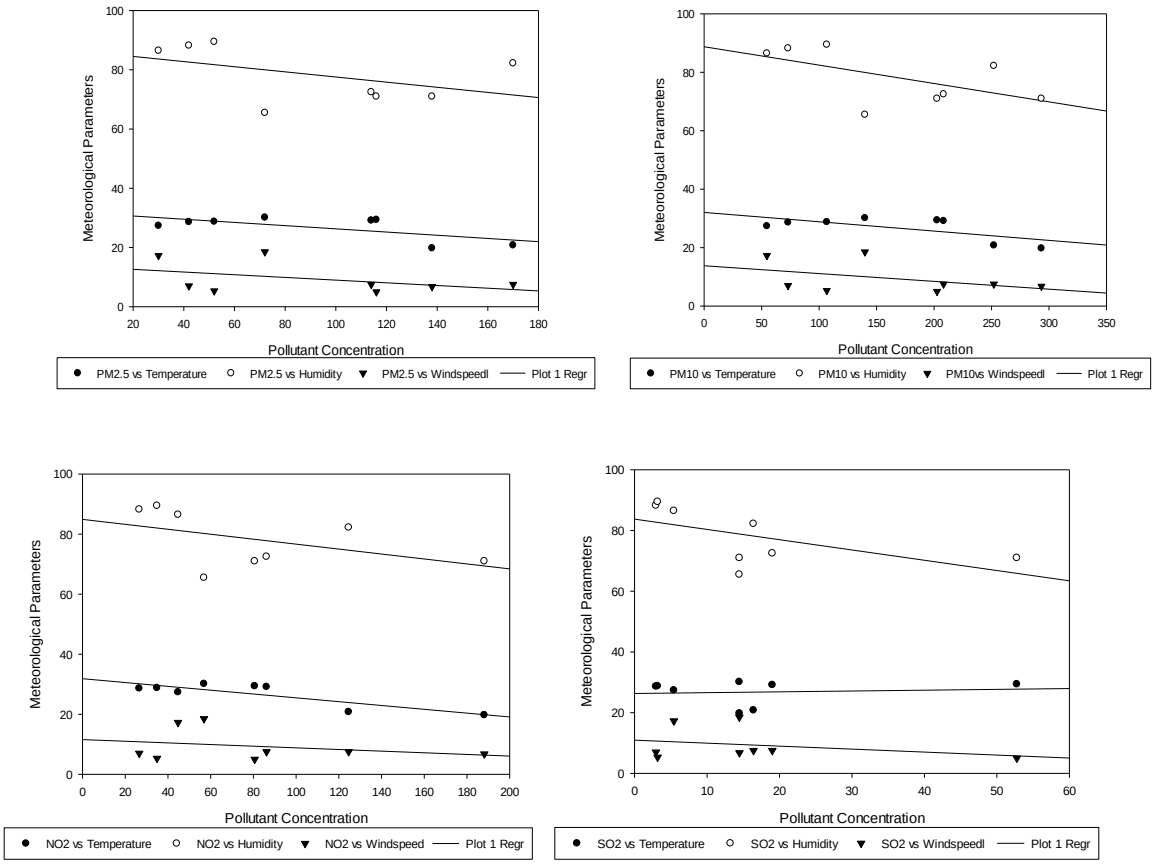

Fig 5: The relationships between Meteorological parameters and Pollutant concentrations

Statistical analysis of the pollutants has revealed very contradictory consequences which showed that the meteorological conditions are inversely proportional to the

pollutant concentrations. Rise in temperature will cause a decrease in pollutant concentration owing to the utilization it caused by increasing vertical dilution and mixing height. Increase in meteorological parameters denotes a decrease in pollutant concentration through coagulation and precipitation. Increase of wind will always wash out the pollutants.(Fig 5)

Discussion: After considering the above explanation and findings it can be said that the sudden projection of pollutant loads are mainly due to the meteorological phenomenon. Although human founts can't be ruled out, the blunt of the effort was provided by weather and climate.

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1918 Spanish Flu:A data driven study

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Abstract

In 1918, a new influenza virus emerged. During this same time period World War I was taking place. The conditions of World War I (overcrowding and global troop movement) helped the 1918 flu spread [1]. The vulnerability of healthy young adults and the lack of vaccines and treatments created a major public health crisis, causing at least 50 million deaths worldwide. This is a data-oriented project to try to understand the Spanish Flu outbreak of 1918 and how it can teach us valuable lessons. We studied the death and mortality related aspects, economic impacts and how social distancing helped mitigate the spread.

Keywords: Spanish Flu, Pandemic, Pathogen, Social Distancing, Disease

Introduction

The Spanish flu pandemic, often regarded as one of the deadliest in history, killed an almost 50million people of the 500 million it infected as it tore through Europe in 1918 and travelled to the US, killing 675,000 Americans. By comparison, the First World War, which ended in 1918, had around 20million deaths.The outbreak was in two waves. The first wave of the 1918 pandemic occurred in the spring and was generally mild with the sick experiencing typical flu symptoms such as chills, fever and fatigue then recovering after several days [2].However, a second, highly contagious wave appeared with a vengeance in autumn of that same year and victims died within hours or days of developing symptoms, their skin turning blue and their lungs filling with fluid that caused them to suffocate.In those times, there were no effective drugs or vaccines to treat it. Citizens were ordered to wear masks, schools, theatres and businesses were shuttered and bodies piled up in makeshift morgues with many having to dig graves for their own family members.It had one difference from other similar diseases. Instead of just young and old people being severely affected, adults in their prime age of 18-40 were also highly affected. By the summer of 1919, the flu pandemic came to an end, as those that were infected either died or developed immunity.

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1. Methodology

We proceed on a model of data-driven analysis. The relevant data have been collected to understand and find patterns in data. We used Python as a Data Analysis tool and several Python libraries like Seaborn, Matplotlib and Plotly have been used to create many of the visualizations. Seaborn was used to create scatterplots, the country wise data was analyzed using Pandas in Python. The line plot was created using Plotly. The context of this report is to understand the Spanish flu as a whole, but adequate numeric data or reports could not be found for regions all across the globe. We researched through many papers and web content to find out accurately what happened in those times. We have tried to understand the pandemic in three ways, how it affected deaths and mortality rates, its economic impact on the world and how effective was social distancing in fighting this invisible enemy. The reason of working on social distancing is that, it occurred in a time before antibiotics. Mankind did not have proper medication to fight against the pathogen. The best weapon in such conditions is social distancing. Hence, we tried to have a look at it.

2. Mortality due to Spanish Flu

"I had a little bird, its name was Enza
I opened the window and In-Flew-Enza.
Obey the laws and wear the gauze,
Protect your jaws from septic paws."
(Popular poem regarding the flu in those days)

Country	Estimated Deaths(UpperLimit)
Australia	15000.0
Brazil	300000.0
Canada	50000.0
China	1280000.0
Denmark	5000.0
France	400000.0
Germany	426600.0
Ghana	100000.0
India(British Republic)	13880000.0
Indonesia	1500000.0
Iran	2431000.0
Iraq	700000.0
Italy	410000.0

Country	Estimated Deaths(UpperLimit)
Japan	390000.0
Korea	200000.0
Mexico	230500.0
New Zeland	8900.0
Norway	15000.0
Portugal	118065.0
Spain	260000.0
Sweeden	34500.0
Switzerland	25000.0
UK	250000.0
US	675000.0
USSR	450000.0
Vietnam	33000.0

Table1:Estimated deaths in countries due to Spanish Flu

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The Spanish flu of 1918, which lasted from January 1918 to December 1920 infected about 500 million people and caused a total death toll of about 50-70 million across the world, making it one of the deadliest pandemics in the history of mankind [3]. The time also coincided with the last phase of World War I and thus to maintain morale many of the participating nations didn't report their deaths. Hence, it's safe to assume that the deadly Flu has affected and resulted in lot more deaths than accounted for. The origin of the virus is believed to be in France, but several other theories exist. Since it was an avian virus and had the ability to spread via air, it spread throughout the world very rapidly infecting and killing people on its way. The above tables show the estimated deaths in major countries around the world. India has the highest number of reported deaths estimated to be about 14 million people. The total deaths including both US and USSR sum up to about 1.2 million.In the 1918 pandemic most deaths occurred among young adults, a group that usually has a very low death rate from influenza [4].One of the hardest hit countries due to the flu was in New Zealand which killed approximately 6500 Pakehas and 2500 Maoris. Japan and Korea also had their fair share of affected cases and mortality. China was hit in two waves. The first wave hit the country around June 1918 and had very less effect whereas the second wave was more devastating and hit the country hard. The flu also caused lots of deaths around the world. The Spanish Flu of 1918 had a huge impact of several Asian countries. Countries like Indonesia, China, Iran and Iraq suffered over half a million people each.The pandemic was probably responsible for >260,000 deaths (1% of the Spanish population), with an excess mortality of close to 1.5% [5].

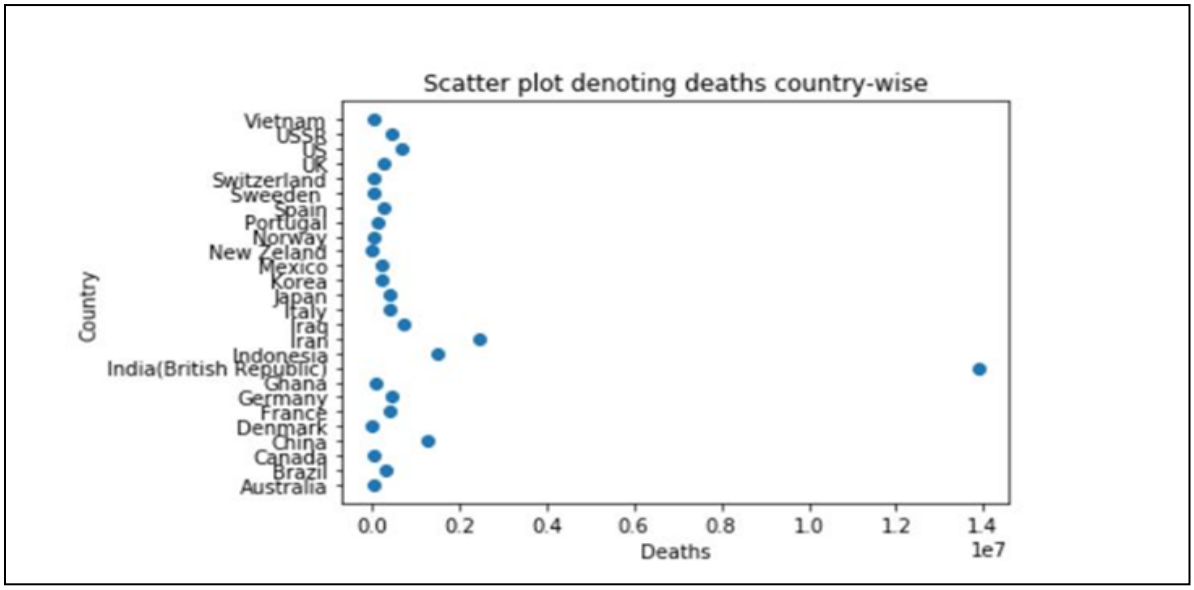

Fig 1**.** The number of deaths as a scatterplot

The above horizontal scatter plot shows the country wise death rates. The countries all around the globe suffered heavy losses both in terms of human lives and money. The Spanish flu taught the world that humanity still had a long way to go. Development in medicines, improve in healthcare and hygiene and proper facilities to treat ill people are the need of the hour.

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4. Life Expectancy during the "Spanish Flu" pandemic

What is Life expectancy?

Life expectancy is basically a calculated figure that tells how long an organism is supposed to live. It is measured using historical data and statistical methods. During measuring the life expectancy of a person, analysts must take several factors into consideration such as demographics, the economic stability of the region, etc.

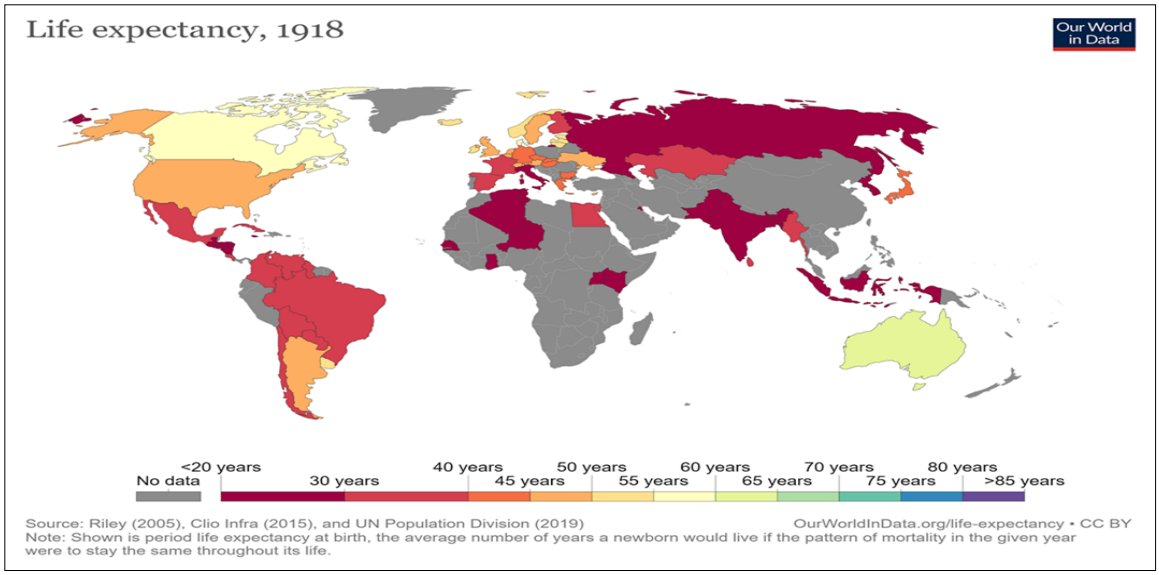

Fig 2**.** Life expectancy during 1918 Spanish Flu.

The flu took place in three waves. The first wave (took place during the spring of 1918) being mild and the patients took a few days to a few weeks to recover [6]. It was during the second wave(broke out during Sep-Nov 1918) that the flu claimed the greatest number of victims. The virus by now had mutated and had enhanced virulence. It affected healthy people who were also immune to the first wave thereby marking the deadliest phase of the flu. At the beginning of 1919, some places reported a "third" wave.

Global Average:-

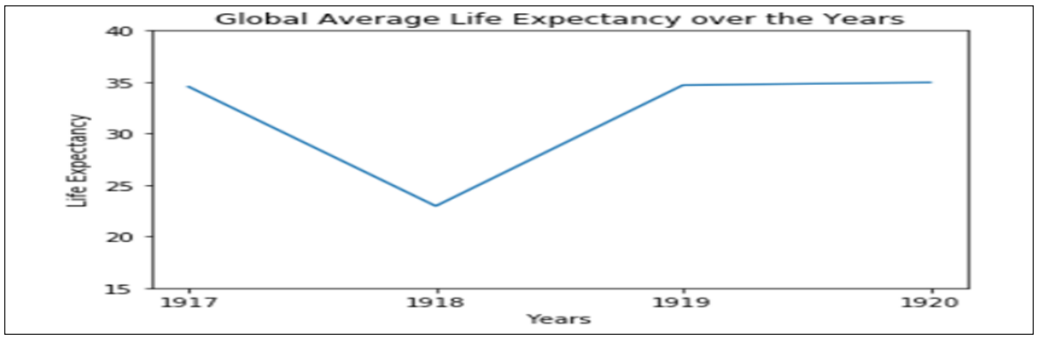

Fig 3. Global Average Life Expectancy

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- From the plot above we see that there's a dip in the life expectancy with an all-time low of 22.5 years globally with the onset of the pandemic,
- Within a year, after the pandemic had passed, the life expectancy was back on track with an average of 35 years
- During 1918, people most vulnerable to the flu was between the ages of 17- 40. [7]

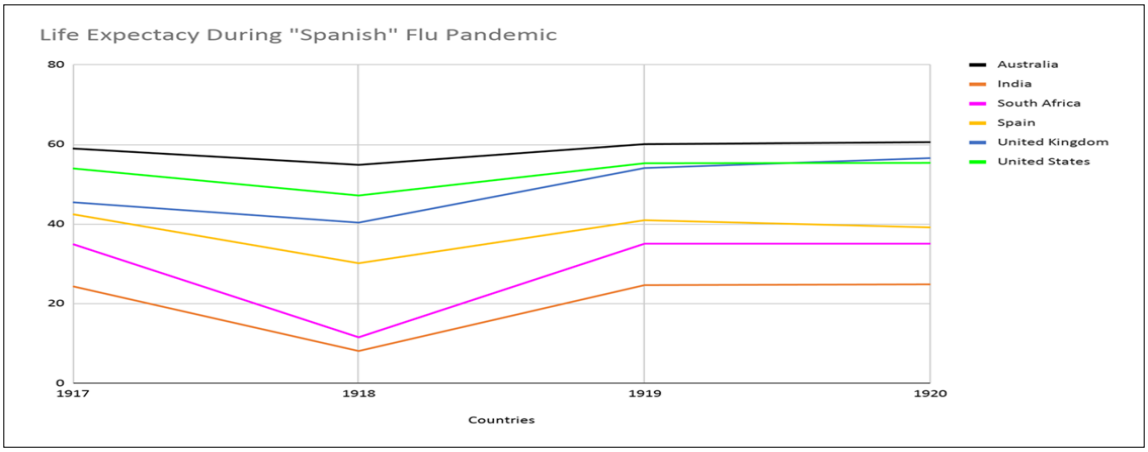

Fig 4. Life Expectancy plot across the countries

From the above plot we can conclude that the Australia had the highest life expectancy amongst all the countries both before and after the pandemic. During the pandemic, India had the lowest life expectancy of around 8 years. South Africa saw a staggering drop from 35 years to 11 years. Unlike other countries, Spain's life expectancy did recover after the pandemic but slowly started going downhill thereafter. After the pandemic passed, the life expectancy in United States rose to 55 years.United Kingdom on the other hand had the least drop in life expectancy, but it was observed that young adults were most vulnerable to the flu in 1918 and the elderly people aged more than 75 had the least death rate of all.

How life expectancy drop and rose again?

- **Aftermath of WW1:** The pandemic hit immediately after the end of the World War 1. Industries, public health centers were disrupted. Millions were getting infected and the medical professionals had no idea on how to treat the patients. On top of that the homebound soldiers took the virus to different parts of the world.
- **Public health measures:** Governments of most countries shut down public community places, schools, offices, etc. Disinfecting the streets were now a common sight. For the financially backward, officials arranged for soap and water and banned anybody to spit on the streets. They also set up centers for checking milk and other food products. These measures helped in curbing the spread of the virus to some extent.

- **Back to Normalcy:** Reports suggest after the virus died out in early 1919, there was an increase in 50% of market value which lasted until the end of 1919. From this we can infer that industries were

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getting back on track and thus people could now find jobs. This in turn let them provide for their families which drastically brought up the life expectancies in countries around the world.

Takeaway

The Spanish Flu was one of the deadliest pandemics in modern history. With no vaccines or medicines, the only viable way people could protect themselves was by avoiding transmission. We can't control the outbreak of pandemics in the future but with the lessons learnt from the "Spanish" flu we can model a better pandemic preparedness plan - which we are doing currently in the case of CoVID-19.

Economic Impact

The Spanish flu outbreak of 1918 had far reaching economic effects. Offices, theatres, businesses were shut down, to contain the outbreak. Important fact worth noting is that males aged 18 to 40 were highly affected, and had serious economic impact, both in the businesses and factories they worked for, and also for their own families [8]. Important thing to understand, in case of trying to assess the economic situation in those days is that, there is not adequate data for those times. In a 2007 research paper by Thomas A. Garrett, Federal Reserve Bank of St. Louis; "Economic Effects of the 1918 Influenza Pandemic", has tried to portray the effects from newspaper articles of that time. Industrial plants are running at limited capacity. Out of a total of about 400 men in the transportation department of the Memphis Street Railway, 124 men were incapacitated the day before due to the pandemic. This led to limitations on the Street Railway service. Influenza mortalities had a direct impact on the worker wages in the US industries. It is based on a simple economic model; large number of deaths in the age group of 18-40 in males led to decrease in number of workers for the industrial sector. This reduced the labor supply, thus increasing the marginal product of labor and capital per worker and led to increased wages. There was already a demand for labor in US, as due to the war many men were drafted into the armed forces. And after the pandemic outbreak of 1918, the demand and pay rate also increased. Let us try to understand this labor hourly pay data.

Book and job printing

Job Printing is printing that uses display type and no more than a sheet or two of paper. Short as that definition is, it encompasses a world of paper items—tickets, letterheads, notices, invoices, vouchers, coupons, cards, labels, posters, receipts, and timetables, to name only a very few. Book Printing refers to the mainstream printing industry.

Payroll manufacturing industries

Manufacturing is one industry that holds a high proportion of unionized labor. Manufacturing pay scales usually come with different pay cycles as well for workers, and this can make payroll administration for manufacturing quite challenging. Here it relates to the manufacturing sector working as workers working in assembly work, machine operation, packaging, shipping, or supervising. This indicates that this sector had high need of labor and hence an increase in hourly wages.

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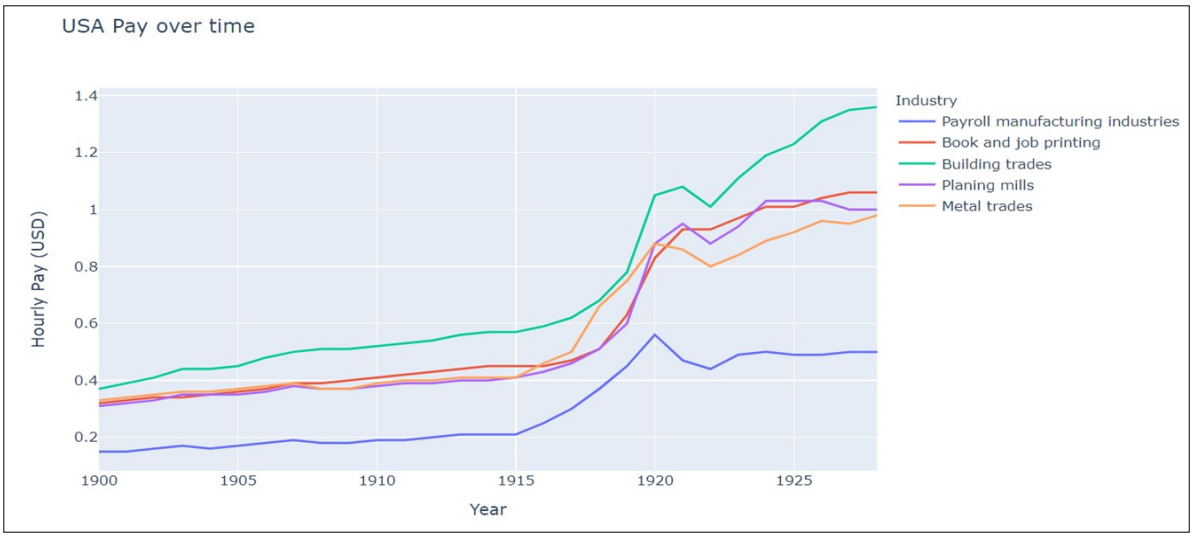

Fig 5. USA Pay over time plot

Payroll manufacturing industries

Manufacturing is one industry that holds a high proportion of unionized labor. Manufacturing pay scales usually come with different pay cycles as well for workers, and this can make payroll administration for manufacturing quite challenging. Here it relates to the manufacturing sector working as workers working in assembly work, machine operation, packaging, shipping, or supervising. This indicates that this sector had high need of labor and hence an increase in hourly wages.

Building trades

Related to the building and construction industry, trades (as carpentry, bricklaying, plumbing) that are essential to and chiefly practiced in connection with building construction. This sector saw a high increase in the hourly wages, indicating a large increase in demand in this sector.

Planning mills

Related to the timber and woodwork industry, a planning mill is a facility that takes cut and seasoned boards from a sawmill and turns them into finished dimensional lumber.

Metal trades

Related to the metal industry. Jobs like blacksmith, foundry workers, metal mine workers, Steel erector, Welder, Boilermaker and so on.The influenza pandemic caused a lot of economic and financial problems for the United States. Coupled with wartime expenditure, this led to various issues in those times. Businesses in entertainment and service sectors faced huge losses. Businesses in the healthcare industry experienced an increase in revenue.

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Urban areas and cities, with most of the business and offices were highly affected by the flu, with death rates increase to many times of death rates. These lead to breakdown in the civil infrastructure of the cities and caused economic and financial problems.

Overall, it hampered the economic growth and led to closure of many businesses and firms. Labor hourly pay rates increased due to increase in demand.

Effects of Social Distancing

The pandemic is thought to have begun in crowded army training camps on the Western Front. The unhygienic conditions especially in the trenches along the French border – helped it to spread and have a far-reaching impact. The war ended in November 1918, but as the soldiers returned home, bringing the virus with them, an even greater loss of life was just around the corner; between 50 million and 100 million people are thought to have died.Thevirus infected 500 million people worldwide and killed an estimated 20 million to 50 million victims— that's more than all the soldiers and civilians killed during World War I combined [9].

Implementation of Social Distancing and its Effects: -

Back in 1918, states and cities across the country told people to stay home. Schools and restaurants were shut down. Public events and community gatherings were canceled. People were told to isolate and quarantine. In some places which lasted for months. All of these led to a huge disruption in American life. Although it worked, things were not so smooth as people didn't always obey what experts and followed Social Distancing [10]. But studies show that the social distancing efforts helped slow the spread of the 1918 flu and reduce the mortality rate overall. Sustained, Layered and Early actions saved lives which was perhaps the most important takeaway from the 1918 flu. The people acted quickly particularly before the flu got to an inflection point in which the virus infected a certain amount of people and spread rapidly. They sustained interventions until the virus truly went away and quickly redeployed if the virus came back. The best thing was that the approaches were layered. Placing restriction on top helped a lot which meant advising against or prohibiting just about every aspect of public life, from schools to restaurants to entertainment venues (with some exceptions for grocery store and medical shops). Cities in which multiple interventions were implemented at an early phase of the epidemic also showed a trend toward lower cumulative excess mortality, but the difference was smaller approximately 20% which was less statistically significant than that for peak death rates.The following chart from the PNAS study, which shows that Philadelphia had a much bigger spike in deaths, while St. Louis kept its death toll down overall due to social distancing measures. Philadelphia waited eight days after their death rate began to take off before banning gatherings and closing schools. They endured the highest peak death rate of all studied. Philadelphia, as just one example, didn't cancel a World War I parade as the 1918 flu picked up, which likely led to thousands of infections

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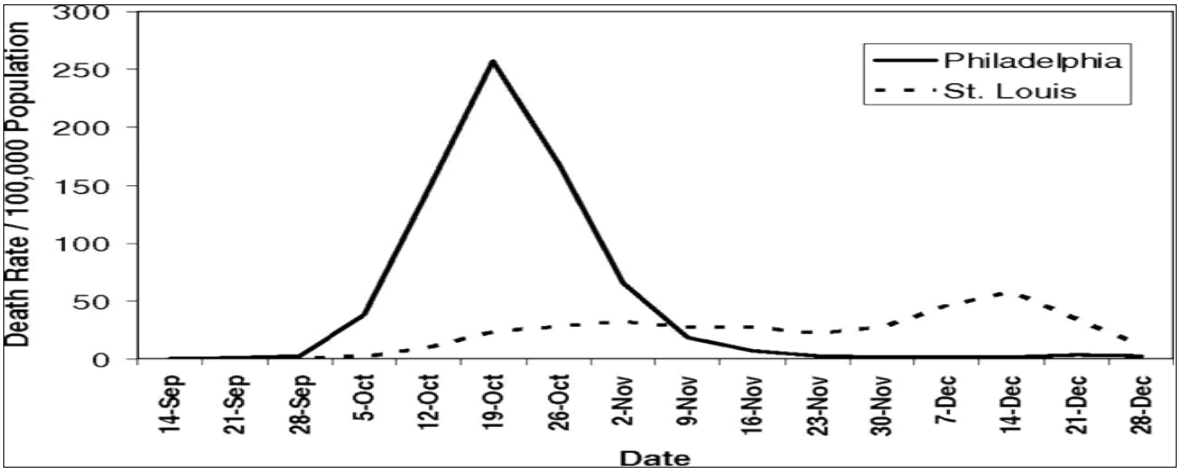

Fig 6**:** Comparative analysis of Weekly deaths per 1,00,000 in Philadelphia & St. Louis.

As the pandemic appeared to subside, St. Louis pulled back its social distancing measures. But it turned out that the pullback was premature — and flu deaths started to rise once again. This graph shows that, with the line chart tracking flu deaths over time and the black and gray bars below showing when key social distancing measures were in place. Thus, we can conclude that St. Louis had strong social distancing measures and a low total death rate. The city successfully delayed its peak in deaths, but faced a sharp increase when restrictions were temporarily relaxed.Notably, the second spike in deaths only appeared when cities removed social distancing measures. The PNAS study, which looked at 17 US cities, reported similar findings that no city experienced a second wave while its main battery of non-pharmaceutical interventions was in place. Second waves occurred only after the relaxation of interventions. Officials instituted social distancing measures, saw flucases fall, then pulled back the measures, saw flu cases rise again, and reactivated the measures.

Seeing the effects of outbreaks drove people to serious action. But people did do social distancing for weeks and months during the 1918 flu pandemic. The city had the lowest death rate on the Eastern Seaboard. After relaxing social distancing measures, San Francisco faced a long second wave of deaths.The following visual on the shows a comparison of death rate per 100,000 population in 1918, based on their strict or lenient lockdown policy.

The rise of globalization, urbanization, and larger, more densely populated cities facilitated the virus' spread across a continent in a few hours—while the tools available to respond have remained nearly the same. Public health interventions were the first line of defense against an epidemic in the absence of a These measures include closing schools, shops, and restaurants; placing restrictions on transportation; mandating social distancing and banning public gatherings. Thus, we can conclude that the cities that ordered Social Distancing measures for shorter periods tended to have spikes in death and higher death rates as compared to the cities that had longer social distancing measures.

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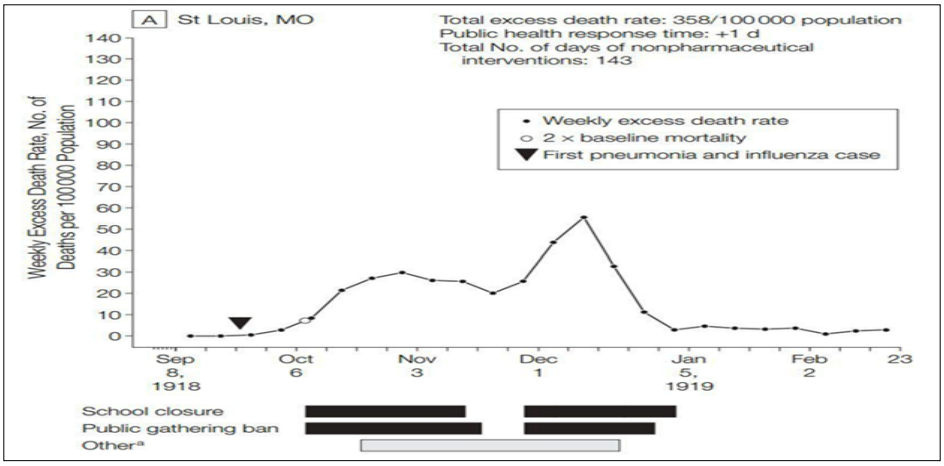

Fig7**:** Weekly Excess Death Rate & Death Rates per 1,00,000 population .

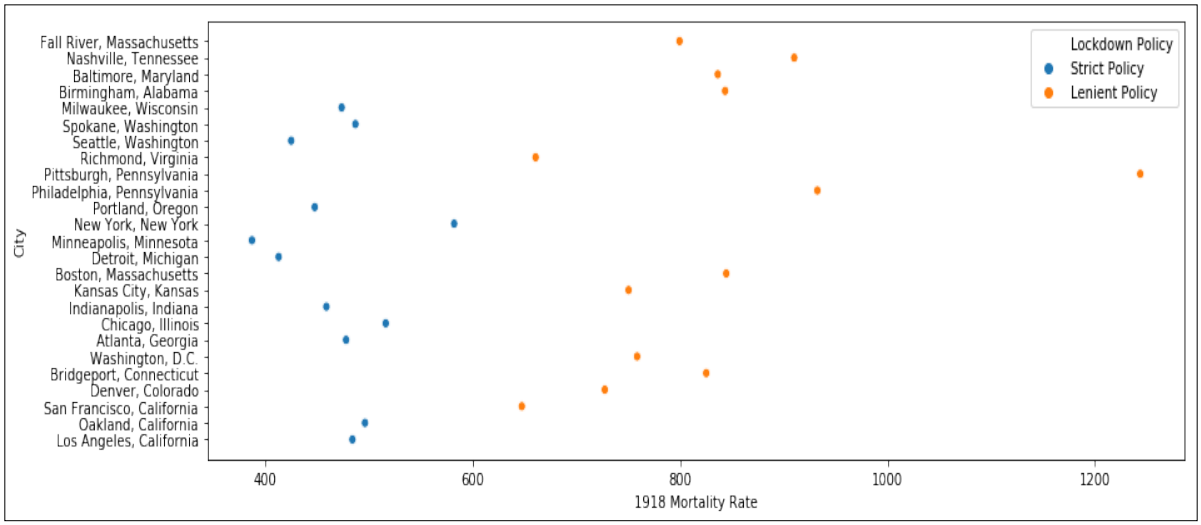

Fig 8**:** - US Cities based on strict versus lenient lockdown policies (per 100,00 people)

Relation to the COVID-19 Outbreak

In comparing the two outbreaks, there a number of things to be considered. The COVID-19 is caused by a corona virus, whereas the Spanish Flu was caused by a influenza virus. There also seems to be a difference in age specific mortality. Spanish flu was seen to be dangerous to young and old alike, but the COVID-19 seems to be more lethal towards the elderly. In case of the Spanish flu, railroads and ships were the only means to carry people over long distances. Hence spread of the virus was gradual. In today's modern world, with planes and shorter ship travel times, the virus was carried to many corners of the world in a short time. Spanish flu hit the world in a time before Sir Alexander Fleming had discovered Penicillin. Without antibiotics, many deaths were perhaps, not caused by virus itself, but by secondary bacterial infections. Spanish Flu reminds us how large can be, the impact of a pandemic. A new unknown

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pathogen can cause terrible devastation and numerous deaths. It served as a motivation, to prepare for such large pandemic outbreaks. The Spanish Flu pandemic did teach us important things. It showed us that, in battles such outbreaks, the most important tool we have is social distancing and lockdowns. Back in 1918, US cities with strict lockdown policies recorded lower deaths. A similar attitude is to be followed in case of the COVID-19 outbreak.

7. Conclusion

In relation to the Covid-19 pandemic governments must decide how much economic disruption to tolerate in order to suppress the disease, or at least to slow its spread [11]. Seeing the overall report, we can say that the economy will be in a bad state after any large disease outbreak, but given time, recovery will happen, and everything will slowly return to normal. There will be large number of deaths, but proper precaution can help in limiting the number of deaths. Proper social distancing can help a lot in spread of disease.

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An Eco Friendly Substitute of Asphalt Binder – Review

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Abstract

The review focuses on the potential use of wood lignin as a partial substitute and performance enhancer in asphalt binders. The aim behind this reviews to fight issues like durability, strength and the reduction in aquifer recharging. Lignin is a dead natural polymer occurring in the secondary cell wall of plant cells. During polymerisation, monlignols accumulate in a non uniform manner, thus leaving behind pores in the structure. Using this property in our asphalt binders would render roads as permeable to water. But lignin being hydrophobic in nature, the material would not corrode due to water actions, unlike commonly used bitumen. Various tests have been conducted to generate aging conditions on test using lignin as asphalt binder. All of them show that lignin addition to binder brings stiffness. Lignin also positively effects high temperature rutting performance, without adversely affecting low temperature. Lignin additionally also shows better recovery than bitumen as binder. The fatigue resistance of surface is negatively affected by lignin. Basically the result of various studies suggests that wood lignin is a promising substitution of bitumen as binder, coming with economic savings and environmental benefits.

Keywords: Asphalt, Lignin, Bitumen

Introduction

The first recorded use of asphalt as paving material has been recorded from Babylonia back in 615 BCE, as stated by author Huge Gillepsie in his book "A Century of progress: The history of hot mix asphalt," published by National Asphalt Pavement Association in 1992. Similar uses have also been recorded in the Greek Civilisation also. Despite these early uses of asphalt, it took modern builders centuries before they actually used asphalt as a paving material. Only in the eighteenth century did Englishman John Metcalf make the first attempts, the 180 miles long Yorkshire Roads. And the binder we have been using for this purpose is the petroleum by-product, bitumen [1].

Though bitumen has served the purpose of a binder quite well for centuries now, there are certain issues that have popped up with time. Continuous questions are being raised now about the durability of bitumen binded asphalt. It is a very common experience where we see the pavement cracking due to fatigue, high temperature, or high rain even.

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Another very important issue that has now come up is bitumen being impervious to water, is hindering the aquifer recharging. So, it is high time that we make some growth hacking to generate a common solution to all these issues.So, to cater to these needs, studies were made to figure out a suitable polymer that can be used as a potential substitute of bitumen as asphalt binder. Lignin, a plant based polymer, is predicted to suffice all the issues stated earlier, by replacing bitumen as asphalt binder, atleast partly.

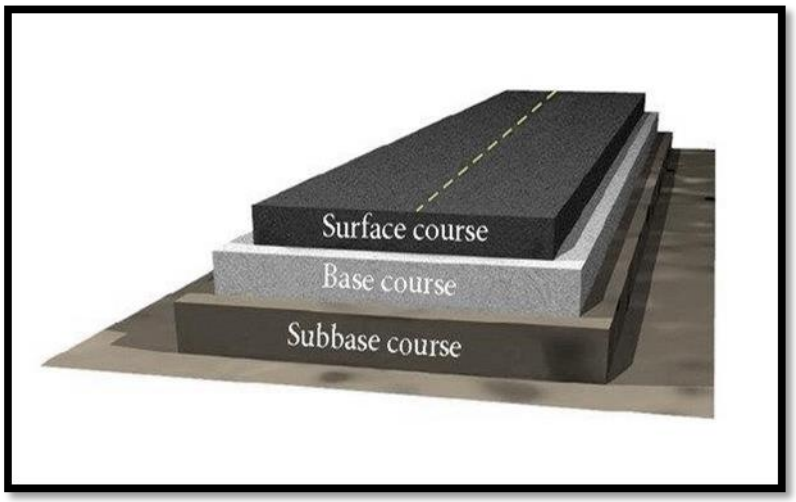

Fig 1: The layers of a normal asphalt road

Water cycle and acquifer recharging

Water cycle is the phenomenon that literally sustains life on earth. It brings back to us the water we lose, by evaporation due to heat. When molecules of water vapor return to liquid form, they create cloud droplets that can fall back to earth's surface as precipitation. Major part of this precipitation lands on the oceans, the part landing on land, flows into rivers and oceans. Only a part, a significant one, seeps into the soil. This seepage goes on through the various layers of soil, fine and coarse, to recharge the under ground water reserve, ie. the aquifer[9].

Occurence and structures

Bitumen is a petroleum by-product, which is hydrophobic in nature. It chemically comprises of Asphaltenes, polar and non polar aromatics, and saturates [6].

Lignin on the other hand is the most abundant organic substance on earth after cellulose. It occurs in amd can be extracted from the cell wall of plants and the middle lamella, where it glues the plant fibres together. Chemically, it is hydrophobic and composed essentially of Guaiacyl, p-hydroxyphenylpropane, Syingyl. These components polymerise in such a manner that they give rise to a structure very much similar to that of bitumen [7-8].

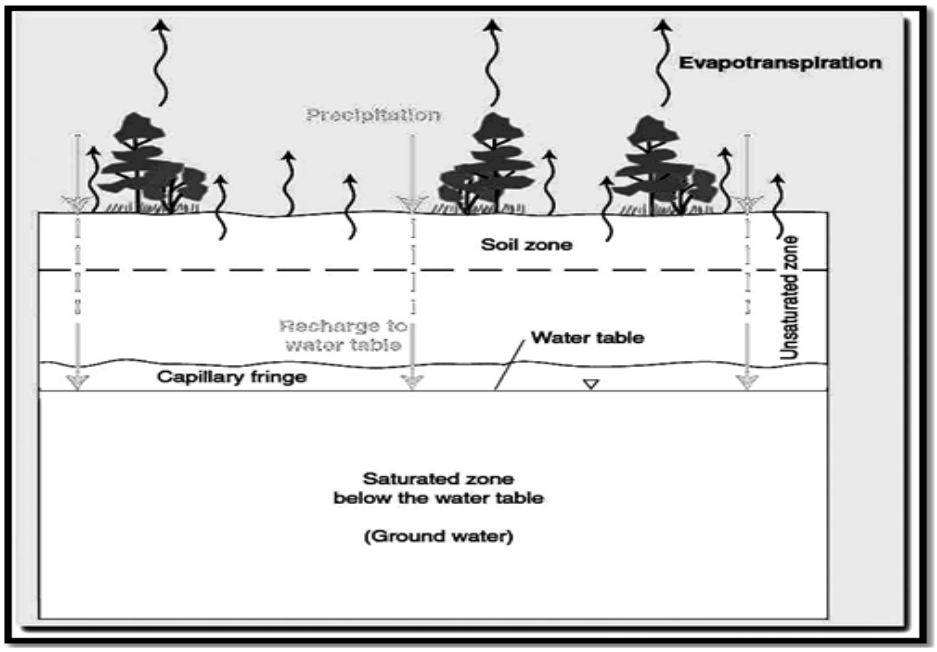

Fig 2: Water table and water cycle (Courtesy: sloarecology.psu.edu)

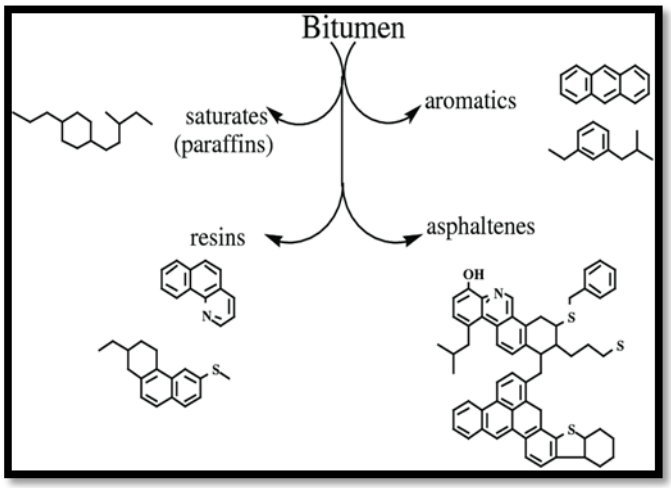

Fig 3: Types of bitumen (Courtesy: researcher.net)

Principle

Lignin is a major component in plant structure. It provides plants with mechanical support. Tissues like bark, vascular bundles, which help tall trees to remain erect, have lignin as a major component. As according to the tissue, there is a regulatory mechanism by which monolignols get polymerized in a plant tissue. And it has been seen that in all tissues lignin shows uneven deposition. Also, tracheids, which help in water conduction in plants, do it through the pores generated in their ends by uneven polymerization of monolignols. So, if we can polymerise monolignols in the asphalt binder as in plant tissues, then the channel sort of structures must feature in our roads also. Now the idea

can be opposed by saying that in plants conduction is facilitated by other factors like root pressure and transpiration pull. But in our case, we need not carry a single drop against gravity, so it won't be as tedious, expectedly. And if the water that pours on the surface can be channelized through the pores to the soil surface, the seepage to the bed rocks for aquifer recharging would be natural [9].

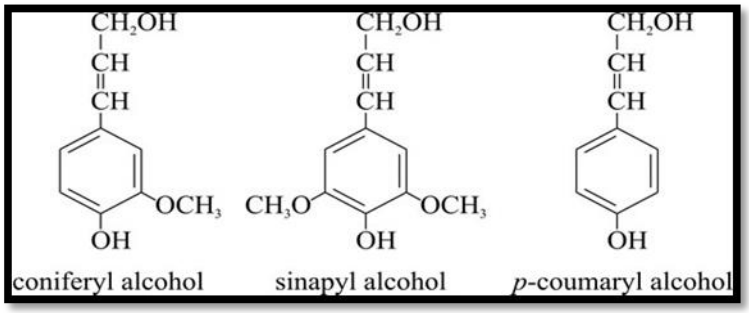

Fig 4: Monolignols (Courtesy: cheresearch.engin.umich.edu)

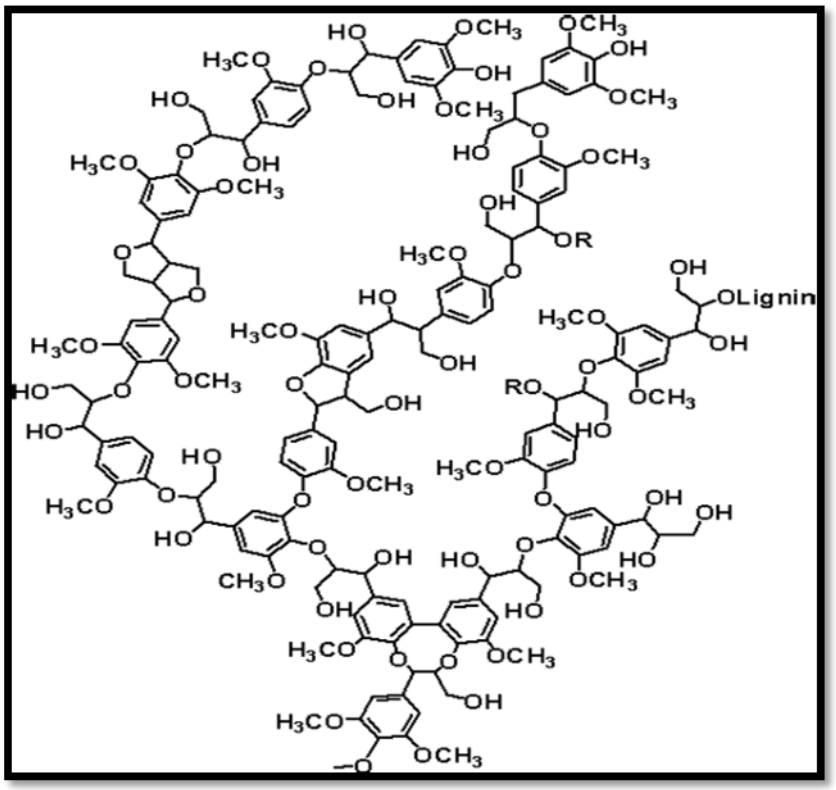

Fig 5: Polymerised lignin (Courtesy: chem.cmu.edu)

Chemically, lignin polymerizes in end wise manner, in which oxidized monolignols undergo cross coupling reactions with radicals formed on the free phenolic ends of growing lignin polymer. In vitro lignin polymerization experiments that produce synthetic lignin polymers via enzymatic or chemical oxidation of monolignols have demonstrated that propensity for lignin linkage formation depends on surrounding conditions, or their chemical structure of monolignols [2].

In plants what happens is, there is a primary, semi permeable cellulose structure. On that lignin gets deposited, unevenly, thus we get the strength and porous features simultaneously. So what we as developers need to do is, initially abandon the idea of replacing bitumen completely with lignin. We need to plan it as a partial substitution, so that we first are able to retain a strong core, over which lignin will be made to polymerise. We may hold on with the age old practice even, where we make the paving in tri layers. The sub base course and base course are basically loose composition layers of stone chunks and fine gravels, which are permeable to water seepage. And then for surfacing, we might use a framework to support the lignin polymerization.

Also the friction coefficient of lignin with rubber tyres is around 0.68, which is comparable to that of bitumen(0.6-0.9). Lignin being strongly hydrophobic, doesn't get damaged due to heavy rain. Lignin also shows better fatigue recovery than bitumen, rendering the roads stronger [3].

Another positive side of using lignin is that it retards litter decomposition, by forming a tough layer over materials. So if we could use the lignin from plant wastes into paving, the decomposition rate would also be facilitated [4].

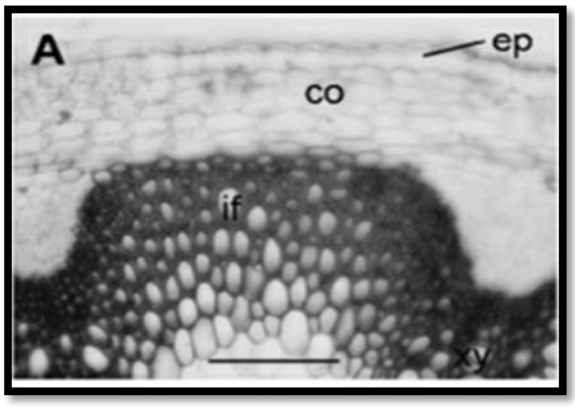

Fig 6: Ectopic deposition of lignin (Courtesy: journal.plos.org)

Conclusion

The challenge that we would face in executing the project is in polymerizing lignin as per our need**.** We need to study the lignin polymerization patterns in various types of tissues and then understand the conditions acting upon to lead to similar polymerization in the tissue pattern which we wish to get featured in our roads[5]. Observing specifically the collenchyma tissues will probably be a good choice as they give a stronger support to their systems too. Basically, the aim behind this study and made proposal is to fight water crisis throughout the world and also make transportation much economical, with introduction of cheaply available lignin, over costly petroleum by product. But even then we remain jammed with another issue. That when we make the crust

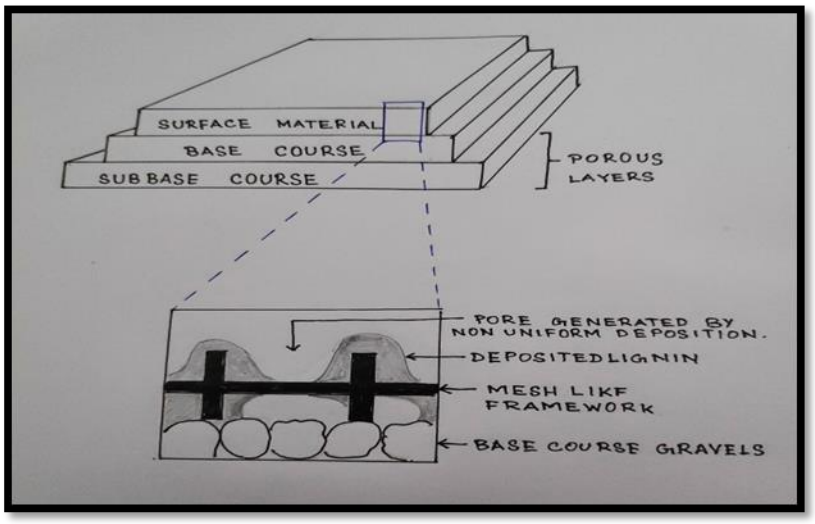

Fig 7: Proposed model

porous, the inner sand core becomes weak. So we need to think of another material that will support the core of our roads.

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Heavy Metal Contamination in Biotic Component: A gradual case study in West Bengal

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Abstract

Fish is a popular human food. Over two-and-a-half billion people globally obtain their daily nutrient intake from fish. In India, it is a major dietary component for over 50 percent, and is a particularly important nutrition source for the poor. Mercury is a deadly environmental pollutant, both in its elemental form and in combination with other chemicals. When released into the environment mercury is transformed into methylmercury through microbial action. Methylmercury is the most pernicious form of mercury. It bioaccumulates in fish and enters human body with the consumption of contaminated fish. Fish in polluted water bodies accumulate methylmercury – a toxic pollutant of high potency that crosses the blood brain barrier and placental barrier, making it an intergenerational toxin. It enters the food chain both from point and non-point sources. Effluent pipes from industrial processes often contain mercury or mercury compounds. Emissions and ash from coal-fired power plants also contain mercury. It is well known that mercury circulates globally and deposits in water, bioaccumulating in the food chain through algae and fish. The higher the pecking order of a fish in the food chain, greater is the amount of mercury it is likely to contain. Advisories on fish consumption are quite common in developed countries, especially for pregnant women. Human exposure to such toxins therefore assumes significance. Contamination of this vital food is a key issue.In developing countries, issues like food contamination rarely draw attention. Mere availability of food is argued to be of foremost concern. In this scenario of poverty and hunger, system of industrial production has largely remained unaccountable to society and the environmental pollution it causes.

Keywords: Contamination, Methyl mercury, bioaccumulation, human exposure

Introduction

Mercury can exist in three oxidation states: Hg^0 (metallic), Hg^{1+} (mercurous) and Hg^{2+} (mercuric). The properties and behaviour of mercury depend on its oxidation state. Mercury in water, soil, sediments, or biota (i.e., all environmental media except the atmosphere) occurs either as inorganic mercury salts or organic forms.

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Mercury in environment

Natural sources of atmospheric mercury are rocks, including coal, from where it enters the atmosphere through weathering and volcanic emissions. Another source is volatilisation from the oceans. Anthropogenic sources of mercury in the environment include coal combustion, mercury uses in cathodes, metal processing, chloroalkali industries, pharmaceuticals and mining of gold and mercury disseminated and can circulate for years, accounting for its widespread distribution.[2] The distances it travels and eventual deposition depends on the chemical and physical form of mercury emissions.

The residence time of oxidised mercury compounds in the atmosphere is uncertain. Even after it is deposited, mercury is commonly emitted back to the atmosphere either as a gas or in association with particulates to be redeposited elsewhere. Mercury undergoes a series of complex chemical and physical transformations as it cycles in the biosphere.

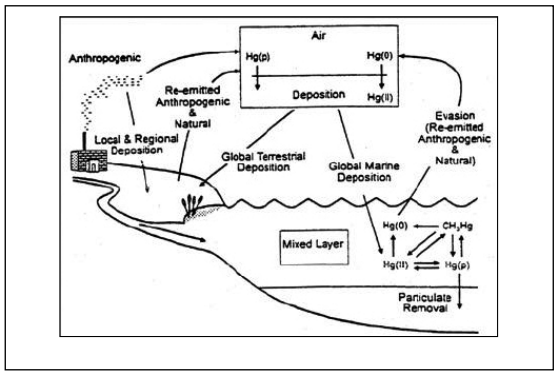

Fig1.A basic diagram of the global mercury cycle

As indicated, mercury is emitted in the atmosphere by a variety of sources, dispersed and transported by air, deposited to the earth, and stored in or transferred between the land, water and air.

Environmental Mercury: Transport and Destinations

Mercury cycle in figure 2 below illustrates the major physical and chemical transformation expected to occur in mercury in freshwater lakes. These processes include a number of infinite and/or indefinite loops

Health impacts of Mercury Humans

The three possible forms of mercury exposure are elemental mercury, inorganic mercury and organic mercury. Each of them has specific effects on human health. Of these, methylated mercury (organic mercury) is of the greatest concern. Methylated mercury is the most toxic of all organic mercury compounds. Of its two common forms – monomethyl mercury and dimethylmercury, the latter is extremely toxic. However, dimethylmercury is very unstable and its occurrence in non-laboratory environment is rare. In nature, it quickly degrades into monomethyl mercury. Monomethyl mercury constitutes the greatest hazard, as it is highly toxic and bioaccumulates in organisms and biomagnifies as it climbs the trophic ladder. It's a neurotoxin that causes a wide array of neurological disorders and can easily be fatal at higher concentrations.

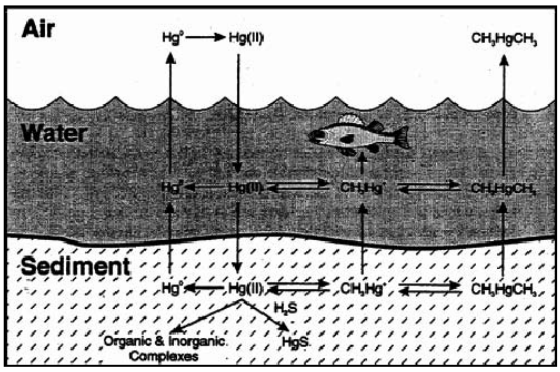

Fig.2

Mercury Cycle in Freshwater Lakes

Cited from EPA Mercury Study Report to Congress. Adapted from Winfrey, M.R. and Rudd, J.W.M. 1990. Review- - Environmental Factors Affecting the Formation of Methylmercury in Low pH Lakes. Environ. Toxicol. Chem. 9:853-869.

Other Organisms

Mercury has adverse effects on a wide range of organisms. Effects of mercury on birds and mammals include death, reduced reproductive success, impaired growth and development and behavioural abnormalities. Sublethal effects of mercury on birds and mammals include liver damage, kidney damage and neurobehavioral effects. Effects of mercury on plants include death, plant senescence, growth inhibition, decreased chlorophyll content, leaf injury, root damage and inhibited root growth and function.

Mercury concentrations in the tissues of wildlife have been reported at levels associated with adverse effects. Toxic effects in piscivorous avian and mammalian wildlife have been associated with point source release

s of mercury in the environment.

Mercury Methylation, Bioaccumulation and Exposure Pathways

Mercury methylation is a key step in mercury absorption in food chains. The biotransformation of inorganic mercury into methylated mercury occurs in the sediments of water bodies. Not all mercury compounds entering an aquatic ecosystem, however, are methylated; demethylation reactions as well as degradation of dimethylmercury occur, and these reactions decrease the amount of methylmercury available in the aquatic environment. There is scientific consensus, however, on the environmental factors that influence variability in mercury methylation in waterbodies.

Often, almost 100 percent of mercury that bioaccumulates in fish tissue is methylated. Numerous factors influence bioaccumulation of mercury in aquatic biota. These include the acidity of the water (pH), the length of the aquatic food chain, temperature and dissolved organic material.

Mercury accumulates in an organism when the rate of uptake exceeds the rate of elimination. Although all forms of mercury accumulate to some degree, methylmercury has a higher propensity for bio-accumulation. Its half-life ranges from months to years in different

organisms. Elimination of methylmercury from fish is extremely slow.

Plants, animals and humans are exposed to methylmercury either by direct contact with contaminated environments or ingestion of mercury contaminated water and food. Generally, mercury builds up more in the higher trophic levels of aquatic food chains (biomagnification). At the top are piscivores, such as humans, eagles, hawks, cormorants and other fish-eating species. These species prey on fish, such as the bronze featherback (Notopterusnotopterus) or the long-whiskered catfish (Sperataaor), which in turn feed on smaller forage fish. Smaller piscivorous wildlife (e.g., kingfishers) feed on the smaller forage fish, which in turn feed on zooplankton or benthic invertebrates. Zooplanktons feed on phytoplankton and the smaller benthic invertebrates feed on algae and detritus. Thus, mercury is transmitted and accumulated through several trophic levels. [5] Accordingly, mercury exposure and accumulation is of particular concern for animals at the highest trophic levels in aquatic food webs and for animals and humans that feed on these organisms.[6]

Methylmercury – Human Exposure Pathways

Humans are most likely to be exposed to methylmercury through fish consumption. Exposure may occur through other pathways as well (e.g., the ingestion of methylmer- cury-contaminated drinking water and food sources other than fish, and uptake from soil and water through the skin). However, for humans and other animals that eat fish, methylmercury uptake through fish consumption dominates these other routes.

There is a great deal of variability in fish-eating populations with respect to fish sources and fish consumption rates. As a result, there is a great deal of variability in exposure to methylmercury in these populations. The presence of methylmercury in fish is, in part, the result of anthropogenic mercury releases from industrial sources. As a consequence of human consumption of the affected fish, there is a risk of human exposure to methylmercury.

Methylmercury is a known human toxicant. Clinical neurotoxicity has been observed following exposure to high amounts of mercury (for example, Mad Hatter's Disease). Consumption of mercury contaminated food has produced overt neurotoxicity. Generally, the most subtle indicators of methylmercury toxicity are neurological changes. The neurotoxic effects range from less immediately observable weakening of motor skills and sensory ability at comparatively low doses to tremors, inability to walk, convulsions and death at very high exposures.[7]

Methylmercury – Absorption and Excretion

Methylmercury is rapidly absorbed through the gastrointestinal tract and distributed throughout the body. It penetrates the blood-brain and placental barriers in humans and animals. It is relatively stable and only slowly demethylated to form mercuric mercury in rats. Methylmercury has a relatively long biological half-life in humans: estimates range from 44 to 80 days. Excretion occurs via the faeces, breast milk and urine. The knowledge

of mercury absorption from inhalation is limited.[8]

Methylmercury – Health Effects

Methylmercury-induced neurotoxicity is of the greatest concern when exposure occurs to the developing foetus, as it easily penetrates the placental and blood-brain barrier. Post-natal brain development continues well into childhood. Methylmercury exposure at early developmental stages adversely affects a number of cellular events in the developing brain both in utero and post-natally. The post-natal age when the development of various regions of the brain is completed varies, and development of many functions continues through the first six years of life.[9]

Methylmercury Disasters

The most notorious methylmercury incident occurred among people and wildlife of Minamata, on the shores of Minamata Bay, Kyushu, Japan. The source of methyl- mercury was a chemical factory that used mercury as a catalyst in the production of acetyldehyde. A series of chemical analyses identified methylmercury in the factory's waste sludge, which drained into Minamata Bay, as a toxicant affecting the people and wildlife in the region. This methylmercury accumulated in the tissue of the Minamata Bay fish and shellfish that were routinely consumed by wildlife and human populations in the region. The symptoms characteristic of nervous system damage. The symptoms included:

Impairment of peripheral vision
Disturbing sensations (feeling of "pins and needles" pricks, numbness) usually in the hands and feet and sometimes around the mouth
Difficulty in movement coordination as in writing
Speech impairment
Hearing impairment
Difficulty in walking
Mental disturbances

It took several years before people realized that they were developing the signs andsymptoms of methylmercury poisoning. Over the next 20 years the number of people known to be affected with what came to be known as Minamata disease increased to thousands. In time, the disease was recognized to result from methylmercury occurring in fish in the Minamata Bay. Deaths occurred among both adults and children. It was also recognized as a potent toxin that could damage the nervous system of growing foetus, if the mother ate fish contaminated with high concentrations of methylmercury during pregnancy.

The nervous system damage from severe methylmercury poisoning among infants was very similar to congenital cerebral palsy. In the fishing villages of this region, the occurrence of congenital cerebral palsy due to methylmercury was very high compared to the incidence for Japan in general. After the source of toxic contamination was identified, mercury release

into the bay was checked. Over time the symptoms were seen to reduce in the local population.

Another methylmercury poisoning outbreak occurred in Japan, in the area of Niigata, in 1965. Again, investigations identified the source to be an acetaldehyde producing chemical factory releasing methylmercury into the Agano river[10].

Effects of methylmercury on nervous system are well established. Consumption of methylmercury contaminated food products (including grains and pork products) has also resulted in severe poisoning with pathological changes in the nervous system and clinical symptoms identical to Minamata disease.

These developments brought to the fore two major points of concern:

Methylmercury in fish is the most prevalent source of mercury poisoning

Methylmercury in fish is the most important source of mercury poisoning among humans.

Methylmercury – safe levels

The concern of methylmercury contamination of food has gradually led to the emergence of permissible or tolerable methylmercury dose standards in different countries including India. Although India now has the Food Safety and Standards Act, specific food standards on the basis of the said Act are not yet in place, and moreover, its standards are not meant to apply to products of farming, fishing and aquaculture.

Food standards in terms of permissible levels of contamination are only available with the Prevention of Food Adulteration Act and Rules, 1954. This gives the limit of mercury in fish as 0.5 ppm by weight and that of methylmercury (calculated as an element) in the case of all foods (including fish) as 0.25 ppm by weight.[11] The fact that the aforesaid Act and Rules mention methylmercury, has tremendous import for this study: for it is the mercury in the methylated form that is of the greatest toxic significance and its presence in our food chain needs to be checked and contained. The study also compares its findings with the PFA standards.

However, it is not enough to determine methylmercury contents in fish, it is also important to know people's average dietary fish intake. It is only when one combines methylmercury contents in fish with the average fish intake that one can assess mercury exposure. This is because the body flushes out methylmercury at a very slow rate, and if the rate of methylmercury intake exceeds the rate of its excretion, it starts building up, causing poisoning. The degree of poisoning per unit intake of methylmercury depends on the body weight: for the same amount of intake, poisoning is less severe in people of higher weight. And finally, young people and pregnant women (the foetus) are most vulnerable, and therefore methylmercury stipulations are of the greatest importance in their case.

Nowadays, standards for the tolerable doses of methylmercury account for its total intake over a period (e.g. per week) or the average daily intake. Of these, the most stringent standard is that of the US EPA, which explicitly factors in the body weight of the recipient. The EPA reference dose for methylmercury is 0.1 μg/kg of body weight/day and this standard has been supported by the US National Research Council as well.[12] The US

Agency for Toxic Substances and Disease Registry (ATSDR) has a less stringent standard or MRL (minimal risk level) of 0.3 μg / kg of body weight / day.[13]

The US FDA has a different standard. It does not speak in terms of body weight of the recipient, but of total permissible dose per week. For one-ppm methylmercury in fish, it advises fish consumption below 198.4465 gm per week and for 0.5-ppm methyl- mercury in fish it advises consumption below 396.893 gm per week. The FDA has been criticised for its relatively lenient standards.[14]

In year 2004, the Joint FAO-WHO Expert Committee on Food Additives developed a norm for tolerable levels of methylmercury in fish. The said Expert Committee reconfirmed this standard in 2006.[15] Its Provisional Tolerable Weekly Intake (PTWI), the tolerable limit of exposure, is given as 1.6 μg/kg of body weight/per week or around 0.228571 μg/kg of body weight/day. Although it is less stringent than the EPA's, is more stringent than that of the ATSDR and far more stringent than that of the FDA.

It is important in this context that the European Food Safety Authority (EFSA) has issued a guideline based on both the Joint FAO-WHO Expert Committee On Food Additives recommendations of PTWI (1.6μg/kg body weight) and the US National Research Council's reference dose of 0.1 μg/kg body weight/day, which is the same as the US EPA's and leads to 0.7 μg/kg body weight PTWI. Essentially the EFSA's recommendations tend to ask vulnerable groups to cut down on their fish consumption.[16]

Objectives

- Quantify the level of mercury in fish and crustacean samples from prominent markets in Kolkata and select waterbodies.
- Study the nature and extent of mercury contamination, and reach a reasonable conclusion through laboratoryanalysis.
- Assesshealthriskfromintakeofcontaminatedfish(basedonlevelofcontamination).
- Provide recommendations on the basis of results andanalysis.

Sampling Locations

Samples for the study were collected from fish markets in Kolkata as well last from various water bodies spread across different area to get a broadview of mercury contamination of fish inKolkata.

After collecting total samples, they were submitted to the EFRAC (Edward Food Research & Analysis Centre Limited) laboratory for total mercury analysis of the fishes collected from Kolkata markets. The sampling strategy required to support thorough going analysis of mercury contamination of edible fish.The locations were selected to represent wide geographical spread, influences of industrial installations and land use practices. Lab results were determined in ppm (mg/kg).

Table 1: List of markets in Kolkata from where samples were collected

Sl. no.	Market	Waterbodies
1	Gariahat	Bantala
2	Sahababu Bazaar	Basirhat
3	Manicktala	Kharibari
4	Sealdah	Nalban
5	Dumdum	Rajarhat
6	Muchipara	Paradwip, Canning
7	Baguihati	Jainagar
8	Ashubabur bazar	Hasnabad, Ghushighata
9	Narayanpur Bazar	Haroa

Materials and Methods:

Mercury analysis is performed as per laboratory internal method, Quantification is performed by ICP-MS.

Microwave assisted wet digestion:

A suitable quantity of sample was weighed accurately and transferred into a clean Teflon digestion tube. Then 7 ml of conc. Nitric acid was added into it and the tube was closed with cap. The tube was kept in microwave tube stand and then kept in microwave digester (CEM

Corp., USA). The door was closed and the digester was switched on. After that the required method was selected and loaded then start button was on. The operating conditions are summarized in Table. After completion of digestion the digester was switched off and allowed to cool the system, then the tube was removed and opened; the content was filtered using Whatman No. 42 filter paper. The filtrate was collected in any graduated vessel and diluted suitably with Milli-Q water

Operating conditions of microwave digester (CEM Corp.)

Ramping stage	Hold time (minutes)	Temperature (^{o}C)	Power (W)
1	20	180	800
2	20	160	800
3	20	160	800
Cool down	10	140	-

INSTRUMENT SPECIFICATION

Inductively coupled plasma mass spectrometry (ICP-MS) 7700 X Make Agilent Technology

Instrumental operating parameters

Plasma condition		Plasma flow (15L /min) Nebulizer pump speed (0.1 rps) RF power 1550 watts
S/C Temperature		2^0C
Detectors parameters		5 mV
TMP Revolution		100 %
Auto sampler conditions	Working mode	Continuous
	wash	Between runs

Fish Intake Survey

The survey was conducted in Kolkata and nearby areas to get a general idea of fish consumption among families with different income levels. No similar survey was conducted in rural areas with ponds, rivers or the sea owing to difficulty in ascertaining actual consumption, as a significant portion of fish intake in such areas comes from non-market sources. However, the necessity of such a survey, conducted in a methodologically rigorous manner, is obvious if one has to get a clear picture of fish intake patterns in West Bengal.

Table 2: Fish intake survey in 200 families in and around Kolkata

Monthly Income (Rs.)	Monthly average fish consumption (kg)
0-10,000	8.5
10,001-20,000	12
20,001-30,000	15.5
30,001-40,000	17.5
40,001-50,000	23
50,001-60,000	25
60,001-70,000	25
70,001-80,000	32
80,001-90,000	32.5
90,001-1,00,000	22

Results and Discussion

Methods

Samples were collected at the point of time and the place where the fishers brought in their catch. This norm was followed in all locations A few other varieties that had been brought in earlier and stocked with the Aaratdar (fish wholesaler) in the market were thus also included. All the samples were taken only after a careful cross-questioning about their sources.

It is important to clarify that the term 'location' here specifies a certain geographical entity and not a particular pond or a river. For instance, the varieties caught from the kharibari have come from different ponds within a radius of about two kilometre. Each pond constitutes a different ecosystem and therefore it can be argued that the fish have come from different locations. But, in this study the term 'location' implies a particular area;

Fish samples were chosen on the basis of the following criteria:

Preference for commonly eaten varieties(mercury in these is the greatest hazard for fish eating people)

Matured specimens (mercury bio-accumulates with age)

To analyse mercury bio-accumulation in different specie

After collection, the samples were identified in the following manner:

By local name of the species /variety

By scientific name of the species (in so far as scientific species identification was possible)

By photographing each sample (for future identification, if necessary)

By weighing and measuring the length of each sample (for estimating age)

Results

The total mercury concentrations of samples collected from Kolkata markets and other locations in West Bengal,including the species average for each location/ market, are given in various tables .There liable detection limit of the instrument and methodology was 0.20mg/kg. That is, for the given methodology and instrumentation, mercury values arrived at below the aforesaid value may not be accepted with a high degree of confidence. Therefore, in this study any value indicated by<0.20mg/kgimpliesavaluex:0<x<0.20 mg/kg (here x is understood to be always, even if slightly, greater than 0, as mercury naturally occurs in the environment and faint traces are present in all organisms).This factor creates obvious problems in working with the data, for example, even at the simplest level of working out mean values.

Since people eat a variety of fish, methylmercury level in an individual fish variety does not give complete picture of their exposure. People's intake of methylmercury depends on a variety of fish in their food and methylmercury contamination levels of these fish. The average methylmercury level of the study samples thus gains significance here. Furthermore, fish in the markets come from variegated sources. A consumer buying her fish from a local market is exposed to contaminated catch coming from different places. Therefore, the state average for mercury contamination of fish would be a good indicator of people's risk of

exposure.

It may be noted here that the two scenarios described above depict relatively low levels of fish consumption, and that fish consumption could easily be higher, particularly in families with higher incomes, costal populations or areas in the vicinity of large waterbodies. The risk of exposure increases with increase in fish-flesh consumption for a given body weight.

The research (Toxic Link and Disha) shows that methylmercury levels in 69 percent samples exceed PTWI for a child weighing 25 kg and consuming 250 gm fish flesh in an entire week. Likewise, 59 percent samples exceed PTWI for women/adolescents of 60 kg consuming 500 gm fish flesh in a week.

It is abundantly clear from the findings that a large number of samples have alarmingly high levels of methylmercury. Especially samples collected from some of the fishing locations across West Bengal show disturbingly high mercury and methylmercury averages. Table 3. Number and percentage of samples exceeding PTWI limits

Given body weight and consumption level	Percentage of samples showing PTWi exceedance
A child of 25 kg consuming just 250 gmof fish flesh in aweek	68.56
An adolescent or pregnantwomanof60kg consuming 500 gm offish flesh in aweek	58.71

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The coastal/estuarine areas of Jharkhali, Kakdwip and Digha show high mercury levels. So does Budge Budge, very close to and downstream of Kolkata in the Hooghly estuary. The Hooghly estuary and the coastal waters of West Bengal are the recipients of industrial effluents, untreated urban sewage and agricultural wash-offs, containing an extraordinarily large variety of toxins from a number of sites across densely populated SouthBengal. Mercury concentration in fish samples from Haldia (Haldi River), an industrial area abutting estuarine site, though high for safe consumption was relatively low in comparison to estuarine samples.The explanation for this anomaly may lie in the fact that Haldi river,which flows into the Hooghly at Haldia and from where many of the samples came, is not as polluted as Hooghly.

The results can be further analysed by comparing the species/variety averages displayed in tables with their feeding habits. It is observed that predatorial and carnivorous species tend to show significantly higher values former cury in comparison to mainly herbivores or omnivores varieties. A striking example is *Harpadonneherus,* described as an ‘aggressive predator’,which shows very high mercury and methyl- mercury values.Other examples are *Epinephelussp.*and *Eleutheronematetradactylum,* which feed on small fish and crustaceans, show high mercury values. On the other hand *Catlacatla*, basically a phytoplankton, detritus

and insect feeder, shows quite low mercury values, and so do *Oreochromis nilotica*, *Labeobata*and *Labeorohita*. This reaffirms that methyl mercury undergoes biomagnification at higher trophic levels, and therefore predator species show higher concentration of mercury. However,a few anomalies also exist. In our study a few herbivorous species like *Liza parsia*werealso found to show high mercury values.

It is interesting to look at the distribution of fish species. The Table 4 shows the situation for Digha, Kakdwip and Budge Budge.Once again there is a predominance of carnivorous types, though perhaps a little les spronouncedthan that ofJharkhali.

Table 4 . Mercury and methyl mercury in sample species from Digha, Kakdwip and Budge Budge (Data Source: Toxic Link and DISHA)

Digha			**Kakdwip**			**Budge Budge**		
s scientific name	**hg (mg/ kg)**	**Mehg(mg/ kg)**	**Species scientific name**	**hg (mg/kg)**	**Mehg(mg/kg)**	**s scientific name**	**hg (mg/kg)**	**Mehg(mg/ kg)**
Otolithoides sp.	0.63	0.504	*Otolithoides sp.*	0.45	0.36	*Ompokpabda*	0.20	0.160
Otolithoides sp.	0.39	0.312	*Otolithoides sp.*	0.50	0.4	*Ompokpabda*	0.20	0.160
Apolectusniger	0.40	0.32	*laginopsispaniju s*	0.42	0.336	*Sillagosihama*	0.37	0.296
Apolectusniger	0.42	0.336	*laginopsispaniju s*	0.36	0.288	*Sillagosihama*	0.56	0.448
Pellona sp.	<0.20	<0.20	*ephaloussp.*	0.48	0.384	*Tenualosailisha*	0.70	0.560
Pellona sp.	<0.20	<0.20	*ephaloussp.*	0.69	0.552	*Tenualosailisha*	0.58	0.464
Devariodevario	0.60	0.48	*Arius sp.*	0.60	0.48	*Eleutheronematetra dactylum*	0.56	0.448
Devariodevario	0.72	0.576	*Arius sp.*	0.58	0.464	*Eleutheronematetra dactylum*	0.82	0.656
Sillagosihama	0.26	0.208	*Racondarussilia na*	0.83	0.664	*lydactylussexfilis*	0.69	0.552
Sillagosihama	0.24	0.192	*Racondarussilia na*	0.71	0.568	*lydactylussexfilis*	0.59	0.472
Liza parsia	0.26	0.208	*Setipinnaphasa*	0.96	0.768	*Harpadonnehereus*	0.45	0.360
Liza parsia	0.29	0.232	*Setipinnaphasa*	1.09	0.872	*Harpadonnehereus*	0.42	0.336
Portumuspelagiu s	0.50	0.4	*Devariodevario*	0.84	0.672	*Panna microdon*	0.61	0.488
Portumuspelagiu s	0.48	0.384	*Devariodevario*	0.96	0.768	*Panna microdon*	0.44	0.352
Eleutheronematetra dactylum	1.14	0.912	*Liza parsia*	0.96	0.768	*Otolithoides sp.*	1.03	0.824
Eleutheronematetra dactylum	1.10	0.88	*Liza parsia*	0.94	0.752	*Otolithoides sp.*	0.46	0.368
Penaeus sp.	1.39	0.556				*Nibea soldado*	0.83	0.664

Penaeus sp.	1.99	0.796				*Nibea soldado*	0.63	0.504
Trichuruslepturus	0.43	0.344						
Trichuruslepturus	<0.20	<0.20						

Table 5. Mercury and methylmercury in sample species from Kolaghat and Durgapur(Toxic Link and DISHA)

Kolaghat			**Durgapur**		
Speciesscientificname	**hg (mg/kg)**	**Mehg(mg/kg)**	**Species scientific name**	**hg (mg/ kg)**	**Mehg(mg/kg)**
Pangasius pangasius	0.41	0.328	*Wallagoniaattu*	0.25	0.2
Pangasius pangasius	0.22	0.176	*Wallagoniaattu*	0.21	0.168
Catlacatla	0.60	0.48	*Sperataaor*	<0.20	<0.20
Catlacatla	<0.20	<0.20	*Sperataaor*	0.22	0.176
Hypophthalmichthysmolitrix	<0.20	<0.20	*Ophisternonbengalense*	0.20	0.16
Hypophthalmichthysmolitrix	0.20	0.16	*Ophisternonbengalense*	0.21	0.168
Cirrhinuscirrhosus	0.27	0.216	*Cyprinus carpio*	<0.20	<0.20
Cirrhinuscirrhosus	<0.20	<0.20	*Cyprinus carpio*	<0.20	<0.20
Labeobata	0.24	0.192	*Eutropichthysvacha*	<0.20	<0.20
Labeobata	<0.20	<0.20	*Eutropichthysvacha*	0.20	0.16
Macrobrachiumrosenbergii	<0.20	<0.20			
Macrobrachiumrosenbergii	<0.20	<0.20			
Oreochromis nilotica	<0.20	<0.20			
Oreochromis nilotica	0.29	0.232			

In the case of Kolaghat, except for two species, all others were herbivorous or mostly herbivorous. But in the case of Durgapur, all varieties except Cyprinus carpio were carnivorous. Yet, the average mercury value for Durgapur is lower than that of Kolaghat .

The other possible factor for variation in mercury concentration in fish across species and locations can be its size and weight. Fish of greater body weight are likely to show higher levels of mercury bioconcentration. It is evident that neither the feeding habits of the species nor the weight of the catch is sufficient to explain the wide range of variation in mercury values across different sampling locations in general.

The other possible explanation may be in the character of the locations. The fish samples from Durgapur, which is a major industrial site, do not show high levels of mercury,

whereas coastal/estuarine sites, often far removed from industrial areas, show high levels. The point is that mercury emitted from thermal power plants may not necessarily end up in the local water bodies. On the contrary, once in the air, mercury is dispersed and transported thousands of kilometre from its likely emission sources.[17]

On the other hand, Mercury used in industrial processes can get into water bodies only if it is discharged as waste with effluents.[18] This is precisely what happened in Minamata and Niigata.

The mean MeHg value for Hugli is considerably high given the fact samples were collected from a purely agricultural zone. A possible source of mercury may be pesticides used in the agricultural fields. Mercury is a known constituent of a large number of fungicides and rodenticides. The known inorganic mercury fungicides are mercurous chloride, mercuric chloride and mercuric oxide, while there are a host of organomercury fungicides.[19]

In order to locate the possible sources of the contamination, a detailed study of the areas is needed – one that investigates mercury concentration not only in the aquatic fauna, but also in the local water bodies.In fact, there are other questions that remain to be explored. When mercury is tested in aquatic fauna, the testing is done on uncooked samples. Yet, there is every likelihood of various changes during the process of cooking. What happens when mercury/ methylmercury contaminated fish is fried, roasted, boiled or curried? These aspects need to be investigated for fuller assessment of possible mercury intake from contaminated fish.

Table 6: Mercury concentration in some fishes available in Kolkata markets

Sl. No	Name of fish	Scientific name	Result	Catch point	Sale point
1	PangashTangra	Pangasius pangasius	0.08	Bantala	Muchipara market
2	Rui	Labeorohita	0.11	Bantala	Maniktala market
3	BagdaChingri	penaeus monodon	0.06	Bashirhat	Dum Dum Bazar
4	BagdaChingri	Penaeus monodon	0.06	Bashirhat	Dum Dum Bazar
5	Magur	Clariasbatrachus	0.08	Bashirhat	Dum Dum Bazar
6	Magur	Clariasbatrachus	0.08	Bashirhat	Dum Dum Bazar
7	Tangra	Mystusgulio	0.06	Bashirhat	AE Market(Saltlake)
8	Tangra	Mystusgulio	0.05	Bashirhat	Dum Dum Bazar
9	Tangra	Arius sp.	0.03	Bashirhat	Ashubabur Bazar
10	Tangra	Mystusgulio	0.04	Bashirhat	Baguihati Market
11	Tangra	Mystusgulio	0.05	Bashirhat	Dum Dum Bazar

12	Telapia	Oreochromis nilotica	0.025	Bashirhat	Ashubabur Bazar
13	Telapia	Oreochromis nilotica	0.05	Bashirhat	Dum Dum Bazar
14	Telapia	Oreochromis nilotica	0.05	Bashirhat	Dum Dum Bazar
15	Vetki	Latescalcarifer	0.07	Bashirhat	Dum Dum Bazar
16	Vetki	Latescalcarifer	0.07	Bashirhat	Dum Dum Bazar
17	Latta	Harpadonnehereous	0.12	Birati	Ashubabur Bazar
18	Bhetki	Latescalcarifer	0.1	Canning	Ashubabur Bazar
19	Khorovetki	Latescalcarifer	0.09	Canning	Muchipara Bazar
20	Pholi	Notopterusnotopterus	0.025	Canning	Muchipara Bazar
21	Rui	Labeorohita	0.04	Canning	Muchipara Bazar
22	Shingi	Heteropneustesfossilis	0.04	Canning	AE Market(Saltlake)
23	Shol	Channasilondia	0.05	Canning	Muchipara Bazar
24	Bhola	Otolithoides sp.	0.05	Digha	AE Market(Saltlake)
25	Bagda	Penaeus monodon	0.04	Ghusighata	Ashubabur Bazar
26	Latta	Harpadonnehereous	0.1	Ghusighata	Ashubabur Bazar
27	Magur	Clariasbatrachus	0.08	Ghusighata	Ashubabur Bazar
Sl. No	**Name of fish**	**Scientific name**	**Result**	**Catch point**	**Sale point**
28	Tangra	Mystusgulio	0.06	Ghusighata	Ashubabur Bazar
29	Telapia	Oreochromis nilotica	0.1	Ghusighata	Ashubabur Bazar
30	Katla	Catlacatla	0.07	Haroa	Dum Dum Bazar
31	Koi	Anabustestudineus	0.05	Haroa	Narayanpur Bazar
32	Parshe	Liza parsia	0.06	Haroa	Dum-Dum bazar
33	Parshe	Liza parsia	0.06	Haroa	Dum-Dum bazar
34	Tangra	Mystusgulio	0.06	Haroa	Dum Dum Bazar
35	Telapia	Oreochromis nilotica	0.1	Haroa	Dum Dum Bazar
36	Koi	Anabustestudineus	0.08	Hasnabad	Dum Dum Bazar
37	Koi	Anabustestudineus	0.08	Hasnabad	Dum Dum Bazar
38	Parshe	Liza parsia	0.06	Hasnabad	Dum Dum Bazar
39	Parshe	Liza parsia	0.06	Hasnabad	Dum Dum Bazar
40	Shingi	Heteropneustesfossillis	0.04	Hasnabad	Dum Dum Bazar

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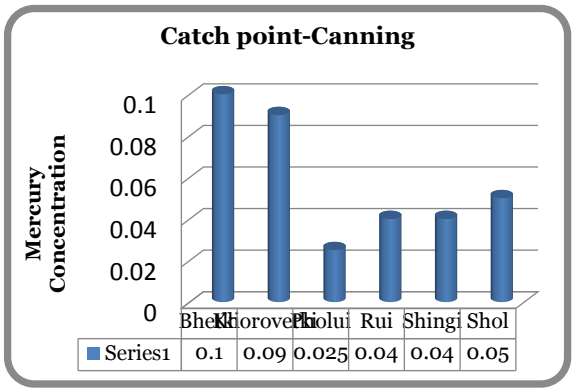
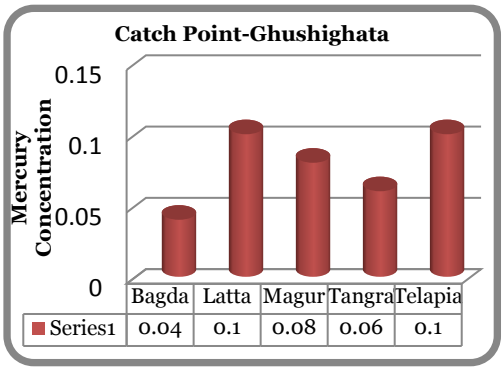
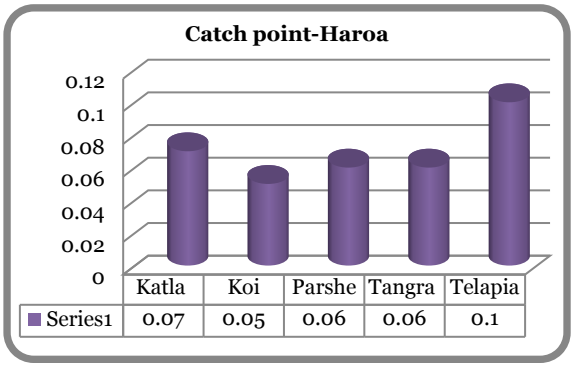
41	Shingi	Heteropneustesfossillis	0.04	Hasnabad	Dum Dum Bazar
42	Koi	Anabustestudineus	0.025	Jainagar	Baguihati Market
43	koi	Anabustestudineus	0.025	Jainagar	Maniktala Market
44	Magur	Clariasbatrachus	0.03	Jainagar	Maniktala Market
45	Shingi	Heteropneustesfossillis	0.09	Jainagar	Baguihati Market
46	Shol	Channasilondia	0.025	Jainagar	Maniktala Market
47	Bata	Labeobata	0.06	Kharibari	Baguihati Market
48	Bata	Labeobata	0.11	Kharibari	Baguihati Market
49	Bata	Labeobata	0.09	Kharibari	Dum Dum Bazar
50	Bata	Labeobata	0.09	Kharibari	Dum-Dum bazar
51	Bhetki	Latescalcarifer	0.04	Kharibari	Narayanpur Bazar
52	Katla	Catlacatla	0.06	Kharibari	Baguihati Market
53	Katla	Catlacatla	0.08	Kharibari	Dum Dum Bazar
54	katla	Catlacatla	0.08	Kharibari	Dum Dum Bazar
55	kholse	CCCccolisafasciata	0.025	Kharibari	Baguihati Market
56	Latta	Harpadonnehereous	0.025	Kharibari	Muchipara Bazar
57	Lilentika	Oreochromis nilotica	0.11	Kharibari	Baguihati Market
58	Lilentika	Oreoghromisnilotica	0.15	Kharibari	Baguihati Market
59	Mrigel	Chirrhinuscirrhosus	0.15	Kharibari	Baguihati Market
60	Parshe	Liza parsia	0.06	Kharibari	Dum-Dum
61	Parshe	Liza parsia	0.06	Kharibari	Dum-Dum bazar
62	Parshe	Liza parsia	0.025	Kharibari	Narayanpur Bazar
63	Rui	Labeorohita	0.05	Kharibari	Ashubadur Bazar
64	Rui	Labeorohita	0.03	Kharibari	Dum Dum Bazar
65	Rui	Labeorohita	0.03	Kharibari	Dum-Dum
66	Sarpnuti	Puntius sarana	0.09	Kharibari	Muchipara Bazar
67	Tangra	Mystusgulio	0.06	Kharibari	Baguihati Market
68	Tangra	Mystusgulio	0.48	Kharibari	Narayanpur Bazar
69	Telapia	Oreochromis nilotica	0.05	Kharibari	Dum Dum Bazar
70	Telapia	Oreochromis nilotica	0.05	Kharibari	Dum-Dum
71	Telapia	Oreochromis nilotica	0.12	Kharibari	Narayanpur Bazar

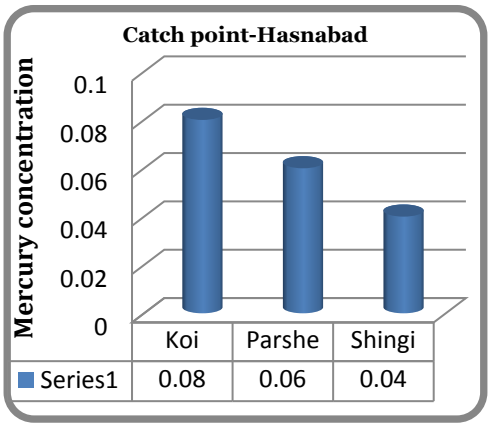
72	Vetki	Latescalcarifer	0.13	Kharibari	Baguihati Market
73	Lilentika	Oreochromis nilotica	0.18	Lowhati	Baguihati Market
74	Mrigel	Chirrhinuscirrhosus	0.18	Lowhati	Baguihati Market
75	Rui	Labeorohita	0.025	Lowhati	Baguihati Market
76	Telapia	Oreochromis nilotica	0.08	Malancha	Dum-Dum
77	Telapia	Oreochromis nilotica	0.08	Malancha	Dum-Dum
78	Vetki	Latescalcarifer	0.06	Malancha	Dum-Dum
79	Vetki	Latescalcarifer	0.06	Malancha	Dum-Dum
80	Magur	Clariasbatrachus	0.08	Mednipur	Ashubadur Bazar
81	sole	Channasilondia	0.05	Mednipur	Ashubadur Bazar
82	Katla	Catlacatla	0.06	Nalban	Maniktala Market
83	Telapia	Oreochromis nilotica	0.07	Nalban	Maniktala market
84	Bhola	Otolithoides sp.	0.06	paradip	Ashubadur Bazar
85	Parsha	Liza parsia	0.04	paradip	Ashubadur Bazar
86	Bele	Platycephalous sp.	0.11	Rajarhat	Rajarhat
87	Mrigel	Cirrhinuscirrhosus	0.11	Rajarhat	Baguihati Market
88	Pabda	Ompokpabda	0.025	Rajarhat	Baguihati Market
89	Sharputi	Puntius sarana	0.1	Rajarhat	Baguihati Market
90	Katla	Catlacatla	0.06	Rajarhat	Narayanpur Bazar
91	Rui	Labeorohita	0.08	Rajarhat	Narayanpur Bazar

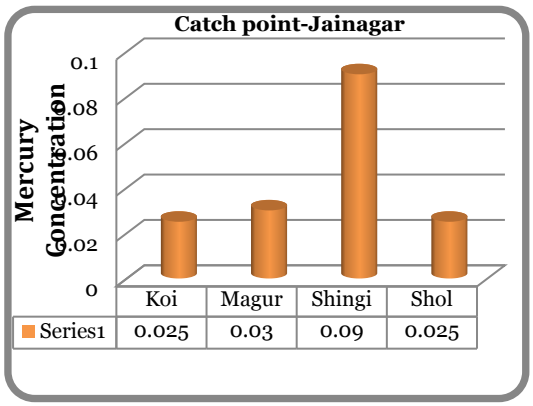
Graphs(Set1): Comparison drawn on different species from same geo- location water body

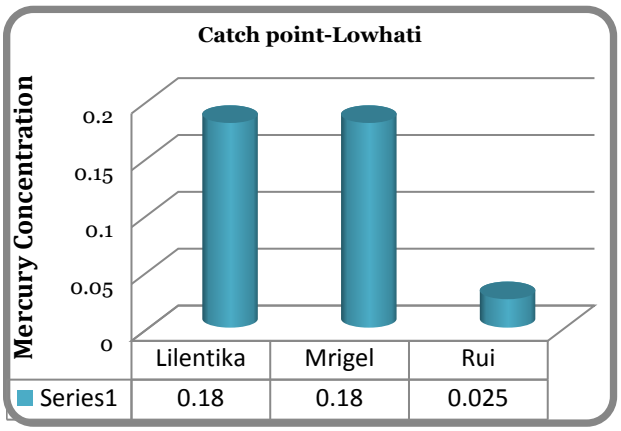












Brief Account of Industrial Belt Locations and Fishing Locations of West Bengal

Durgapur Asansol Region

This is the most important heavy industry region in the state. The western part of the districtis dry and has a large number of industries and mines;agriculture dominates in the eastern part.

Steel plants and coal miningare the most important features of this region. Apart from DPL thermal power plant of 395MW there are several captive powe rgenerating stations. Many heavy industries are situated near the river Damodor. There is also a barrage on Damodor connecting Bardhaman with Bankura district.

Hugli

Hugli districtis adjacent to Kolkata. Eastern part of the district, lying on the western side of the Hooghly river,is under Kolkata Metropolitan Area. A large number of industries are situated in the district, mostly by the side of the river.The eastern part of the district,which has wonderfully richalluvial deposits as well as excellent irrigation facilities, is famous for

al fertilizers and pesticides are used.

Kolaghat

Kolaghat is in East Midnapore district ,adjacent to western border of Howrah dis trict. It is on the bank of Rupnarayan River, which is the border line of Howrah andEast Midnapore district. Kolaghat has 1260 MW thermal powerplant.

Kolkata

Kolkata is one of the most densely populated cities in the world. Once the capital of India, it is one of the earliest industrial hubs in Asia. A large number of heavy, medium and small industries are situated in and around the city.

EastKolkataWetland(EKW):

It is situated in the eastern side of the city ,where the city sewage flows into Bidyadhari river. The area has a large number of sewage fed ponds. These ponds also act as settling tanks.

BudgeBudge

It is an industrial hub adjacent to southern Kolkata by the side of the Hooghly river. The area has several oil depots of different companies and a thermal power plant of 500 MW are capacity.

Haldia

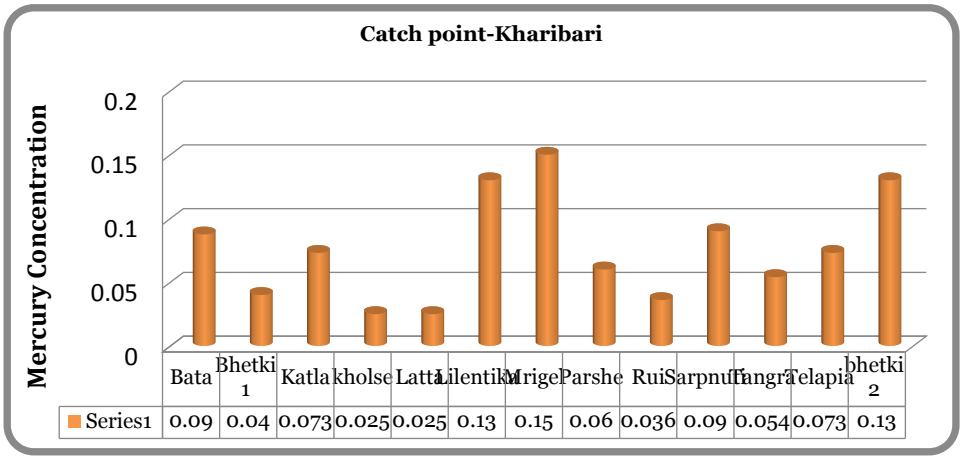
Haldia is an industrial port town in EastMidnapore district. It is situated on the western bank of Hooghly river,where the latter meets the Haldi river. The town has a number of petro-chemical, chemical, oil refinery units.

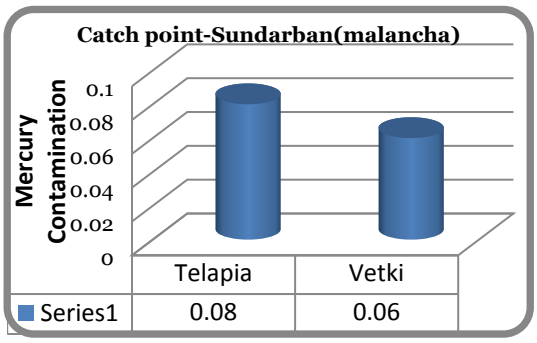
Kakdwip

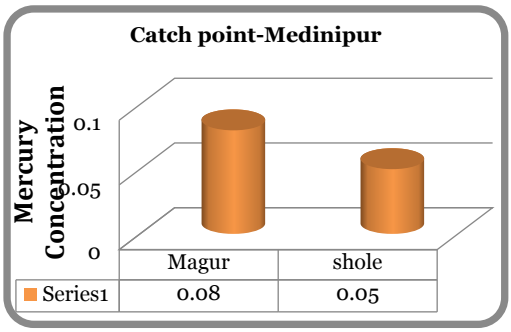
Kakdwip is situated on the eastern bank of the Hooghly estuary and is almost on the Bay of Bengal. The area is in South 24 Parganas district, one of the gateways to the Sundarban. There is no big industry. Agriculture and fishing are the main occupations.

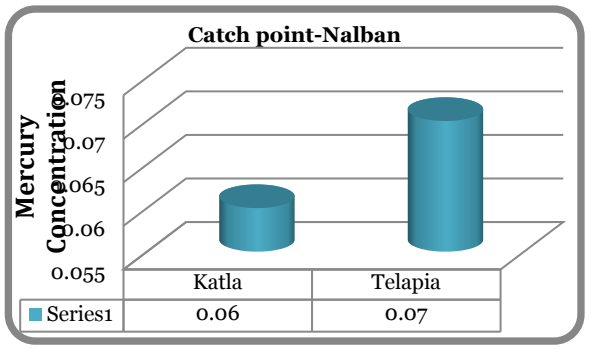
Digha

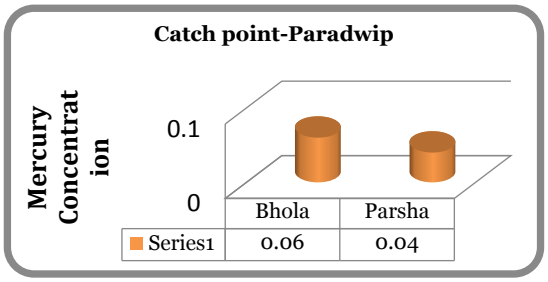
Digha is the most important sea resort of West Bengal,situated in East Midnapore district, adjacent to Orissa border. It has a fishing harbour.

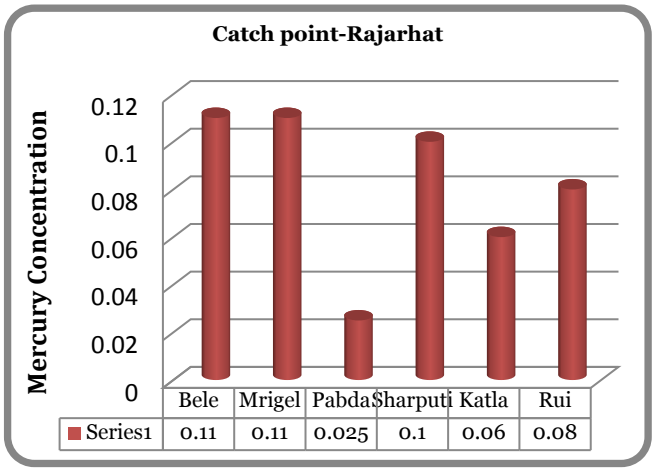

Catch point-Kharibari
Mercury Concentration
0.2
0.15
0.1
0.05
0
Bata
Bhetki 1
Katla
kholse
Latta
Lilentik
Mrigel
Parshe
Rui
Sarpnuti
Tangra
Telapia
bhetki 2
Series1
0.09
0.04
0.073
0.025
0.025
0.13
0.15
0.06
0.036
0.09
0.054
0.073
0.13

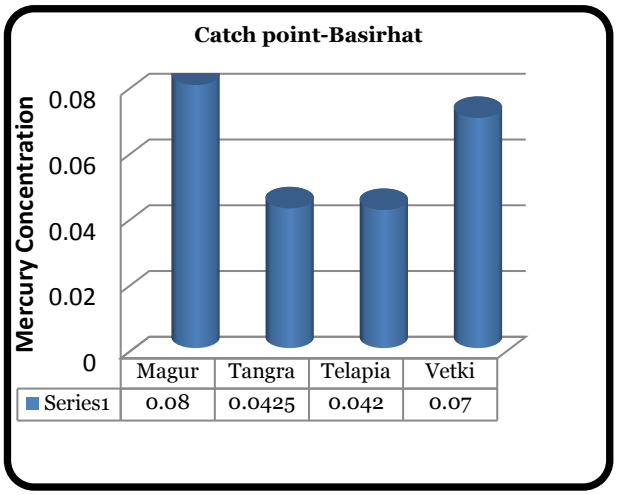

Catch point-Sundarban(malancha)
Mercury Contamination
0.1
0.08
0.06
0.04
0.02
0
Telapia
Vetki
Series1
0.08
0.06


Catch point-Medinipur
Mercury Concentration
0.1
0.05
0
Magur
shole
Series1
0.08
0.05


Catch point-Nalban
Mercury Concentration
0.075
0.07
0.065
0.06
0.055
Katla
Telapia
Series1
0.06
0.07


Catch point-Paradwip
Mercury Concentrat ion
0.1
0
Bhola
Parsha
Series1
0.06
0.04


Catch point-Rajarhat
Mercury Concentration
0.12
0.1
0.08
0.06
0.04
0.02
0
Bele
Mrigel
Pabda
Sharputi
Katla
Rui
Series1
0.11
0.11
0.025
0.1
0.06
0.08


Catch point-Basirhat
Mercury Concentration
0.08
0.06
0.04
0.02
0
Magur
Tangra
Telapia
Vetki
Series1
0.08
0.0425
0.042
0.07

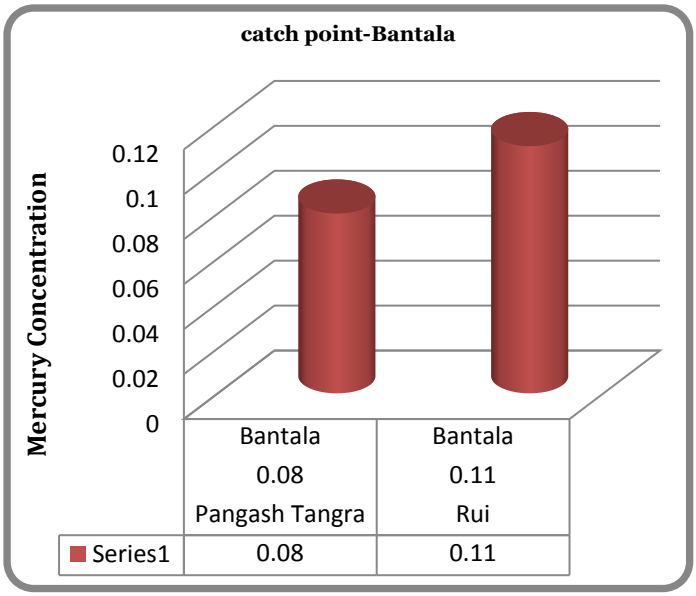

catch point-Bantala
Mercury Concentration
0.12
0.1
0.08
0.06
0.04
0.02
0
Bantala
0.08
Pangash Tangra
Bantala
0.11
Rui
Series1
0.08
0.11

Table7:ComparativeTable of mercury concentrationdepending on catchpoint				
Code No.	**Name of fish**	**Result**	**Catch point**	**Average**
24	BagdaChingri	0.04	Ghusighata	
5	BagdaChingri	0.06	Bashirhat	
10	BagdaChingri	0.06	Bashirhat	
				0.053
47	Bata	0.06	Kharibari	
48	Bata	0.11	Kharibari	
49	Bata	0.09	Kharibari	
50	Bata	0.09	Kharibari	
				0.0875
18	Bhetki	0.1	Canning	
51	Bhetki	0.04	Kharibari	
				0.07
23	Bhola	0.05	Digha	
85	Bhola	0.06	Paradip	
				0.055
52	Katla	0.06	Kharibari	
53	Katla	0.08	Kharibari	
54	katla	0.08	Kharibari	
82	Katla	0.06	Nalban	
90	Katla	0.06	Rajarhat	
29	Katla	0.07	Haroa	
				0.06833
34	Koi	0.05	Harowa	
35	Koi	0.08	Hasnabad	
36	Koi	0.08	Hasnabad	
41	Koi	0.025	Jainagar	
42	koi	0.025	Jainagar	
				0.052

17	Latta	0.12	Birati	
25	Latta	0.1	Ghusighata	
56	Latta	0.025	Kharibari	
				0.081
57	Nylontikka	0.11	Kharibari	
58	Nylontikka	0.15	Kharibari	
73	Nylontikka	0.18	Lowhati	
				0.147
6	Magur	0.08	Bashirhat	
26	Magur	0.08	Ghusighata	
43	Magur	0.03	Jainagar	
80	Magur	0.08	Mednipur	
11	Magur	0.08	Bashirhat	
				0.07
59	Mrigel	0.15	Kharibari	
74	Mrigel	0.18	Lowhati	
88	Mrigel	0.11	Rajarhat	
				0.147
86	Parshe	0.04	paradip	
30	Parshe	0.06	Haroa	
31	Parshe	0.06	Haroa	
37	Parshe	0.06	Hasnabad	
38	Parshe	0.06	Hasnabad	
60	Parshe	0.06	Kharibari	
61	Parshe	0.06	Kharibari	
62	Parshe	0.025	Kharibari	
				0.053
2	Rui	0.11	Bantala	
21	Rui	0.04	Canning	
63	Rui	0.05	Kharibari	
64	Rui	0.03	Kharibari	

65	Rui	0.03	Kharibari	
75	Rui	0.025	Lowhati	
91	Rui	0.08	Rajarhat	
				0.05214
66	Sarpunti	0.09	Kharibari	
83	Sarpunti	0.1	Newtown	
				0.095
39	Shingi	0.04	Hasnabad	
40	Shingi	0.04	Hasnabad	
44	Shingi	0.09	Jainagar	
46	Shingi	0.04	canning	
				0.0525
22	Shol	0.05	Canning	
45	Shol	0.025	Jainagar	
81	Shol	0.05	Mednipur	
				0.042
3	Tangra	0.06	Bashirhat	
7	Tangra	0.05	Bashirhat	
12	Tangra	0.03	Bashirhat	
13	Tangra	0.04	Bashirhat	
27	Tangra	0.06	Ghusighata	
67	Tangra	0.06	Kharibari	
68	Tangra	0.048	Kharibari	
14	Tangra	0.05	Bashirhat	
32	Tangra	0.06	Haroa	
				0.05
4	Telapia	0.025	Bashirhat	
8	Telapia	0.05	Bashirhat	
15	Telapia	0.05	Bashirhat	
28	Telapia	0.1	Ghusighata	
33	Telapia	0.1	Haroa	

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69	Telapia	0.05	Kharibari	
70	Telapia	0.05	Kharibari	
71	Telapia	0.12	Kharibari	
76	Telapia	0.08	Malancha	
77	Telapia	0.08	Malancha	
84	Telapia	0.07	Nalban	
				0.07
9	Vetki	0.07	Bashirhat	
16	Vetki	0.07	Bashirhat	
72	Vetki	0.13	Kharibari	
78	Vetki	0.06	Malancha	
79	Vetki	0.06	Malancha	
18	Vetki	0.1	canning	
51	Vetki	0.04	Kharibari	
				0.075
87	Bele	0.11	Rajarhat	
55	kholse	0.025	Kharibari	
19	Khorovetki	0.09	Canning	
89	Pabda	0.025	Rajarhat	
1	PangashTangra	0.08	Bantala	
20	Pholoi	0.025	Canning	

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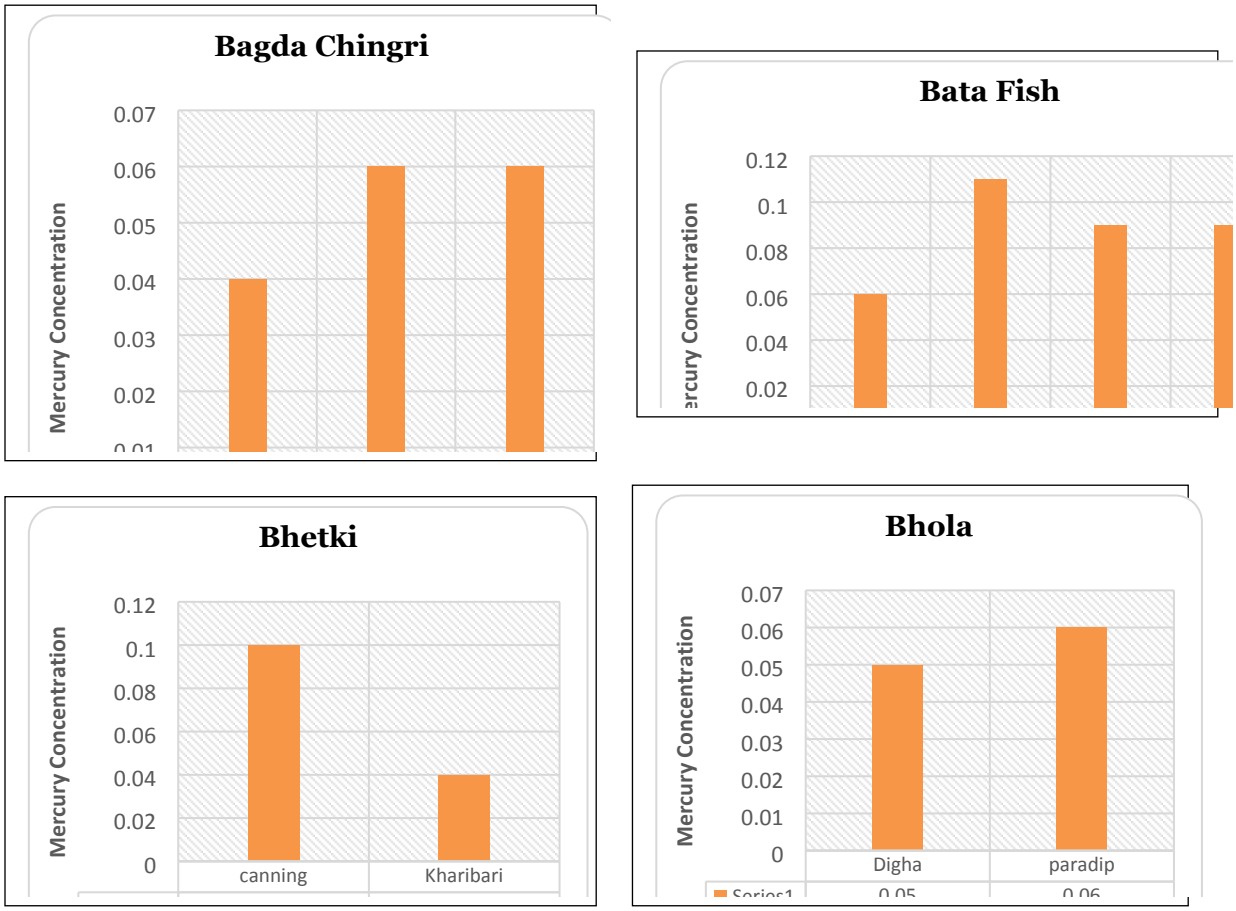
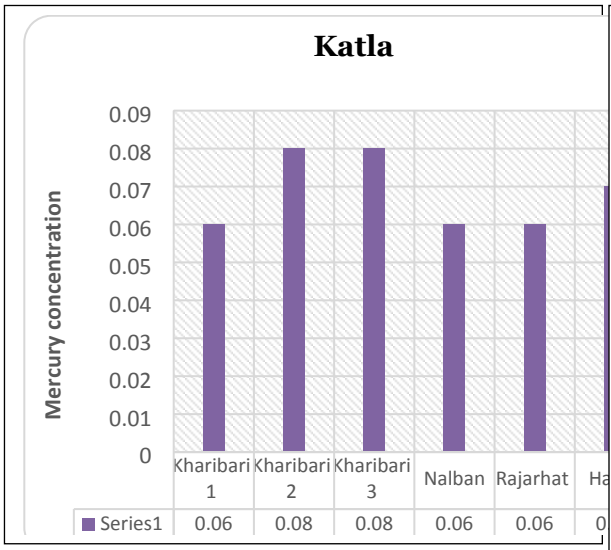
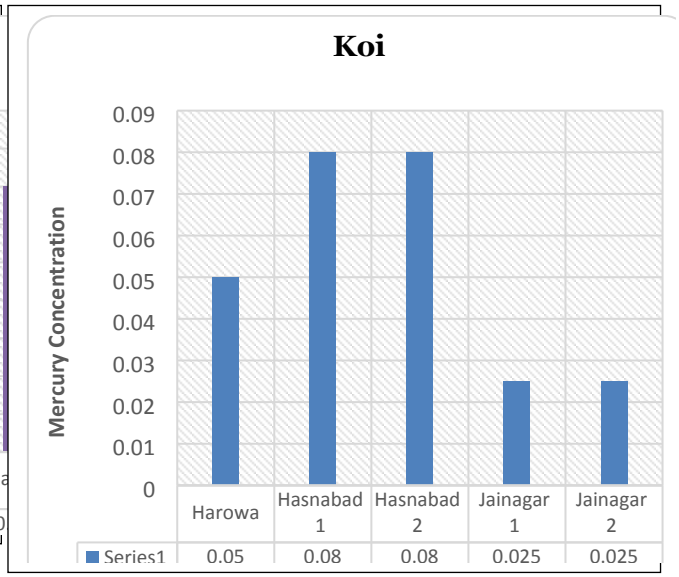
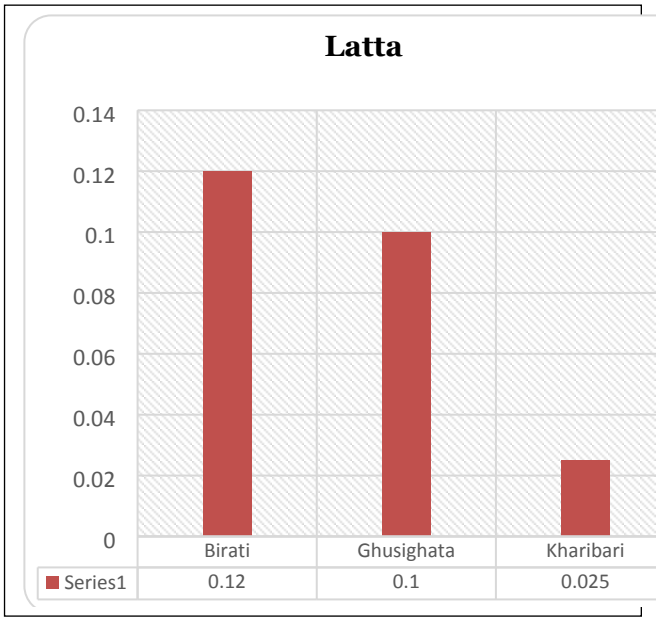
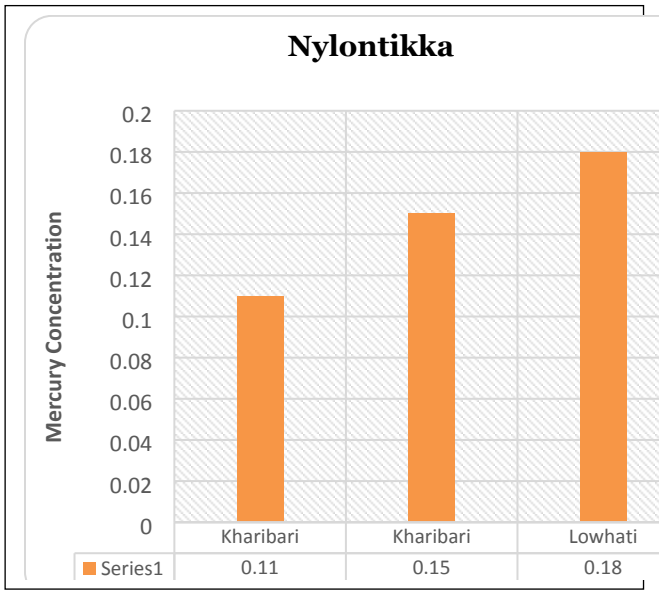
Graph (Set2): Comparison of same fish species from different waterbody

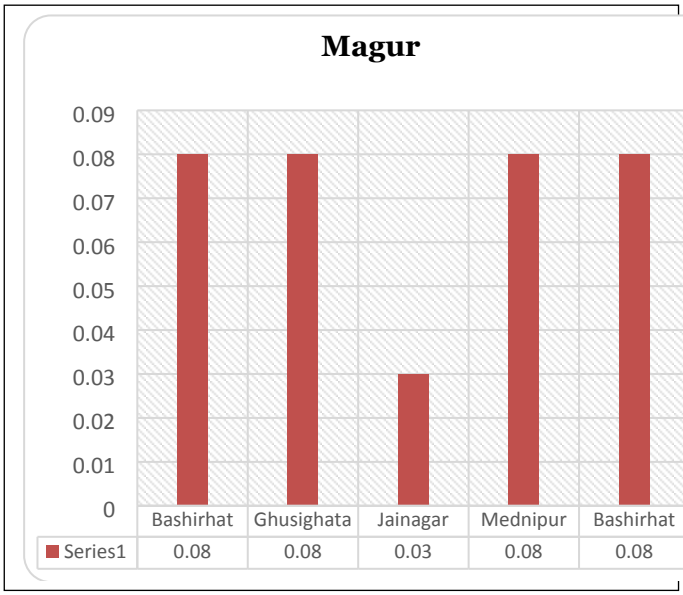


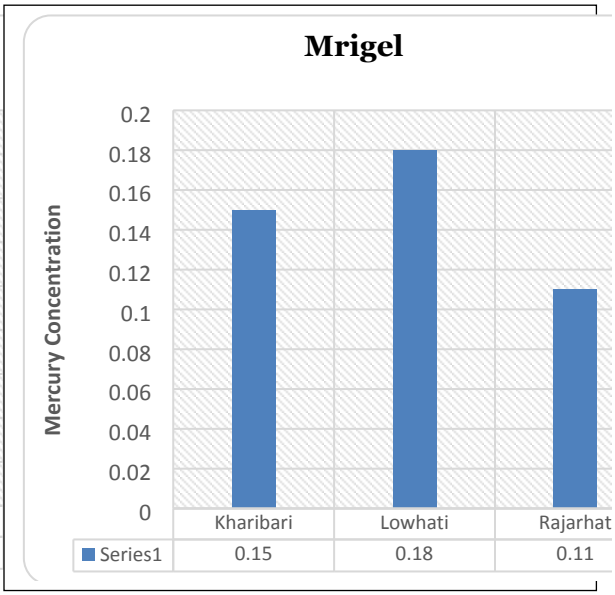

Katla
Mercury concentration
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Kharibari 1
Kharibari 2
Kharibari 3
Nalban
Rajarhat
Series1
0.06
0.08
0.08
0.06
0.06

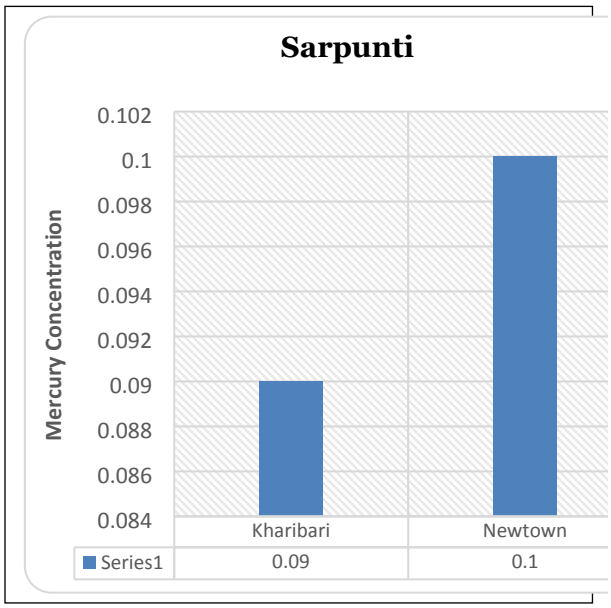

Koi
Mercury Concentration
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Harowa
Hasnabad 1
Hasnabad 2
Jainagar 1
Jainagar 2
Series1
0.05
0.08
0.08
0.025
0.025

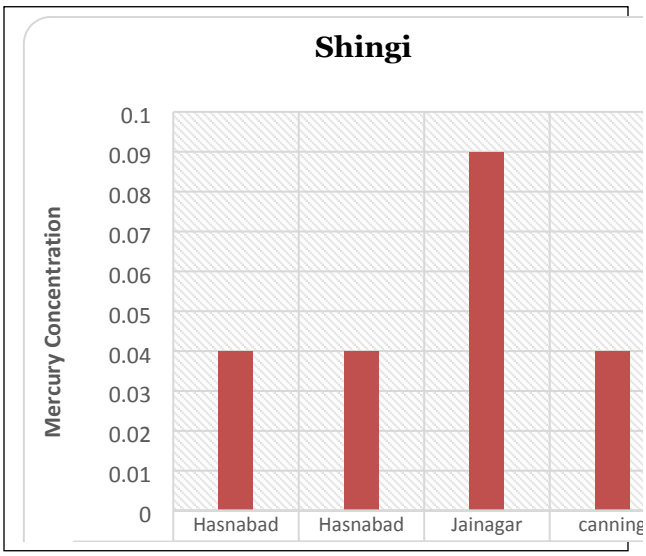

Latta
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Birati
Ghusighata
Kharibari
Series1
0.12
0.1
0.025

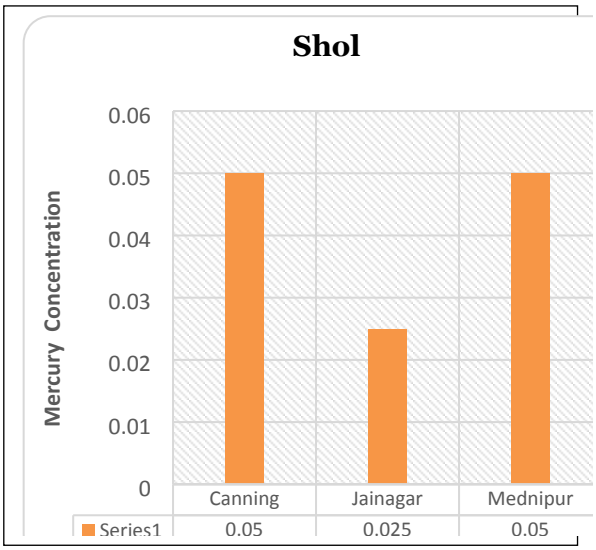

Nylontikka
Mercury Concentration
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Kharibari
Kharibari
Lowhati
Series1
0.11
0.15
0.18

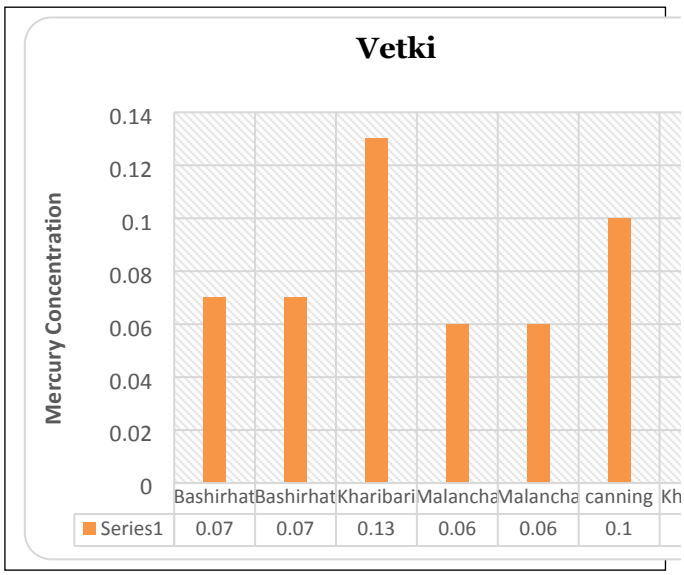

Magur
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Bashirhat
Ghusighata
Jainagar
Mednipur
Bashirhat
Series1
0.08
0.08
0.03
0.08
0.08

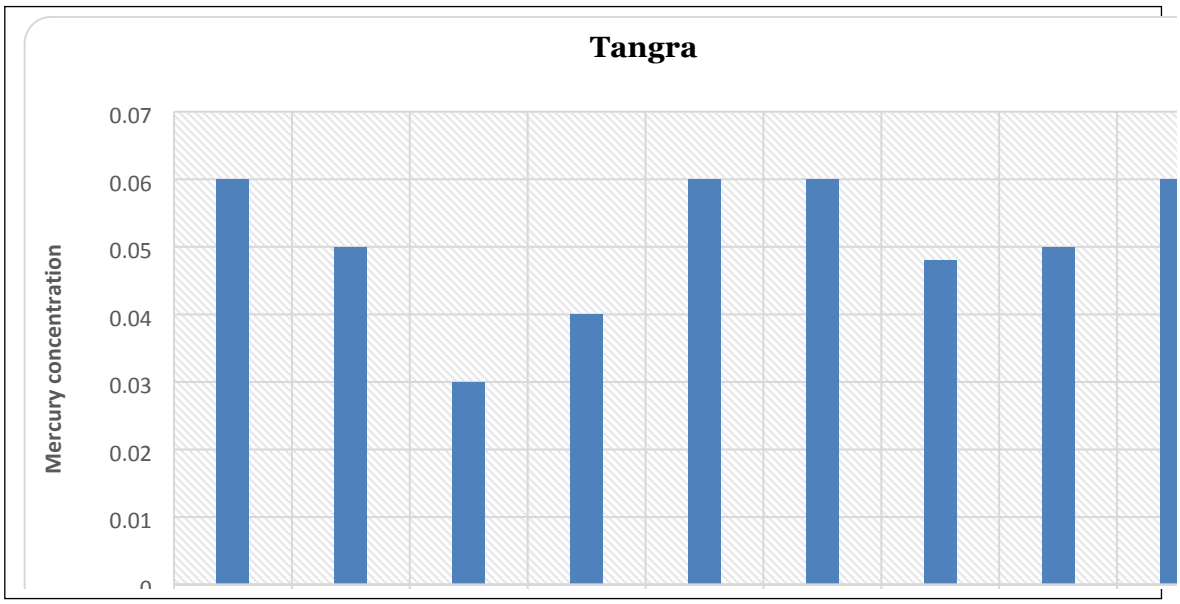

Mrigel
Mercury Concentration
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Kharibari
Lowhati
Rajarhat
Series1
0.15
0.18
0.11

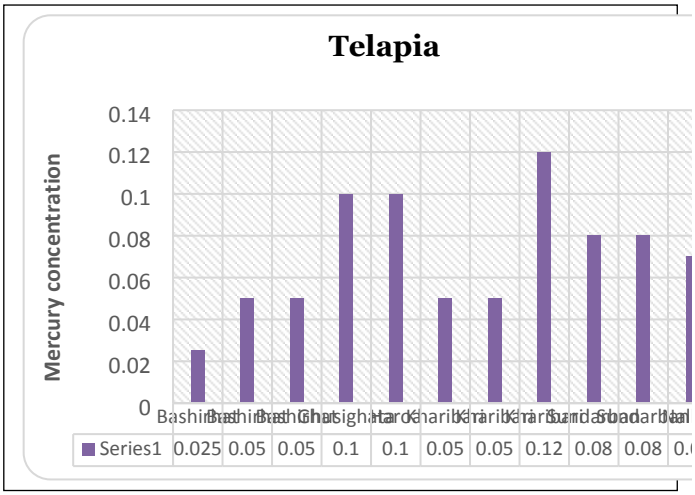

Sarpunti
Mercury Concentration
0.102
0.1
0.098
0.096
0.094
0.092
0.09
0.088
0.086
0.084
Kharibari
Newtown
Series1
0.09
0.1

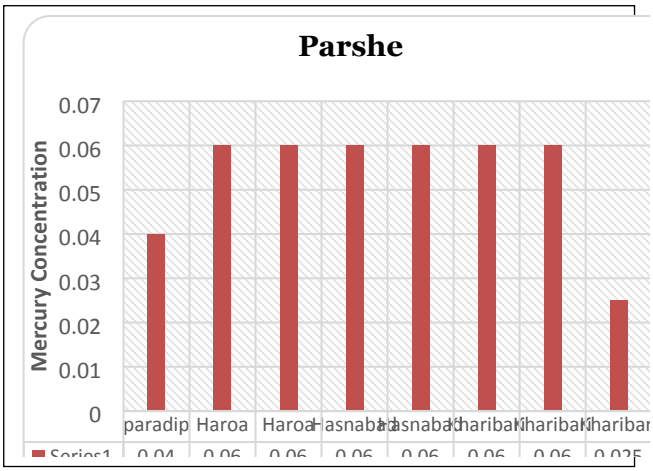

Shingi
Mercury Concentration
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Hasnabad
Hasnabad
Jainagar
canning


Shol
Mercury Concentration
0.06
0.05
0.04
0.03
0.02
0.01
0
Canning
Jainagar
Mednipur
Series1
0.05
0.025
0.05


Vetki
Mercury Concentration
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Bashirhat
Bashirhat
Kharibari
Malancha
Malancha
canning
Kh
Series1
0.07
0.07
0.13
0.06
0.06
0.1


Tangra
Mercury concentration
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0


Telapia
Mercury concentration
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Series1
0.025
0.05
0.05
0.1
0.1
0.05
0.05
0.12
0.08
0.08


Parshe
Mercury Concentration
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Haroa
Haroa
Kharibari
Kharibari

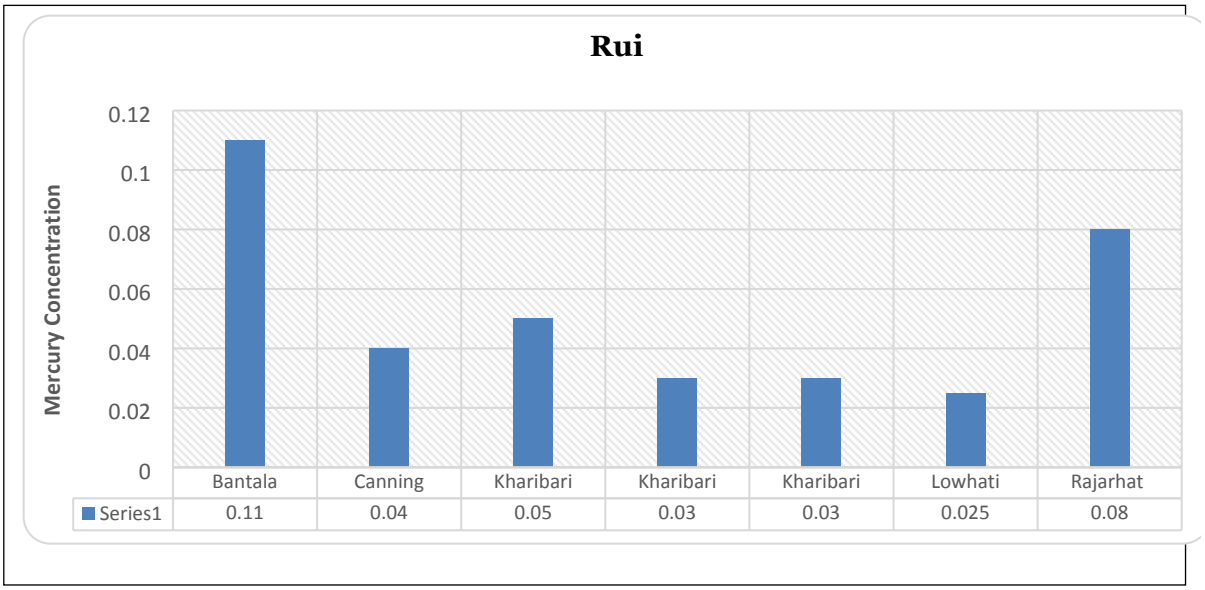
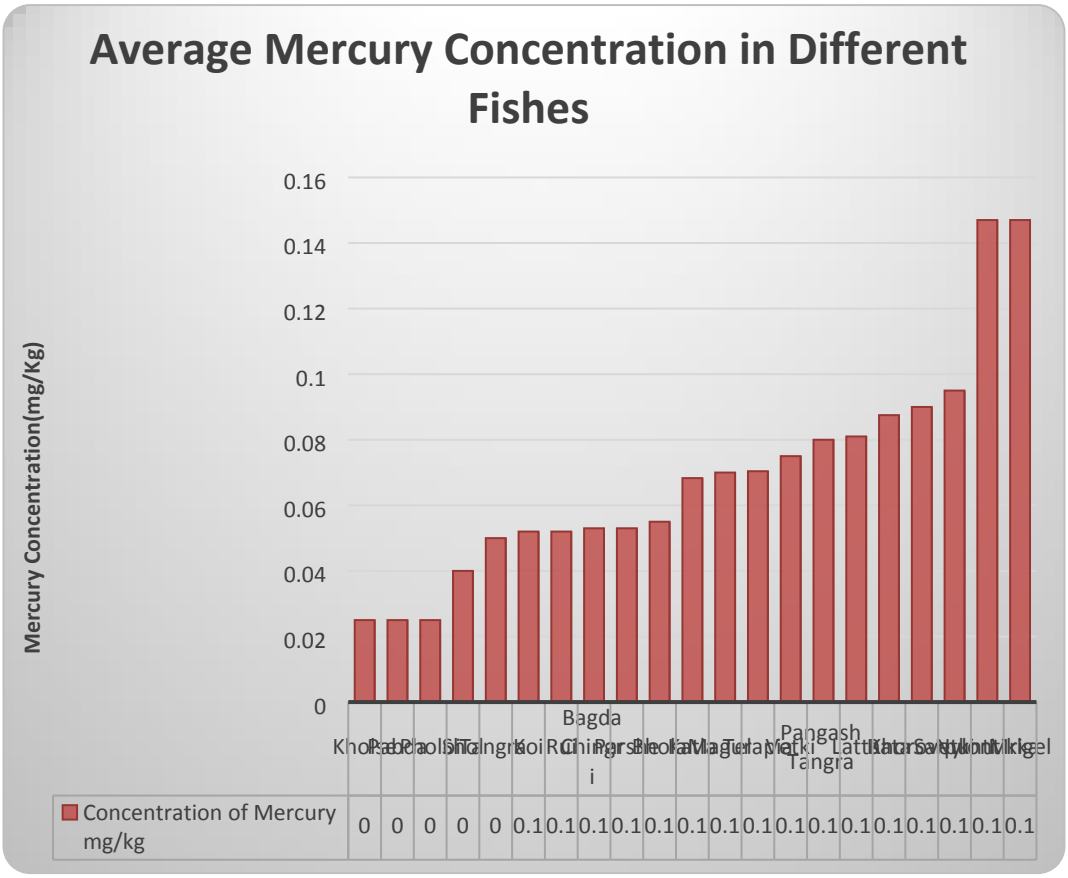

Rui
Mercury Concentration
0.12
0.1
0.08
0.06
0.04
0.02
0
Bantala
Canning
Kharibari
Kharibari
Kharibari
Lowhati
Rajarhat
Series1
0.11
0.04
0.05
0.03
0.03
0.025
0.08

Table 8: Average Mercury Concentration		
Sr. No	**Type**	**Concentration of Mercury mg/kg**
1	**Kholse**	**0.025**
2	**Pabda**	**0.025**
3	**Pholoi**	**0.025**
4	**Shol**	**0.04**
5	**Tangra**	**0.05**
6	**Koi**	**0.052**
7	**Rui**	**0.052**
8	**BagdaChingri**	**0.053**
9	**Parshe**	**0.053**
10	**Bhola**	**0.055**
11	**Katla**	**0.0683**
12	**Magur**	**0.07**
13	**Telapia**	**0.0704**
14	**Vetki**	**0.075**
15	**PangashTangra**	**0.08**
16	**Latta**	**0.081**
17	**Bata**	**0.0875**
18	**Khorovetki**	**0.09**
19	**Sarpunti**	**0.095**
20	**Nylontikka**	**0.147**
21	**Mrigel**	**0.147**



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Conclusion

That fish in kolkata have significant, and often alarming, levels of mercury contamination is evident from this study. Both the government and civil society should wake up to this problem. The Health and Environment Departments of the government should undertake a thorough investigation of the scale, intensity and sources of mercury pollution.

Not only fish, but water and soil samples as also blood and hair samples of the population need to be tested to judge the levels of contamination.

- Immediate release of advisories on fish consumption guiding citizens about relatively safe/unsafe fish species and sources.
- The scientific community should independently and in collaboration with the government, undertake such investigation.
- Once the sources of pollution are identified, efforts must be made to bring mercury pollution down to safe levels.
- Mercury and other pollutants of similar severity should be come an important item in civil society initiatives.
- Medical practitioners should include pollutant-induced pathology as a key item in their diagnostic and therapeutic procedures.

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19 .Fishing toxics: A study report by Toxic link and Disha

Extraction of Antioxidants from spices

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Abstract

Spices are an important part of our diet mainly because of their taste. They add a great taste to the prepared food. Since the ancient days spices and herbs are also known for their medicinal properties because they are used for treating various kind of illness like cough and cold, diabetes, common cold, gastric ulcer, different types of skin diseases and so on. In the present days, spices are available in different forms and different kinds of non permitted dyes are added to it mainly to protect its texture. These non permitted dyes are harmful and can cause serious illness if consumed regularly.The aim of this project is to estimate, extract and characterize the antioxidants and bioactive molecules from spices. Not only this, the presence of artificial colours are also tested in the spices. Six different spices were taken that we use in our daily life and they are also available easily in the market. They are taken as three different forms (the organic form, the whole spice and the powdered form available in the market) for the experiment.All the spices were treated with aqueous ethanol and polyphenols were estimated with the help of Folin Ciocalteau reagent. The absorbance are recorded using spectrophotometer. The presence of dye like sudan and rhodamine are tested with the help of ethyl acetate and methanol respectively. The functional group are also identified with the IR spectroscopy.

Keywords: Spices, Medicinal propertires, Folin CIocalteau reagent, Artificial colours

Introduction

An antioxidant can be defined as a molecule capable of inhibiting the oxidation of other molecules. Oxidation produces free radicals which can then start chain reactions which ultimately lead to damaging of cells. Antioxidants prevent these chain reactions by removing the free radical intermediates and also inhibit other oxidation reactions. Antioxidants are sometimes called “free-scavengers.” The source of antioxidants can be natural such as caretonoids or artificial such as BHT. The antioxidants are also produced in the body and they are known as endogenous antioxidants and the antioxidants that come from outside the body and they are known as exogenous antioxidant. Antioxidants can protect lipids and oils in food from oxidative

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degradation. When antioxidants are added to food they control the rancidity development, retard the formation of toxic substances. Antioxidants are often added to food to maintain the nutritional quality and to increase the shelf life. Natural antioxidants can be obtained from edible products such as spices and herbs, which are easily available. Oxidative stress, which is caused by the high concentration of free radicals can be reduced by natural antioxidants [1]. Natural antioxidants can be induced by various factors like UV rays, gamma rays, X-ray radiation, polluted food, psycho emotional stress, smoking, intensive physical extension, adverse environmental condition, alcoholism, drug addiction and so on. Oxidative stress is often linked to heart disease, cancer, stroke, arthritis, respiratory problems, immune deficiency. Parkinson's disease and so on[2].

A spice may be defined as a dried part of the plant which may be a root, leaf, bark which is primarily used for flavoring, colouring or preserving food substances. Not only these, spices also have other properties like antimicrobial properties. Spices can also be used in the manufacture of medicine, cosmetics, perfumes and so on. Spices are an important diet constituent all across the world. They are mainly used because of their flavour, but since the ancient times, spices and herbs are also known for their medicinal properties. Almost 80% of the world's population mainly depends exclusively on the plants and herbs for their medicinal properties for healing, where as in the developed world, preference Synthetic food colours are being used widely in food as well as in spices mainly in the powdered form to avoid the loss of original color and also to make the product attractive to the customer. Some food colours are permitted for use while some are not used. Some food colours may even cause serious illness. Some food colours that are permitted under the provision of Food Adulteration Act(1945), which includesis given more to the pharmaceutical medicine and surgery.

Three Red shades- Carmoisine, ponceau 4R, Erythrosine
Two yellow shades- Sunset yellow-FCF and Tartrazine
Two blue shades- Brilliant blue FCF, carmine
Green shade- Fast green FCF

But other than these permitted dyes some unpermitted dyes such as rhodamine, sudan III, IV, metanil yellow are commonly used in food industeies and scientific research. Scientific research include staining of lipid with sudan IV for visualization and analytical purpose. Sudan IV is commonly used as a food adulterant in red chilli powder and foods haivng red chilli like curry powder, meat masala, frozen mix and spice mixes. It is commonly used as it is cheaper and it gives an intense orange-red colour. Not only these, sudan dyes are also used in cosmetic products and animal testing and it is found that sudan III causes allergic reaction (International Agency for Research on cancer, 1975). These dyes can be an origin of allergic reaction, eczema, skin dermatoses (Jaskot and Costa, 1994) which affects liver, lungs and the vasculatory system, immune system and the reproductive system.

Another dye which is commonly used in food processing industries is rhodamine. It is banned by the Government of India as per PFA act(1954) beacaue it was found carcinogenic to humans.

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Not only carcinogenic but the dye also has some other serious harmful effect such as reproductive and developmental toxicity, neurotoxicity, and acute toxicity. It is used in food industries, sweet and confectionary items.

METHODS :-

Preparation of gallic acid calibration curve:

Gallic acid was used as a standard polyphenol to express total polyphenol content in the samples. For this purpose, a gallic acid calibration curve was constructed by taking a range of standard gallic acid concentrations from 0-100µg/ml.

Each of the standard gallic acid solutions were reacted with Folin-ciocalteau reagent sodium carbonate using the above mentioned procedure and absorbance was recorded at 760nm.

Preparation of spices for the extraction:

For the preparation of the extracts, about 100mg from each of the samples was finely crushed and homogenized and dissolved in 1ml of 50% aqueous ethanol(HPLC grade, Merck, Germany).

Then it was taken in graduated tube and centrifuged at 10000g for 5 minutes at room temperature. The supernatant was collected in fresh autoclaved tube and volume was made up to 1 ml with 50% aqueous ethanol.

Preparation of Sodium 10% Carbonate

To prepare 10%(w/v) sodium carbonate solution 1g sodium carbonate(SRL, Mumbai, India)was weighed and dissolved in 6 ml of distilled water.

The solution was shaken vigorously to dissolve the salt completely and volume was finally made up to 10 m.

The extraction of polyphenols

For determination of total polyphenol content, 50µl of plant extract was mixed with Folin-ciocalteau reagent and 750 µl of 10% sodium carbonate solution in a micro centrifuge tube.

All the tubes were wrapped with aluminium foils to avoid exposure to light. The mixture in each tube was shaken well and incubated at room temperature for 30 minutes in dark.

The absorbance was recorded at 760nm in UV-VIS spectrophotometer. Total phenolic content was expressed µg Gallic acid equivalent .

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Chemical test:

Test for rhodamine:

This test was carried out for the whole red chilli and red chilli powder.2 gm of sample was taken in a test tube and to that 5 ml of actone was added. Then the colour change was observed.

Test for rhodamine:

This test was also carried out for the whole red chilli and red chilli powder.½ spoon of the sample was taken in a test tube and to that 3 ml of distilled water was added and then 10 drops of carbon tetrachloride was added. The test tube was shaken vigorously so that the content mixes properly. The red colour disappears. And then dropwise HCL was added.

Test for lead chromate:

This test was carried out for turmeric powder.1 g of sample was taken in a test tube and to that 3 ml of distilled water was added, and then 10 drops of HCL was added.

Test for Sudan III,IV:

This test was carried out for whole red chilli and red chilli powder.1 g of sample was taken in a test tube and to that 2 ml of hexane was added and then it was shaken well. After it was settled down, the upper layer was decanted in another test tube. 2 ml of Aceto nitile reagent was added and then it was shaken well. The colour change was observed0.1 g of sample was taken in a test tube and to that 1 ml of hexane was added and kept to dissolve. After that 5-10 drops of HCL was added to the sample. The colour of the sample was observed.

Test for Metanil yellow:

0.1 g of sample was taken in a test tube and to that 1 ml of hexane was added and kept to dissolve. After that 5-10 drops of HCL was added to the sample. The colour of the sample was observed.

IR spectroscopy

IR spectroscopy consists of the following steps:

About 50mg of KBr was added to 5 mg of the sample and was mixed properly.

After that 7-10 ton(about 8 ton) of pressure is applied manually for a time period of 1 min, then a disc like structure is formed.

And then the absorbance is measured.

Transmittance is changed into the absorbance mode to check the result

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RESULT

$V_1S_1=V_2S_2$
S_1= 1 mg/ml
S_2= (150,300,450,600,750,900)
V_2= 1ml

Table1 : Stock solution preparation of gallic acid:

Sl No.	***V1***	***S1 (mg/ml)***	***V2 (ml)***	***S2 (microgram/ml)***	***Ethanol (ml)***	***Concentration***
1.	0.15	1	1	50	0.85	150
2.	0.30	1	1	100	0.7	300
3.	0.45	1	1	150	0.55	450
4.	0.60	1	1	200	0.4	600
5.	0.75	1	1	250	0.25	750
6.	0.90	1	1	300	0.1	900

Table2 : Optical Density of Gallic Acid :-

Sl No.	***Concentration Of Gallic Acid (microgram/ml)***	***Optical Density(OD)***
1.	150	0.280
2.	300	0.485
3.	450	0.620
4.	600	0.760
5.	750	0.933
6.	900	1.220

Table3 : The absorbance of spices recorded at 760nm:

SPICES	ABSORBANCE

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Whole Black Pepper	0.973
Organic Black Pepper	0.863
Local Black Pepper	0.518
Whole Cinnamon	2.420
Organic Cinnamon	1.998
Local Cinnamon	0.903
Whole Cumin	0.650
Organic Cumin	0.985
Local Cumin	0.828
Whole Turmeric	1.573
Organic Turmeric	0.995
Local Turmeric	0.497
Whole Red Chilli	0.934
Organic Red Chilli	1.085
Local Red Chilli	0.995
Whole Coriander	0.318
Organic Coriander	0.858
Local Coriander	0.620

After plotting the calibration curve, OD vs Concentration , the concentration of root and shoot tissues were found out from the curve by plotting their respective OD.

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Table 5: Spices Vs Concentration

Spices	Concentration
Organic , Local Black pepper	751 , 325
Organic , Local Cinnamon	955, 752
Organic , Local Cumin	820, 655
Organic, Local Turmeric	835, 323
Organic, Local Red chilli	855, 880
Organic, Local Coriander	751, 450

Calculation :-

$$C = C1 * (v/m)$$

C= total phenolic content in microgram/g.

C1= Concentration of gallic acid calculated from the calibration curve or standard curve of Gallic acid in microgram/g.

v = Volume of extract in ml.

m = weight of the spices in gram.

Total phenolic content of

Organic Black pepper is 0.03004 microgram/g. **Organic Cinnamon** is 0.0382 microgram/g. ; **Organic Cumin** is 0.0328 microgram/g. ; **Organic Turmeric** is 0.0374 microgram/g ; **Organic Red chilli** is 0.0342 microgram/g. ; **Organic Coriander** is 0.03004 microgram/g. ; Local Black pepper is0.0130 microgram/g. ; **Local Cinnamon** is 0.03008 microgram/g. ; **Local Cumin** is 0.0262 microgram/g.**Local Turmeric** is 0.01292 microgram/g. ; **Local Red chilli** is 0.0352 microgram/g. ; **Local Coriander** is 0.0180 microgram/g

Table**4: CHEMICAL TEST :-**

Spice	Adulterant	Rapid test	Observation	Interference
Whole red chilli & red chilli powder	Rhodamine	2 gm of sample was mixed with 5 ml of acetone		Immediate appearance of red colour in red chilli powder indicates the presence of dye

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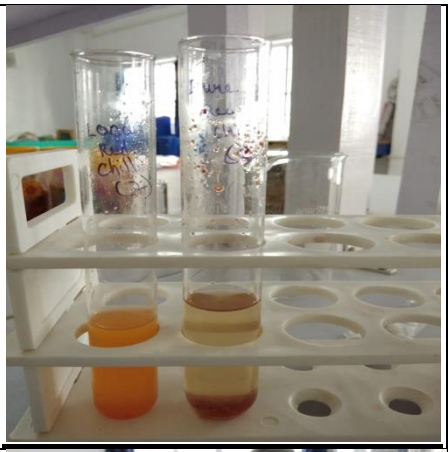
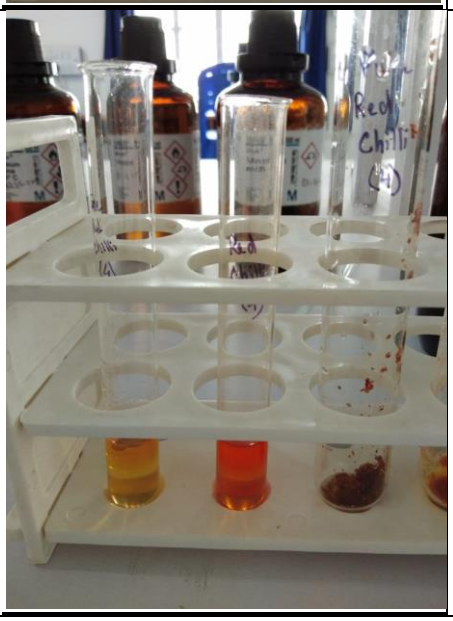
Whole red chilli & chilli powder	Rhodamine	½ spoon of sample was mixed with 3 ml of distilled water. Test tube was shaken vigorously		The reddish appears after addition of HCL, indicates the presence of dye
Whole red chilli & red chilli powder	Sudan III,IV	1 gm of sample +2ml of hexane. Upper layer was decanted and 2 ml of acetonitrile reagent was added and shaken		Appperance of red color after addition of acetonitile reagent indicates the presence of dye

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Turmeric powder	Lead chromate	1g of sample+ 3 ml of distiiled water and 10 drops of HCL		The absence of pink color indicates the the absence of lead chromate in the sample.

Conclusion

From the experimental work, it is seen that red chilli contains the highest amount of antioxidant. Cinnamon and red chilli also contains huge amount of antioxidants. It is better to use the whole form of spice or the organic ones as they are less likely to contain unpermitted dyes which are not only harmful but also carcinogenic. Spices should ben important part of our life and to be consumed not only for their taste but also for their enormous health benefits.

From the absorbance it can be said that the organic form of the spice contains more antioxidant than the other forms and so is the purity. From the chemical test it is confirmed that the local powder form of the spice contain dyes like rhodamine and sudan III,IV, where as the local turmeric powder is free from lead chromate, which is dangerous in nature, though there is a possibility of the presence of other dyes in minor amount.

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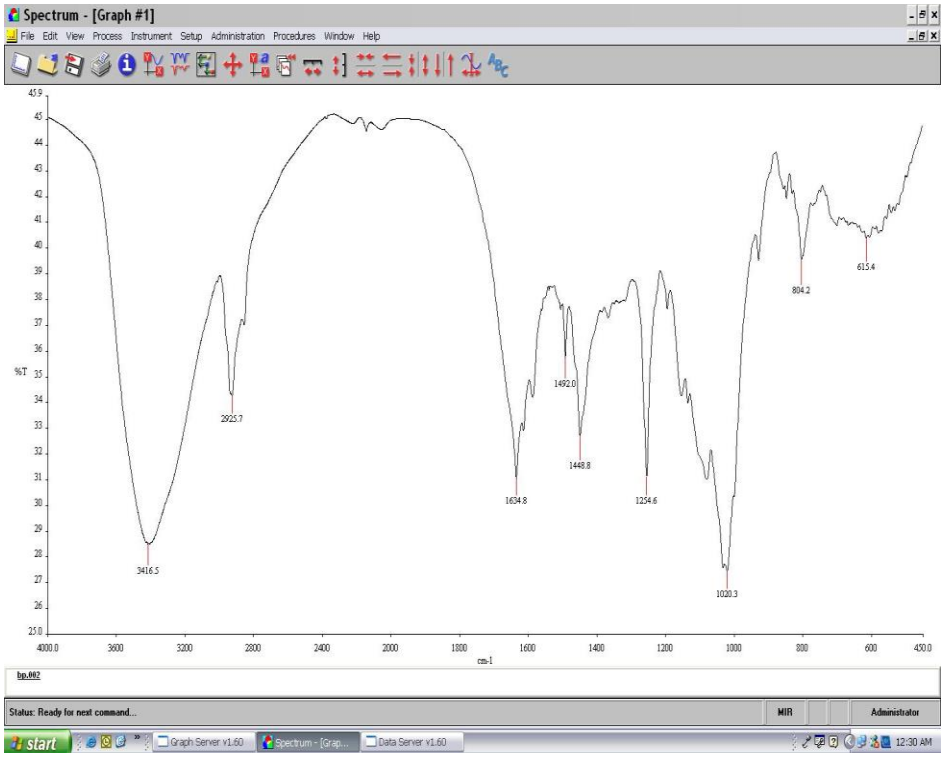
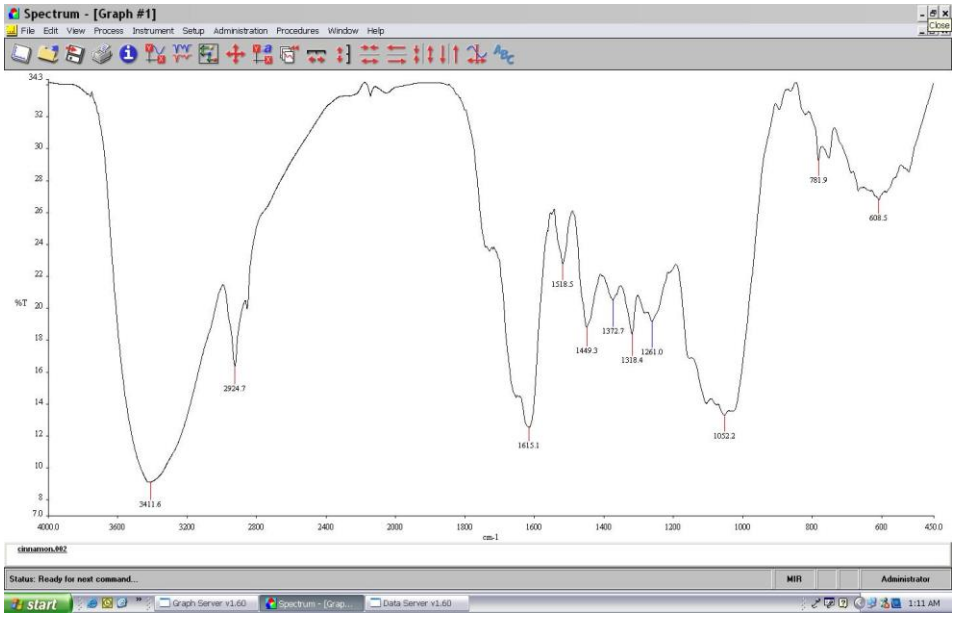
5. Infrared Spectrometry observation :-

5.1 IR Graph of Black pepper :-



5.2 IR Graph of Organic Cinnamon :-



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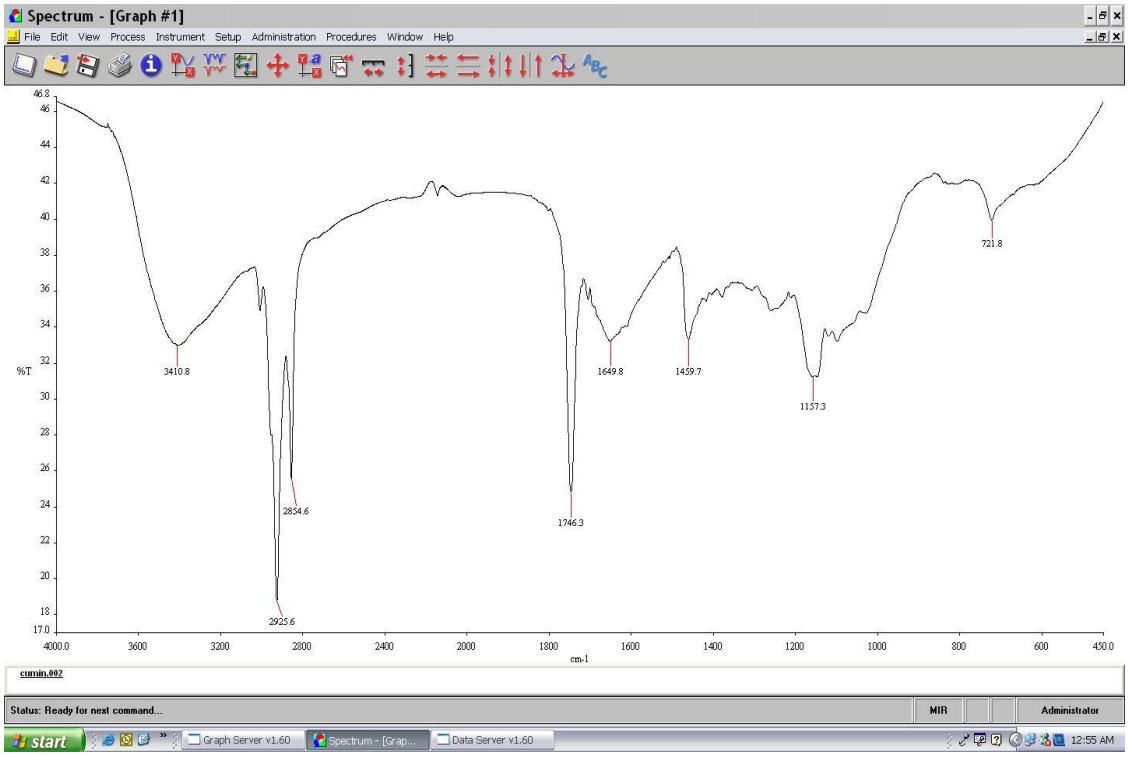
5.3 IR Graph of Organic Cumin :-



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