IMPACT OF POLLUTANTS ON ECOSYSTEMS AND HUMAN HEALTH

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PREFACE

The book contains a compilation of peer-reviewed papers presented at the 5th International Conference on Ecotoxicology and Environmental Sciences (ICEES-2016), held in Kochi, India in February 2016, sponsored by the World Academy of Sciences, Italy. Like the four former meetings the first one of which took place in 2007, The Institute of Ecotoxicology and Environmental Sciences based in Kolkata organized the ICEES-2016 international event. After taking great care in the compilation process, the Institute is proud to present this edited volume to our readership, containing 14 peer-reviewed articles from over 50 papers presented at the ICEES-2016 international conference. International experts authored five of these chapters.

Environmental Ecotoxicology is a multidisciplinary field of science concerned with the study of harmful effects of various biological and physical agents on living organisms at the population and ecosystem level. In ecosystems, all organisms (animals, plants and microbes) and compartments (air, water and soil) are mutually interconnected and form a system together. The sources of environmental contaminants are diverse and they are largely generated by human activities such as waste product discharge in a solid, liquid or gas form into the atmosphere, soil or surface water from industrial factories, agricultural products, incinerators, sewage plants, etc. Many of these contaminants are known to harm the ecosystems and adversely impact human health. A substantial numbers of these pollutants are known mutagenic, teratogenic or carcinogenic substances, or act as endocrine disruptor, harm the immunosystem, or have other effects. The damaging effects may not only be temporary but may also have long-term effects, causing also problems for next generations.

In this volume, the authors address a variety of issues concerning the adverse impact of contaminants on ecosystems and human health. Identification, management, reduction of contaminants, discharge from sources as well as pollutant effects on environment and health are covered, while remediation, reutilization of waste products as well as regulations and monitoring the release of waste materials into the environment are also included.

Because of the complexity and multidisciplinary nature of the subject, both national and international collaborations are relevant in order to exchange ideas among experts on ecotoxicology and related subjects. International conferences like the one organized by The Institute of Ecotoxicology and Environmental Sciences in Kochi in 2016 do not

only enhance interest in ecotoxicology research but also foster national and international scientific collaborations. The organizers of the next ICEES-20018 conference in New Delhi, India in February 2018 are planning to involve more international experts in the areas of ecotoxicology and environmental sciences.

The book will be of interest to a wide audience including ecotoxicologists, environmental scientists, professionals, educationists, undergraduate and graduate students as well as interested public since adverse effects of environmental pollutants on ecosystems and human health are universal problems. The editors express their appreciation to the contributors for their quality work and cooperation. Gratitude is extended to The Institute of Ecotoxicology and Environmental Sciences for organizing the Kochi event and to Ms. Shreyasee Roy of St. Xavier's College (Autonomous), Kolkata, Mrs. Aditi Bose of Jadavpur University, Kolkata and Dr. Sarmila Pal of Hooghly Hohossin College, Hooghly, West Bengal who were directly or indirectly involved in the preparation of this volume.

Any findings, opinions, conclusions, or recommendations expressed in the book are those of the authors and do not reflect the views of the editors or The Institute of Ecotoxicology and Environmental Sciences. Likewise, the editors and the Institute cannot take any responsibility or liability for errors and misrepresentation of data this book may contain.

Prof. Badal Bhattacharya, Ph.D.

Prof. Jacob de Boer, Ph. D.

Prof. Pasquile Avino, Ph. D

18th January, 2018

CONTENTS

CHAPTER - I

ASSESSMENT OF MEDIAN LETHAL CONCENTRATION (LC_{50} 96 h) AND BEHAVIOURAL MODIFICATION OF NONYLPHENOL IN THE CICHLID FISH, *Etroplus maculatus* (BLOCH, 1795)

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ABSTRACT

Nonylphenol is a toxic xenobiotic compound widely used as an element of detergents, paints, pesticides, pulp and paper processing, plastics and personel care products. In the present study median lethal concentration (LC_{50}) of nonylphenol in the cichlid fish *Etroplus maculatus* was determined by probit analysis. Eight different concentrations of nonylphenol (0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.5 mg/L) were exposed to fish with ten animals per group for 96 h maintaining a control group. Mortality of the fish in each group was monitored throughout the experiment and the results of probit analysis indicated that the percentage of mortality is positively correlated (r = +0.94) against the concentration of nonylphenol. The result showed that 0.89 mg i.e., 890 µg/ L of nonylphenol kills 50% of the exposed fish. In the experiment, the body weight of all treated groups remained unchanged as compared with the control group. However, mucous deposition was significantly increased in nonylphenol-treated groups in dose-dependent manner. The movement and the behaviour of the fish were closely examined throughout the study. Fish exposed to different concentrations of nonylphenol showed slow and restricted movements, loss of equilibrium, decreased opercular movements and hemorrhage in gills and throughout the body surface. In conclusion, nonylphenol at 890 µg/L is considered as 96 h LC_{50} value and the modification in normal behavioural pattern due to nonylphenol can be used as an indicator of ecologically relevant monitoring of environmental contamination.

KEY WORDS: Nonylphenol, Median lethal concentration, *Etroplus maculatus*, Probit analysis, Behaviour

1. INTRODUCTION

Nonylphenol (NP) is the commercially most important member of the group of alkyl phenols and has large number of isomeric compounds which are varying in the point of

attachment of the nonyl group to the phenol molecule and in the degree of branching in the nonyl moiety. NP is used as a starting material in the synthesis of nonylphenol ethoxylates (NPEs), and as a monomer in polymer production. NPEs are non-ionic surfactants and used in detergents, personal-care products, textile processing, agricultural chemicals, pulp and paper processing, metal and mineral processing, latex paints, wetting agents, emulsifiers, foaming agents, inks, adhesives, and pharmaceuticals. The breakdown of nonylphenol ethoxylates in the environment may give rise to significant quantities of nonylphenol. During degradation, NPE's ethylene oxide units are cleaved off and leave several degradation products such as short-chain ethoxylates, their carboxylic acids, and nonylphenols. As for NP, the major part (85%) is released to water, while the release to soil amounts to 13%, and to air 2.5%. The main routes of calculated exposure of humans to background pollution from the environment are from plant roots (70 to 80%) and fish (1 to 29%). It is clear from the available data that nonylphenol bioconcentrates to a significant extent in aquatic species, with bioconcentration factors (BCFs) of up to 1,300 in fish (US-EPA, 1996).

The occurrence of nonylphenol in the environment is clearly correlated with anthropogenic activities such as wastewater treatment, landfilling and sewage sludge recycling. Thus nonylphenol is found often in different environmental compartments such as surface water, sediment, groundwater, soil, air, sewage sludge and effluents from sewage treatment works. Due to its low solubility and high hydrophobicity, nonylphenol accumulates in these matrices and where it persists (Soares et al., 2008). A wide range of acute and chronic studies has been reported for nonylphenol exposure in aquatic species. Fish are considered as the most susceptible species to pesticide after being exposed through gills, skin or food. In the present study *Etroplus maculatus*, an indigenous cichlid fish was used as an experimental model to evaluate the median lethal concentration of nonylphenol for 96 h by using probit analysis method. In aquatic ecotoxicology the nexus of behavioural sciences with the study of toxicants have become predominant in recent years. Therefore, the study also incorporates the behavioural endpoints because there was a lack of understanding of behavioural modification with respect to ecologically relevant issues such as growth, prey capture, stress resistence, reproduction and longevity. Behavioural endpoints in aquatic toxicology consequently provide a valuable tool for toxicity assessment of nonylphenol in *Etroplus maculatus*.

2. MATERIALS AND METHODS

2.1. Animal

The cichlid fish, *Etroplus maculatus* weighing 7.5 ± 1.5 g and length 7.5 ± 1.5 cm were collected from fish farm, KKF Nursery, Manjeri, Vaniyambalam, Malappuram District, Kerala. Fishes were acclimatized to the laboratory conditions prior to experiments and were exposed with constant supply of water and good lighting system. They were maintained in well-aerated aquarium tanks (40 L capacity) and was dechlorinated.

2.2. Preliminary tests

The physico-chemical features of the tap water were estimated as per APHA (1998). Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100%, pH was 7.6 which were monitored using a standardized procedures.

2.3. Chemical

Technical grade Nonylphenol, 4-(2, 4-dimethylheptan-3-yl) phenol of 97% purity was purchased from SISCO Research Laboratories Pvt. Ltd., Mumbai, India.

2.4. Evaluation of median lethal concentration (LC_{50}-96 h)

The LC_{50} value in 96 h time interval was determined by probit analysis, with a confident limit of 5 % level (Finney, 1971). The fishes were not fed a day prior to and during the test to reduce fecal and excess food contaminating the test solution. Five specimens were placed in each tank of replicates so that ten fishes were maintained in each test and aerated using tubed motorized pumps. Monofilament netting was used to cover the tanks to prevent the specimens from jumping out of test solutions. For determining LC_{50} concentration of nonylphenol, separate aquarium tanks of 40 L of water capacity with 10 animals were taken and eight different concentrations (0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.5 mg/L) of nonylphenol were added. Control tub with 40 L water capacity having 10 fishes were maintained along with treatment groups without the addition of toxicant. The lethal concentration for 50 % killing (LC_{50}) values was computed on the basis of probit analysis (Finney, 1971) for 96 h exposure. The mortality as well as behaviour of fishes in each group was monitored throughout the experiment at varying concentrations.

2.5. Statistical Analyses

Median lethal concentration or 96 h-LC50 value were analyzed with SPSS statistical analysis software (Version 19.0) using Probit Analysis Statistical Method. The LC50 values (with 95% confidence limits) were calculated using MS Excel 2007, the correlation between mortality on Y-axis and concentrations on X-axis and the best-fit line was also obtained.

3. RESULTS AND DISCUSSION

In the present study each group of fishes were exposed to different concentrations of nonylphenol for 96h. In the experiment, the body weight of all treated groups remained unchanged as compared with the control group, but when the weights observed along with mucous secretion showed significant increase ($P<0.05$) in dose-dependent manner than that of control groups (Figure. 1 and 2).

Different concentrations of nonylphenol showed different percentage of mortality at different time interval as shown in Table 1. Mortality of the fishes in each group were continuously monitored throughout the experiment and it was observed that 0.5 and 0.6 mg/L concentrations of nonylphenol did not caused mortality as that of control group. Nonylphenol at concentrations, 0.7, 0.8, 0.9, and 1mg/L showed death of 2, 4, 5 and 6 animals respectively at the end of 96 h exposure. All fishes were killed at 1.2 and 1.5 mg/L concentrations after 24 and 5 h of nonylphenol treatment. The results of probit analysis indicated that the percentage of mortality is positively correlated ($r = +0.94$) against the concentration of nonylphenol which shows high degree of positive correlation.

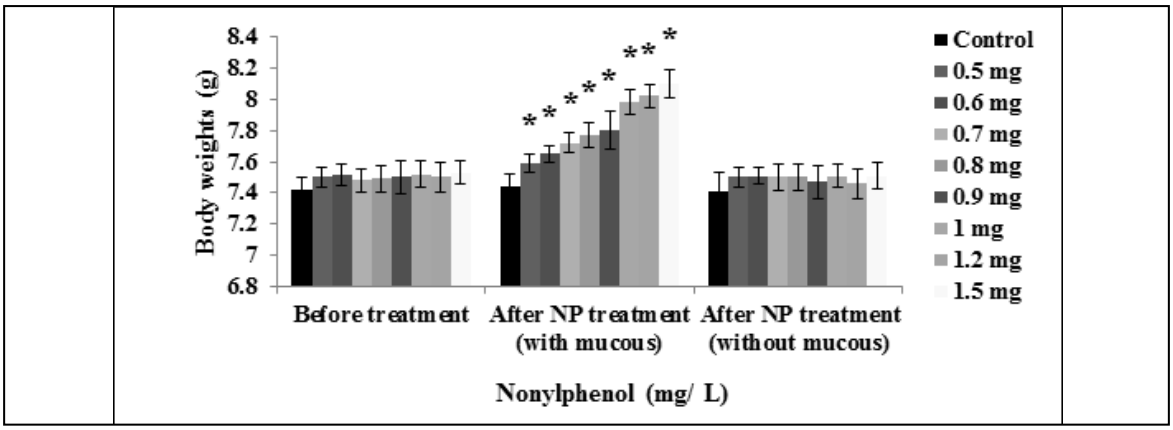

Figure. 1: Effect of nonylphenol on the body weight of fish, *Etroplus maculates*

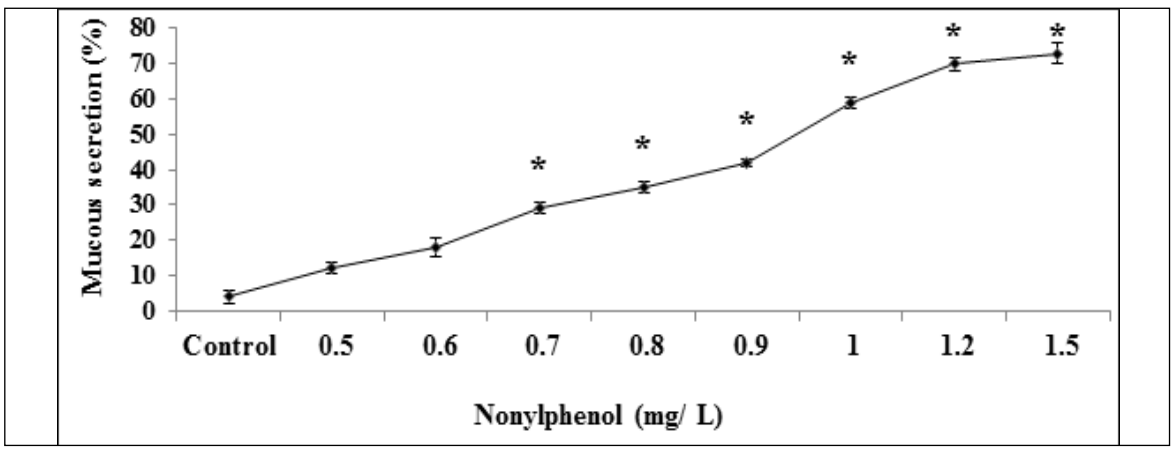

Figure. 2: Effect of nonylphenol on mucous secretion in Etroplus maculates

Table 1: Percentage of fish mortality exposed to different concentrations of nonylphenol in cichlid fish, *Etroplus maculatus* for 96 h

Concentrations (mg/L)	Mortality (%)	Hour of mortality
0.5	0	96
0.6	0	96
0.7	20	96
0.8	40	96
0.9	50	96
1	60	96
1.2	100	24
1.5	100	5

Table 2: Probit analysis of 95% confidence limits for effective concentrations of nonylphenol in *Etroplus maculatus*

Prob	Concentration (mg)	95% Confidence Limits	
		Lower	Upper
.01	0.49378	0.00802	0.67244
.02	0.54045	0.09835	0.70595
.03	0.57005	0.15541	0.72745
.04	0.59233	0.19818	0.74379
.05	0.61044	0.23286	0.75718
.06	0.62586	0.26228	0.76868
.07	0.63938	0.28801	0.77884
.08	0.65149	0.31097	0.78800
.09	0.66250	0.33180	0.79639
.10	0.67263	0.35091	0.80416
.15	0.71459	0.42942	0.83701
.20	0.74794	0.49088	0.86404
.25	0.77655	0.54274	0.88810
.30	0.80225	0.58847	0.91056
.35	0.82605	0.62994	0.93226
.40	0.84865	0.66833	0.95382
.45	0.87050	0.70442	0.97574
.50	0.89002	0.73874	0.99850
.55	0.91353	0.77173	1.02259
.60	0.93538	0.80372	1.04860
.65	0.95798	0.83506	1.07721
.70	0.98178	0.86612	1.10934
.75	1.00748	0.89740	1.14624
.80	1.03609	0.92968	1.18987
.85	1.06944	0.96437	1.24368
.90	1.11140	1.00440	1.31500
.91	1.12153	1.01358	1.33271
.92	1.13254	1.02337	1.35214
.93	1.14465	1.03394	1.37370
.94	1.15817	1.04551	1.39800
.95	1.17359	1.05846	1.42597
.96	1.19171	1.07336	1.45915
.97	1.21398	1.09128	1.50033
.98	1.24358	1.11455	1.55563
.99	1.29025	1.15020	1.64381

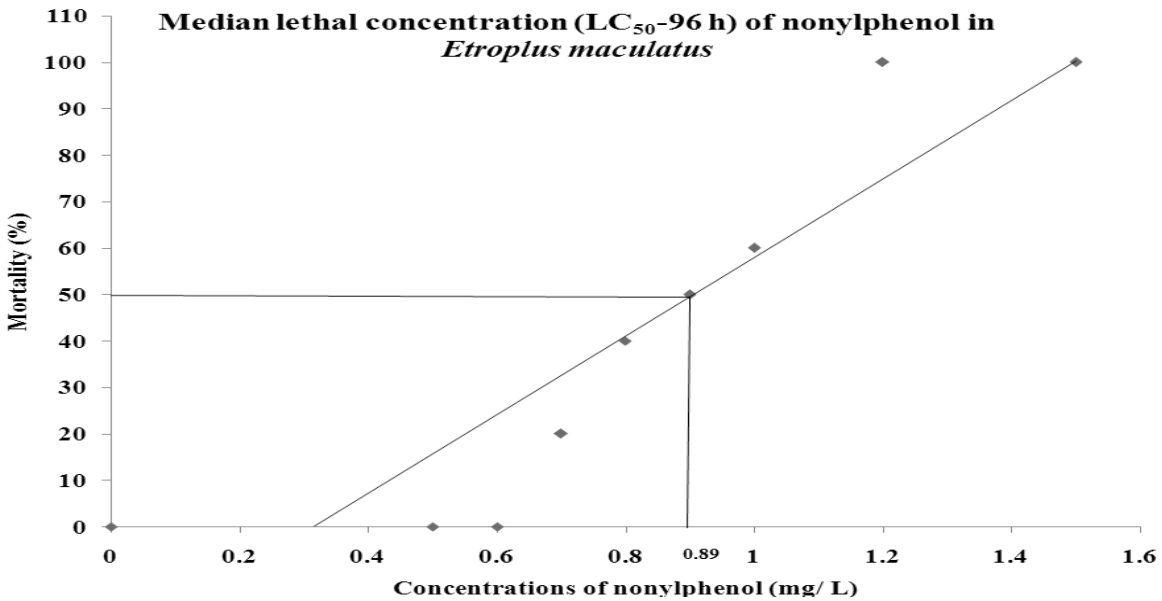

Figure. 3: Median lethal concentration (LC_{50}-96 h) of nonylphenol in *Etroplus maculates*

The median lethal concentration (LC_{50}) of nonylphenol was computed on the basis of probit analysis for 96 h exposure, which is 890 µg/L (Table 2 and Figure. 3). The median lethal concentration (LC_{50}) value of nonylphenol in male and female Medaka were reported as 0.85 and 0.87 mg/L, respectively for 72 h (Kashiwada et al., 2002). However the LC_{50}-96 h of nonylphenol in *Oreochromis mossambicus* was determined as 1.5 mg/L by probit analysis (Balakrishnan et al., 2014).

Aquatic behavioural toxicology is the recently growing and recognized field in toxicology that provides perspective link to the physiology and ecology of an organism and its environment. Behavioural endpoints may often contribute an additional utility or biologic significance to morphologic and physiologic adaptation of an animal in the ecosystem. Behaviour also allows an organism to adjust to external and internal stimuli in order to meet the challenge of surviving in a changing environment. Fish models are widely used in behavioural studies as it offers important indices for ecosystem assessment. In the present study the behaviour of fish was continuously monitored in all treatment groups. Control fish maintained in toxicant-free water were found to be active throughout the experiment. Nonylphenol exposure irrespective of varying concentrations showed abnormal behaviour in fishes when compared to control group. Immediately after the toxicant exposure fish showed aggressive swimming for few hours as the toxicant triggers the stimulus response by avoiding the area containing the contaminant. Since the animal was maintained in tanks, the fishes are unable to avoid the toxicant area which would resulted in slow and restricted swimming activity with decreased rate of opercular movement thereby altering respiratory pattern.

After 24 h of nonylphenol exposure body surfaces become reddened and hemorrhagic, unable to maintain normal posture or equilibrium and large amount of mucous secretion were also observed. As a result, fishes become lethargic and at the time of death they exhibited transient hyperactivity before collapsing. Similar results were observed by different

researchers in different fish species in response to various toxicants. Erratic activity followed by restricted movements, haemorrhage in body surface, reddening of fins and finally loss of equilibrium in *Etroplus maculatus* were observed after bisphenol A exposure (Asifa and Chitra, 2015). Therefore, behavioural toxicology testing provides biologically relevant endpoints to evaluate sub lethal effects of nonylphenol and may compliment traditional toxicity testing.

4. CONCLUSION

The 96 h median lethal concentration of nonylphenol (LC_{50}-96 h) by probit analysis for Etroplus maculatus was determined as 0.89 mg/L (890 µg/L). The study gives further evidence that assessment of fish behavior can constitute a highly sensitive method to indicate the toxic effect of nonylphenol.

5. ACKNOWLEDGEMENT

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FATE, MOBILITY AND TOXICOKINETICS OF NANOPARTICLES IN THE ENVIRONMENT

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ABSTRACT

Use of nanoparticles (NPs) has gained a momentum in recent days due to their unique range of properties. NPs find wide applications ranging from commercial products to medicine and cosmetic, adsorbent to catalyst, missile to agriculture. Newer Nps are being engineered, as material of demand, to explore further applications utilizing the novel properties seen at the nanoscale following nanotechnology. Thus, nanotechnology is a fast growing, interdisciplinary field of research covering engineering, physics, chemistry and biology.

With their quantum of usage a proportionate quantity of NPs also put into the environment via water and soil. It is worth to assess the fate of NPs in the environment, whether these may pose any threat to the environment. Depending on their behavior, if they develop any toxicity, the level is very crucial to determine.

It is again of great concern that whether the remarkably small size of NPs facilitates their entry in the living cells. In such a situation their reactivity in biological matrices is expected to enhance leading to harmful physiological effects. Both the manufacturers and consumers of NPs may become susceptible to health disorders. Moreover, discharged NPs, both crude and degraded, if persist in the water-soil compartments may damage the environmental quality and even enter the food chain. The release as well as dispersion and dissolution ability of NPs are the most critical factors developing and controlling their toxic properties. The kinetics of movement from water to biota is expected to play the decisive role for manifestation of NPs toxicity.

1. INTRODUCTION

The present day research on science and technology is mainly for the design and synthesis of useful materials including nanoparticles (NPs). The range of applications of NPs is considerably wide covering different sectors such as energy, paint, lubricant, optics, food, medicine and cosmatics. As per the US National Science Foundation (NSF) market for nanotechnologies estimated as $700 billion on 2008 will be more than $1 trillion within 2015. With such huge production a proportionate amount of NPs come in contact with

several environment compartments such as air, water and soil as well as sediment. NPs according to their origin may be classified as natural, incidental and engineered [1].

Among the common natural NPs soil colloids and airborne nanocrystals of sea salts are most important. Incidental nanoparticles may be obtained during grinding of primary or secondary minerals, from wear of metal or mineral surfaces or combustion of fossil. Smoke and fire from volcanoes and carbon black also contribute NPs to environment. Engineered nanoparticles (ENPs) synthesised as per design and need are particularly important to study in terms of their fate, mobility and ecotoxicity due to their use for multiple purposes.

The environmental compartments such as air, water and soil may act as both the source and sink of NPs. Both indoor and outdoor air containing NPs may pose a threat to health. Sometimes gas transformation of atmospheric/stratospheric ozone, NOx, etc. via catalytical action of NPs may become the prime cause for air quality degradation. Content of natural NP in water is mainly from organic and inorganic colloids, mostly originating from soil. The discharge of synthesised NPs as well as degraded materials is expected to increase the NP load enormously in water. Soil contains huge amount of natural NPs. Soil colloids are constituted of silicate clay minerals, iron- or aluminum oxides/-hydroxides and humic organic matter, including black carbon. Weathering and re-arrangement of soil constituents and biological transformation of dead organics, humic acids and minerals increase the load of NP. High porosity and extremely high specific surface area (tens to hundreds of square meters per gram) together with charged and hydrophobic adsorption sites of soil/sediment induce interactions of NP with all introduced dissolved (solutes) and suspended (particles) materials.

Mostly synthesized NPs are disposed to soil, sediment and aquatic systems. The fate, residence character and behavior of NP in different environment compartments are of very crucial importance. A large amount of nanomaterials synthesised may come in contact with the environment, either during production, transport, use or when they end up as waste. The NPs ultimately may put some stress or hazard to the environment and the habitats. The risks associated with NPs arise as a combined role of mobility, transport and transformation as well as load in the concerned matrix [2].

The NPs compared to the larger particles show high chemical reactivity due to large relative surface area and increased surface to volume ratio. It is estimated that while surface of a 30 nm particle contain 5% of its atoms a 3 nm particle has 50% of its atoms on the surface [3]. Therefore, for a given mass of material the atoms availability is much higher in NPs than in larger particles. Thus NPs become more reactive and interact with the environment more efficiently than the larger particles [4]. The possible consequences are the changed electrical properties and strength. Moreover, with the reduced size, to a few tens of nanometers, the material properties are dominated by quantum effects. As the electrons become confined the electrical, magnetic and/or optical properties of material are altered [5]. However, extent and nature of such changes in particle behavior with reduced size depend on the kind of NP, viz. elemental metal NP (e.g., Ag(0), Au(0), Cu(0)), metal oxide NP (e.g., TiO_2, Al_2O_3, CeO_2, ZnO), quantum dots and carbon-based NP (e.g., carbon nanotube and fullerene). Therefore, different NPs need to be studied differently.

2. **FATE**

The important parameters that may govern the fate of NPs in the environment are chemical composition, surface charge, surface area and ability for redox transformation. Depending on the surface charge and due to high specific surface area some NPs are expected to adsorb both inorganic and organic pollutants. Thus, NPs may generate induced toxicity influencing mobility, bioavailability and degradation of some potentially harmful substances viz. heavy metal, polyaromatic hydrocarbon and so on. Wiesner et al. proposed that redox reactions may influence the transformation and fate of engineered nanoparticles [6]. Precipitation, dissolution and mobility of metals as well as degradation of organic matter are influenced by redox condition of the matrix. So, fate of NP is determined by transformation and persistence. Persistence has a great impact on the behavior of a particular NP. The persistent NPs sometimes develop toxicity or the degraded residues of NPs may show toxicity and result long term environment quality degradation. So degradation kinetics has much influence determining the toxicity level. Again, the persistence may be directly correlated with the level of exposure that results into hazard. It is demonstrated that carbon nanotubes (CNTs) and fullerenes, are extremely persistent even under high temperature [7], in presence of strong acid and resist photolytic/ozonation attack [8]. Metal oxide NPs such as Zn, Al and Fe facilitate mechanical degradation of aggregates in soil. However, organic (surfactant) coated NPs are easily degradable in the environment. Thus, these kinds of NPs easily release their core particles upon such degradation. Roberts et al assessed the possible release of some NPs from lipid coated CNTs during ingestion by the crustacean *Daphnia magna* in the intestine [9].

3. **MOBILITY**

Mobility of NPs includes transport, transfer and passage from one environmental compartment to other (soil to water and vice-versa) and one environmental compartment to organism (water to microorganism or plant). The rate of transfer in biotic and abiotic environment determines their availability and hence the possible interaction with the matrix constituents. Moreover, the possible modification on interaction with other phases also governs the mobility. Sometimes deposition and aggregation (inter and intra) influence the particle transport. While deposition varies with the surface nature, aggregation depends on the nature of particle itself. The stability of particle itself and its suspension is very important determining its mobility. A destabilized particle suspension has a higher tendency to aggregate and deposit to some suitable surface [10]. Several factors such as surface potential, solution pH and ion concentration, nature and concentration of stabilizing agents (surfactants or coatings), etc. [11] influence the stability and mobility of NPs. The matrix nature also has some direct influences on mobility of NPs.

Contrary to water, soil and sediment act as porous media that are likely to constitute natural barriers against transport and remobilization of NPs. The extent of NP mobility in such matrices depends on porosity, surface charge, nature of matrix, composition of matrix, reactivity of each matrix constituent and the prevailing physico-chemical conditions. The fate and bioavailability of NPs dispersed in these systems thus strongly depend on the filtering properties resulting from these conditions [12]. The organic and mineral content

as well as complex structural composition of natural matrix need elaborate investigation for determining transport mechanism and residence behavior of nanoparticles under natural conditions. A variety of operations may proceed simultaneously with different rates. The predominance of any mechanism depends on the nature of the matrix. Along with dispersion and permeation sometimes adsorption onto solid and dissolved matter that present in soil, sediment and water becomes prominent. In some other situation binding of polar NPs to hydrophilic constituents of matrix and non polar NPs to hydrophobic constituents of matrix prevail. Thus fullerenes and carbon nanotubes (CNTs), being non-polar do not easily disperse or dissolve in water. Among the several environmental factors pH and ionic strength [13, 14] govern the mobility.

The possibility, extent and pathway of NPs mobility i.e whether they are bound within or transported out of soils and sediments largely depend on the physico-chemical properties, structure and concentration of NPs [15]. However, interactions with dissolved constituents may also affect their mobility. Hyung studied that dissolved organic matter is a constituent of both surface waters and soil and sediment pore water, and interact with CNTs in a way that may enhance their dispersion and transport [16].

4. **TOXICOKINETICS**

A proper understanding of toxicological behavior of NPs in abiotic and biotic environment is of great importance in terms of ecotoxicity. In abiotic environment the study encompasses the total ecosystem and its mutual balance. The study for biotic environment is rather complex yet much important as it involves all the habitats and their interactive and cooperative influences on each other. Once the NP enters in a certain environmental compartment its interaction depends on:

(1) physico-chemical state and specific form,

(2) kind and mechanistic pathway of interaction,

(3) bioavailability,

(4) kind and extent of changes in structure and functioning of biotic communities and

(5) consequences on ecological bablance.

An increasing number of ecotoxicity studies are reported for nanomaterials, including nanoparticles (NP). In the aquatic environment the NPs are shown to pose hazards or at least risk to organisms such as plants, fungi, algae, invertebrates and fish [17-20]. Assessment of risk of NP is necessary not only as a control measure for its use but also to find alternatives as protective measure. Traditionally the hazard and risk is due to dose-response relationship and extent as well as route of exposure. In considering the potential of NP to cause any harm several aspects that need to be consider are:

q mode of toxicological studies viz. in-vivo or in-vitro for biological species (biotic environment) and ecotoxicity for environmental compartments (abiotic environment),

q route of entry to biotic and abiotic environment,

q transport to the target including the metabolism for biological species,

q nature of disorder viz. physiological, genetic or functional and

q kind of damage viz. physical such as inflammation, irritation, stress or permanent viz. mortality, mutation change, growth reduction, reproductive disorder.

The spread and extent of damage are governed by the toxicodynamics and toxicokinetics of NP interaction with the speices of concern. The toxicokinetic behavior, including their entry into the environment, their movement and dispersion in different environmental compartments, the process of uptake by biota and fate within the organism decide whether (and to what degree) nanoparticles reach the site of toxic action (exposure). The toxicodynamic behavior includes all harmful effects in the living organism, including biochemical or physiological changes that adversely affect reproduction, growth or mortality rates of the organism. Although of utmost necessity there is a lack of systematic research on the study of hazards and risk of NPs, as it faces several challenges. To fill up the knowledge gap it is needed to —

develop protocol for precise determination of NPs in different environment matrices,

standardize characterization procedures of NPs,

establish dose-response relationships for NPs of different composition and particle size for different organisms,

study toxicokinetics, toxicodynamics and toxicity mechanisms for NPs of different composition and particle size,

design procedures for real time exposure of organisms to NPs,

estimate production volume, applications and load of discharge as waste for NPs,

assess the specific form, stability and fate of NPs in consumer products,

develop models for site specific risk assessment of NPs and finally

compile the data and make a data base on toxicity of NPs of different composition and particle size.

In conclusion it may be mentioned that more and systematic research on the risk assessment of NPs is needed. This will help to understand fate and behavior of nanomaterials in the environment and human food chain as well as to protect our environment.

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CHAPTER - II

HEAVY METAL RELATED RISK ASSESSMENT FOR POPULATION HEALTH VIA CONSUMPTION OF GREEN LEAFY VEGETABLES IN DHAPA, KOLKATA, INDIA.

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ABSTRACT

Consumption of vegetables grown in heavy metal rich soil poses a great threat as a major food chain route for human exposure. A vast area of Dhapa, a well known municipal solid waste (MSW) landfill site located in the eastern fringes of the city of Kolkata is presently used for cultivation of vegetables. This study assessed Lead (Pb), Nickel (Ni), Zinc (Zn), Chromium (Cr), Manganese (Mn), Copper (Cu), Cobalt (Co) and Cadmium (Cd) levels in green leafy vegetables (Red Spinach, Radish etc.) collected from 5 sites surrounding the core MSW dumping zone in Dhapa. AAS was used to analyze theconcentration of these metals in a total of 16 test vegetables. Results showed a substantially higher accumulation of heavy metals in leaves of these vegetables than in their roots. The order of accumulation of metal toxins in the leaves was Mn> Cr>Pb> Cu> Zn> Ni> Co> Cd. For the leaves as well as the roots the order of transfer factors (TF) were $TF_{Cd}>TF_{Ni}>TF_{Cr}>TF_{Co}>TF_{Cu}>TF_{Pb}>TF_{Mn}>TF_{Zn}$, indicating Cd is the most bio-available among all the metals and Zn the least. Furthermore, this study highlights that Pb, Ni andCd contained in the vegetables exceeded the Indian and World health Organization (WHO/FAO) reference values. However, the Health Hazard Quotient (HHQ) value of <1indicates a relative absence of health risks associated with the ingestion of contaminated vegetables.

KEY WORDS: Dhapa, MSW, Toxicity, Plant uptake, Risk assessment.

1. INTRODUCTION

Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities, and as a result we are exposed to toxic substances through various pathways,especially food chain. Vegetables are common in diet of mass throughout the

world and are the sources of essential nutrients, antioxidants and metabolites (Thompson et al., 1990). They also act as buffering agents for acid substances produced during the digestion process. However, both essential and toxic elements are present in vegetables with a wide range of concentrations as they are said to be good absorber of metals from the soil (Lokeshwari et al., 2006; Eslami et al., 2007).Reports have shown that vegetables grown in heavy metal rich soils are mostly contaminated (Kawarta et al., 2008; Sharma et al., 2007). Vegetables absorb these metals from contaminated soils as well as from polluted environmental deposits through the roots and incorporate them into the edible parts of plant tissues or deposit on the surface of vegetable (Haiyan et al., 2003;Nwajei et al., 2009). Within permissible limits, heavy metals are important for human body as essential metallo-enzymes, but their overexposure can lead to serious health hazards. A number of factors influence the accumulation and concentration of heavy metals on and within plants. These factors include climate, deposition of SPM on plants, nature of soil on which the plant is grown, application of fertilizers and irrigation with waste water (Anyanwu et al., 2004; Khairiah et al., 2004).

Heavy metal contamination in vegetables predominantly due to anthropogenic activities has been widely reported. Many of these studies report the effect of wastewater irrigation on agricultural produce (Wang et al., 2012; Harmanescu et al., 2011; Arora et al., 2008; Muchuwetti et al., 2006), while others have studied the fate andbehaviour of municipal solid waste (MSW)-compost amended soils (Smith, 1992; Zinatiet al., 2001; Liu et al., 2006). In this context, considering Dhapaas the main solid waste disposal site for many decades for the entire Kolkata Municipality, the progressive deterioration of the soil quality(may be called contaminated land) has been recorded in a few available literature references. Early reports by Gupta et al. (1990) and Mitra and Gupta (1999) found significant accumulation of heavy metalsin sewaged vegetables of Dhapa. In a study done in 2006–2008 (Banerjee et al., 2010) high Cu (71.16 ppm), and Pb (70.66 ppm) were found in red IndianRed Spinach grown in Dhapa. The effect of garbage farming and the practice of sewage-fedaquaculture prevalent in East Calcutta Wetlands (ECW, a Ramsar designated site), resulting in high Cu and Mn values has also been reported by Raychaudhuri et al. (2007).

In the present study, an attempt has been made to assess the health riskof heavy metals for the roots and edible parts of two locally grownvegetables, radish and red Indian Red Spinach. Dhapa being a partof the famous Kolkata wetlands, and one of the 22 such wetlands worldwide accorded theRamsar status, the long term implications are important for the ecological management ofsuch priceless natural habitats.

2. MATERIALS AND METHODS

2.1. Background of the study area

An area on the eastern fringes of the city of Kolkata, West Bengal, India, known as *Dhapa*, has been earmarked since the middle of the 19th century for dumping of municipal solid waste (MSW) generated in the city and its suburbs. Dumping of MSW is still continuing in certain parts of Dhapaand the rest of the vast stretch of land is used by the farmers to grow vegetables. This region has a collection of small vegetable growing farms, the total area of which is about 2000 acres. The topsoil of these vegetable cultivating farms has evolved

from the MSW. This MSW was found to contain higher concentrations of essential elements as well as heavy metals. Thus, the Dhapatopsoil and vegetables grown there are also expected to contain elements in higher concentrations than those in uncontaminated soil. Five small villages of Bainchtola, Arupota, Durgapur, Sahebabad and Khanaberia sorrounding the Dhapa waste disposal area were selected as study sites.

Figure.1: Images of Dhapa disposal area.

Figure. 2: Images of agricultural lands adjacent to the MSW disposal area in Dhapa.

2.2 **Sample collection and preparation**

Standing food crop samples including Radish (RaphanusSativus) and Red Spinach (AmaranthusDubius) were collected from the sites. At harvest, plants were divided into leafy shootand root, properly washed with water to remove the soil particles adhered to the surface of the vegetables. Samples were then sliced into pieces and air dried for 2 days to remove moisture content. Once air dried, each sample was weighed and further oven-dried at 800C, ground and stored at room temperature for analysis.

2.3. **Sample digestion and analysis of heavy metal**

For the heavy metal analysis of vegetable samples, 1 gm dried sample was digested in 15 ml of HNO3, H2SO4 and HClO4 mixture (5:1:1) at 80°C until a transparent solutionwas obtained. After cooling the digested sample was filtered using Whatman No. 42 filter paper and the final volume was made up to 50 ml with Milli-Q water and analysed for heavy

metals using a Varian Spectra 220 FS spectrometer. All the concentration of the metals is expressed in mg kg-1 in dry weight.

3. DATA ANALYSIS

3.1. Transfer factor

Metal concentrations in the extracts of soils and plants are calculated on the basis of dry weight. The transfer factor (TF) was calculated as follows:

$$TF = C_{plant} / C_{soil} \text{ (Cui et al., 2005)}$$

where, C*plant* and C*soil* represent the heavy metal concentration in extracts of plants and soils on dry weight basis, respectively.

3.2 Daily intake of metals

The daily intake of metals (DIM) is determined by the following equation.

$$DIM = [C_{metal} \times C_{factor} \times D_{food\ intake}] / B_{average\ weight}$$

where C*metal*, C*factor*, D*food intake* and B*average weight* represent the heavy metal concentrations in plants (mg kg^{-1}), conversion factor, daily intake of vegetables and average body weight, respectively. The conversion factor 0.085 was used to convert fresh green vegetable weight to dry weight, as described by Rattan et al. (2005). The average daily vegetable intakes for adults and children were considered to be 0.345 and 0.232 kg $person^{-1}day^{-1}$ respectively, while the average adult and child body weights were considered to be 55.9 and 32.7 kg respectively.

3.3 Health risk index

The health risk index (HRI) for the locals through the consumption of contaminated vegetables was based on the food chain and the reference oral dose (R_fD) for each metal. The HRI <1 means the exposed population isassumed to be safe.

$$HRI = DIM / R_fD \quad \text{(US-EPA; 2002)}$$

4. RESULTS AND DISCUSSIONS

4.1 Metal accumulation in plants

Heavy metal concentration (mean and ranges in ppm) of the heavy metals for both the leaves and roots of Radish and Red Spinachare presented in Table 1, 2 and Figure 1. Results revealed variable metal levels in different types and parts of the vegetable samples under investigation.In general, the average values of Cu, Pb, Ni, Zn, Cr and Mn were much higher in the leaves of the sampled vegetables than in their roots. From the mean values, the order of accumulation of metal toxins in the leaves of Radish was Mn> Cr >Pb> Cu > Zn > Ni > Co > Cd whereas in roots the order was Mn> Cr > Cu >Pb> Zn > Ni > Co > Cd. The Radish leaves had the maximum concentration of Cu (62.80), Pb (84.81), Zn (43.89), Cd (7.66), Co (4.65) and Mn (121.79) while Ni (30.88) and Cr (70.42) were maximumin the Red Spinach leaves. In the case of the roots of thevegetables, Radish sample had the heavy metals in the sequence of Cu > Cr >Mn> Zn >Pb>Ni > Co > Cd and Red Spinach had Cr >Mn> Cu > Zn >Pb>Ni > Co > Cd. Radish root had the maximum concentration of Cu (34.96),Mn (22.9), Pb (18.81) and Cd (1.61) while the highest values for Cr (55.66),Zn (19.91), Ni

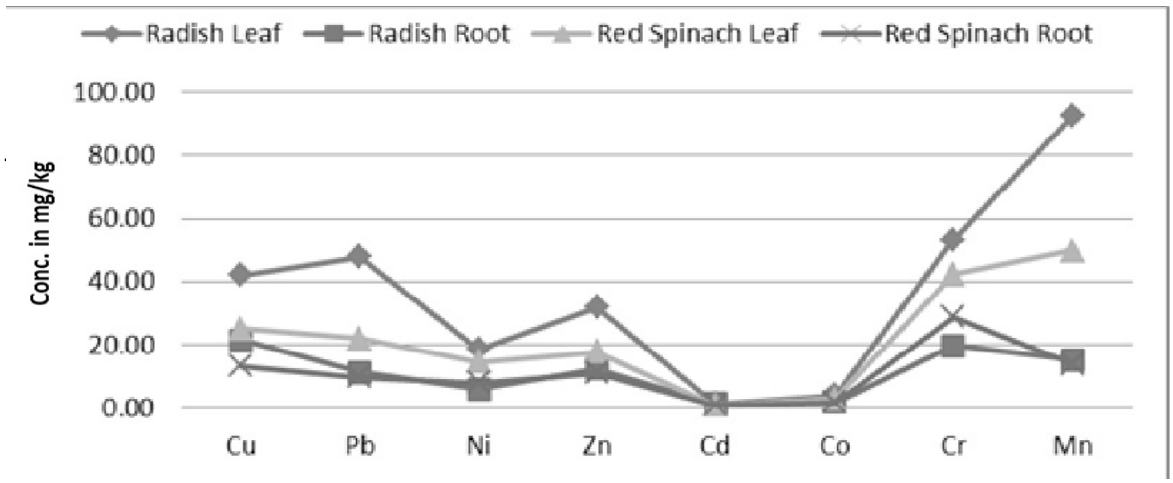
(13.99) and Co (2.63) werefound in the Red Spinach roots.Leafy vegetables accumulate much higher contents of heavy metals as compared to other vegetables. This is because leafy vegetables have higher translocation and transpiration rate in comparison to other vegetables in which transfer of metals from root to stem and then to fruit is longer which results in lower accumulation than leafy vegetables (Itanna et al.)

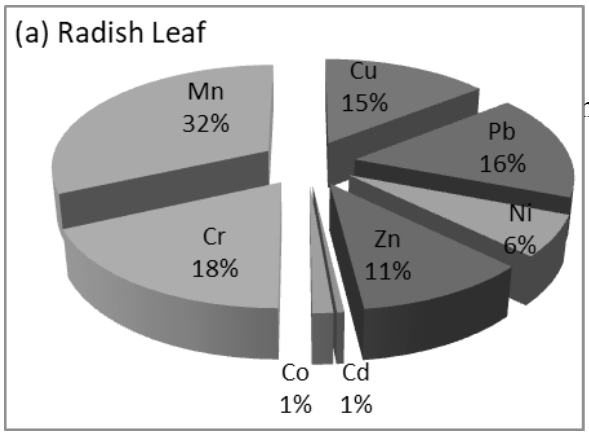
Table 1: Concentration of heavy metals and transfer factor (TF) in Radish.

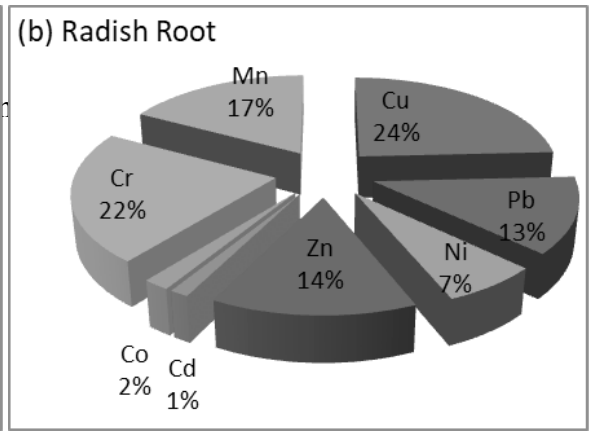
Heavy Metal	LEAF				ROOT			
	Concentration		TF		Concentration		TF	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Cu	30.66 - 62.80	41.98	0.07 - 0.20	0.12	14.40 - 34.96	21.51	0.04 - 0.11	0.06
Pb	24.74 - 84.81	47.73	0.04 - 0.17	0.09	6.00 - 18.81	11.30	0.01 - 0.04	0.02
Ni	16.32 - 20.21	18.55	0.27 - 0.33	0.31	5.33 - 6.59	5.97	0.09 - 0.11	0.1
Zn	25.19 - 43.89	31.79	0.02 - 0.05	0.03	8.36 - 17.43	12.37	0.01 - 0.04	0.02
Cd	1.98 - 7.66	1.36	0.97 - 1.31	1.09	0.54 - 1.61	1.17	0.54 - 1.12	0.92
Co	3.07 - 4.65	3.83	0.13 - 0.26	0.19	1.38 - 1.82	1.64	0.06 - 0.08	0.07
Cr	49.17 - 58.77	53.30	0.12 - 0.18	0.16	18.15 - 21.74	19.58	0.04 - 0.08	0.06
Mn	73.98 - 121.79	92.45	0.09 - 0.10	0.1	11.03 - 22.90	15.27	0.01 - 0.03	0.02

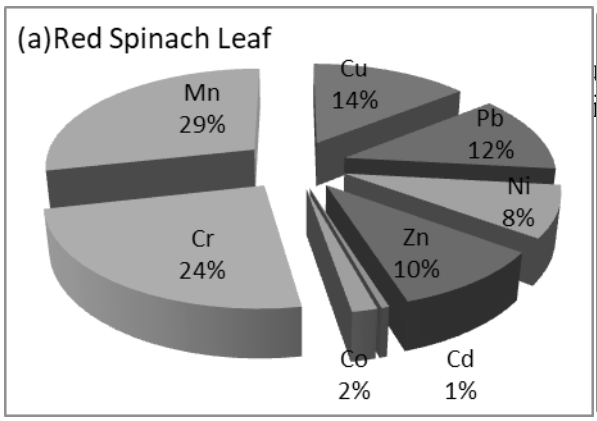
Table 2: Concentration of heavy metals and transfer factor (TF) in Red Spinach.

Heavy Metal	LEAF				ROOT			
	Concentration		TF		Concentration		TF	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Cu	13.49 - 59.24	24.91	0.04 - 0.19	0.08	7.82 - 20.84	13.29	0.03 - 0.07	0.04
Pb	10.13 - 63.25	21.77	0.02 - 0.15	0.06	5.83 - 15.93	9.66	0.01 - 0.07	0.03
Ni	4.99 - 30.87	14.50	0.08 - 0.63	0.28	4.34 - 13.99	7.80	0.07 - 0.29	0.15
Zn	9.87 - 28.01	17.63	0.01 - 0.04	0.02	5.94 - 19.91	10.95	0.01 - 0.04	0.02
Cd	0.74 - 0.98	0.87	0.59 - 1.21	0.90	0.51 - 1.16	0.89	0.40 - 1.26	0.92
Co	1.89 - 3.32	2.84	0.09 - 0.20	0.15	0.90 - 2.63	1.70	0.05 - 0.16	0.09









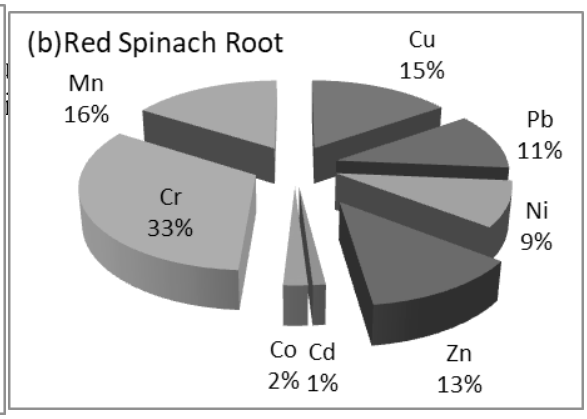


Figure. 3: Percent contribution of individual metal in (a) leaf and (b) root sample of Red Spinach.

In any case, the mean values for Cr, Ni and Pb in all the samples exceeded the limits set by the Indian (Awasthi, 2006) and WHO/FAO (WHO/FAO 2007) standards given in Table 3. Among the metals Pb and Cr content in the leaves were found to be 3-4 times greater than their respective concentration in the roots, suggesting aerial deposition as a point source for pollution may be possible.

Table 3: Indian and WHO/FAO reference values of plants.

Sample	Standards	Cu	Pb	Ni	Zn	Cr	Mn	Cd	Co
Plant (µg/g)	Indian standard (Awasthi, 2000)	30	2.5	1.5	50	20	-	1.5	-
	WHO/FAO	40	5.0	-	60	-	-	0.2	-

4.2 Transfer Factor

The ability of a metal species in its different forms to migrate from the soil through the plant parts and makes itself available for consumption was represented by the transfer factor (TF). The transfer factor is a function of different attributes such as the soil pH, soil organic matter, availability of the metal and soil particle size. The transfer factors of heavy metals for both the leaves and roots of the vegetables, Radish and Red Spinach are shown in Table 1, 2 and Figure 4. For the leaves as well as for the roots, the order of TFs was Cd > Ni > Cr> Co > Cu >Pb>Mn> Zn, indicating Cd is the most bioavailable among all the metals and Zn the least. These values in the leaves were higher than the corresponding values found in theroots of the vegetables. While the highest transfer factor for Cd (1.31) was obtained in Radish leaves, Red Spinach leaves had the highest values for Ni (0.63) and Cr (0.28). In case of roots, highest transfer factors for Cd (1.26), Ni (0.29) and Cr (0.22) were found in the Red Spinach. Transfer factor of 0.1 indicates that plant is excluding the element from its tissues. The greater the coefficient value than 0.50, the greater is the possibility of vegetables being contaminated by metal through anthropogenic activities (Sajjad et.al, 2009). In view of this fact,transfer factor of Cd (1.31) and Ni (0.63) implies vegetables may be contaminated in a decreasing order by these metals through anthropogenic activities. However the transfer factor does not present therisk associated with the metals in any form. The degree of toxicity of heavy metals to human metabolic system depends upon their daily intake by the latter.

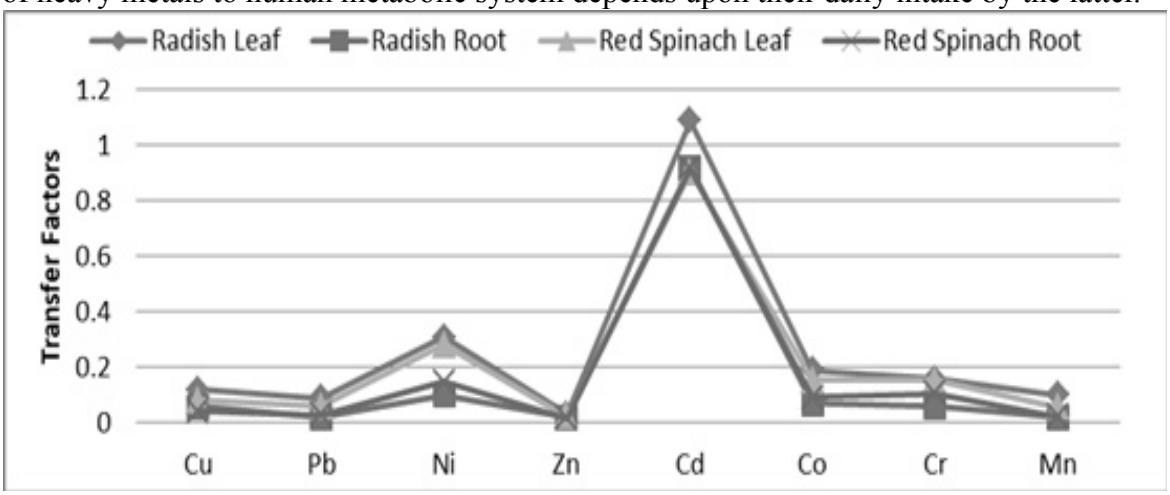


Figure.4: Transfer factor (TF) of individual metals in vegetables

4.3 Daily intake of Metals

The daily intake of heavy metals was estimated according to the average vegetable consumption. The estimated DIM through the food chain is given in Table 4 for both adults and children. The DIM values of heavy metals among the leafy parts of the vegetables were in the order Mn> Cr >Pb> Cu whereas for the roots the order was Cu >Mn> Cr >Pb. The range of values for the different metals in leaves were Cu ($7.08x10^{-3}$ to $3.57x10^{-2}$), Pb ($5.31x10^{-3}$to $3.81x10^{-2}$), Ni ($2.62x10^{-3}$ to $1.86x10^{-2}$), Zn ($5.18x10^{-3}$ to $1.69x10^{-2}$), Cd ($3.88x10^{-4}$ to $5.91x10^{-4}$), Co ($9.91x10^{-4}$ to $2.00x10^{-3}$), Cr ($9.94x10^{-3}$ to $4.25x10^{-2}$) and Mn ($1.43x10^{-2}$ to $5.20x10^{-2}$). Corresponding values for the roots were Cu ($7.55x10^{-3}$ to $2.11x10^{-2}$), Pb ($3.15x10^{-3}$ to $1.13x10^{-2}$), Ni ($2.79x10^{-3}$ to $3.97x10^{-3}$), Zn ($4.39x10^{-3}$ to $1.05x10^{-2}$), Cd ($2.83x10^{-4}$ to $9.69x10^{-4}$), Co ($7.22x10^{-4}$ to $1.10x10^{-3}$), Cr ($9.52x10^{-3}$ to $1.31x10^{-2}$) and Mn ($5.79x10^{-3}$ to $1.38x10^{-2}$).

Table 4: DIM (daily intake of metals) and HRI (human health risk index) for individual heavy metals caused by consumption of different vegetables.

Heavy Metal	Radish				Red Spinach			
	Child		Adult		Child		Adult	
	DIM	HRI	DIM	HRI	DIM	HRI	DIM	HRI
Cu	1.30E-02	3.24E-01	1.13E-02	2.82E-01	1.50E-02	3.76E-01	1.31E-02	3.27E-01
Pb	6.81E-03	1.95E+00	5.93E-03	1.69E+00	1.31E-02	3.75E+00	1.14E-02	3.26E+00
Ni	3.60E-03	1.80E-01	3.13E-03	1.57E-01	8.74E-03	4.37E-01	7.61E-03	3.80E-01
Zn	7.46E-03	2.49E-02	6.49E-03	2.16E-02	1.06E-02	3.54E-02	9.25E-03	3.08E-02
Cd	7.07E-04	7.07E-01	6.15E-04	6.15E-01	5.26E-04	5.26E-01	4.57E-04	4.57E-01
Co	9.91E-04	NA	8.62E-04	NA	1.71E-03	NA	1.49E-03	NA
Cr	1.18E-02	7.87E-03	1.03E-02	6.85E-03	2.54E-02	1.70E-02	2.21E-02	1.48E-02
Mn	9.21E-03	NA	8.01E-03	NA	3.00E-02	NA	2.61E-02	NA

4.4 Human health riskassessment (HRI)

In order to assess the health risk or impact of any chemical pollutant, it is essential to estimate the level of exposure by quantifying the routes of exposure of a pollutant to the target organism. There are various possible exposure pathways of pollutants to human body but the food chain is one of the most important pathways. As mentioned earlier, food crops were contaminated with heavy metals and the consumption of such foodstuffs can lead to human health risks. In the study area, the vegetables produced are mostly sold in the local urban market and therefore the risks associated was assessed by calculating HRI in the leaves and roots of different vegetables and the values are shown in Table 4. The data indicated that there is no significant risk from all metals studied, as revealed by the HRI values <1 except for Pb(as shown in Figure 5) which ranged from 1.69 to 3.75. The HRI values for the metals studied showed that the consumption of leaves of Radish and Red Spinach poses a greater health threat than consuming the roots.

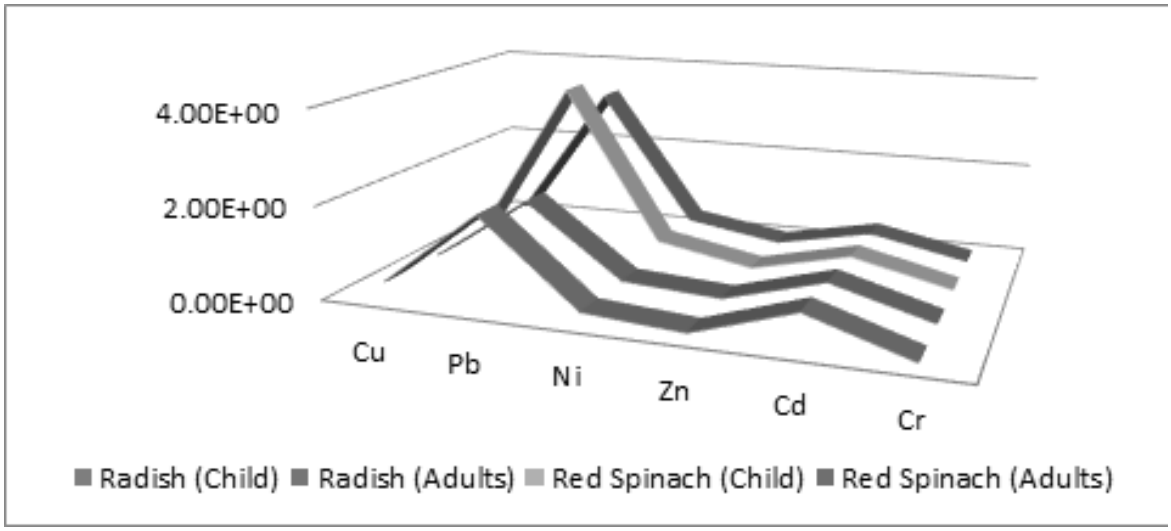


Figure. 5: HRI values for child and adults by consumption of vegetables.

5. CONCLUSION

The findings of the study regarding DIM and HRI suggest that the consumption of plants grown in MSW contaminated soil is nearly safe now and free of major risks, except Pb.This study also indicates that though transfer factors for Ni, Cu and Cr were high, only Pb had a significant health hazard quotient. Consumption of leaves had more heavy metal contamination health risk associated than the roots of both Radish and Red Spinach. In conclusion, this study on the health risks associated with the consumption of heavy metal enriched vegetables grown and consumed there locally provided a foundation for a more detailed, long term and systematic investigation of the same. It is therefore suggested that regular monitoring of heavy metals in vegetables and other food items should be performed in order to prevent excessive build-up of these heavy metals in the human food chain. Future studies have to be incorporated other sources of heavy metal exposures such as dust inhalation, dermal contact and ingestion of metal contaminated soils and waste waters especially by the Dhapa children.

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HEPATIC HISTOPATHOLOGY OF *Oreochromis mossambicus* (PETERS, 1852) UNDER SILICA NANOPARTICLES TOXICITY

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ABSTRACT

Nanotechnology is the manipulation of matter on the atomic scale, to develop tiniest new products for use in engineering, cosmetics, science and medicine. However, the studies on nanotoxicology provide pathologic effects of nano-based particles. Silica nanoparticles (SiO_2-NPs) at 5 mg/ L was exposed to the freshwater fish, *Oreochromis mossambicus* for 24, 48 and 96 h. Histopathological changes in the liver tissues were observed in treated fishes maintaining a control group. Control liver tissues showed normal architecture having granular parenchymatous cells with clear cytoplasm and spherical nucleus. Exposure to nanoparticles for 24 h showed disorganized hepatic parenchyma when compared to control hepatocytes. At 48 h of treatment vacuolar degeneration and enucleated hepatocytes were noted and at 96 h of nano-silicon dioxide exposure showed necrotic hepatocytes with leucocyte infiltration. The severity of hepatic lesions was observed to be increased in time-dependent manner. Histopathology is the reliable biomarker and valid biomonitoring tool that detect cellular changes due to silica nanoparticles in the liver of exposed fish.

KEY WORDS: Silica nanoparticles, liver, histopathology, *Oreochromis mossambicus.*

1. INTRODUCTION

The recent development of technology for reducing material size has provided innovative nano particles. The unique properties of engineered nano particles such as small size, surface area etc made them useful in a wide range of industrial applications and for making a large variety of commercial products. The production and use of manufactured nano scale materials is growing significantly in every year. The increase in demand and production of nano particles worldwide leads to the enormous exposure of organisms. The fate and

behaviour of nano particles in aquatic environment depends on various physic-chemical properties such as surface area concentration, size distribution, stability, solubility etc. Among the physicochemical properties of nano materials, particle size plays an important role in interactions with biological system.- Once the particles enter into the body, they finally reach the blood circulatory system and from there nano particles can easily enter tissues, and cross cell membranes, allowing them to harm the biological system. It has been stated that nano structured materials has the ability to pass through biological barriers such as the blood brain barrier or cell membranes and also cross the placenta. Moreover, they can move along the nerve pathways and arrive at organs like liver and kidney (Kulvietiset al., 2011; Shilo et al., 2015).

Silica (SiO_2) nano particles are one of the most widely used nano materials developed for a broad spectrum of biomedical and biotechnological applications such as biosensors for DNA, cancer therapy, disease labelling, gene and drug delivery (Hirsch et al., 2003,Dhruba et al., 2005). Nano silica is an emerging new and promising class of nano particles that have an increasing concern on its biosafety impact. In the liquid phase, nano particles exist as emulsions or suspensions and they can easily enter into the secondary lamellae of gill or to the mucous on gill surface layer according to t he access on water flow. Nano particles through the blood or systemic circulation can reach the distant vital organs, such as the liver, kidney, brain, lungs, spleen etc. Liver is one of the most important organs in the body that detoxifies foreign substances or toxins, where it counteract a wide range of toxic chemicals, both endogenous and exogenous, and therefore, has efficient neutralizing mechanisms (Hinton and Lauren, 1990). Fish hepatic pathology is widely well understood by histological observations, one of the reliable tools in toxicological studies. The present study was thus focused on the hepatic histopathology of fish, *Oreochromis mossambicus* under silica nano particles toxicity.

2. MATERIALS AND METHOD

2.1. Chemical

Silicon dioxide nanoparticle (size 7-14 nm; purity >99.8%) was purchased from Reinste Nano Ventures Pvt Ltd, New Delhi, India. The test concentration of silicon dioxide was prepared just before the exposure by sonication (100 kHz for 30 min) using double distilled water and was maintained as stock.

2.2. Test animal

Oreochromis mossambicus weighing 6 ± 1.5 g and length 6.5 ± 1cm were collected from a fish farm, Safa Aquarium, Kozhikode,Kerala. Fishes were acclimatized to the laboratory conditions for two weeks prior to experiments. They were exposed to good lighting system and with constant supply of water maintained in well-aerated tubs (40 L capacity), which was regularly dechlorinated.

2.3. Preliminary tests

The physico-chemical features of the tap water were estimated as per the procedure given in APHA (1998). Water temperature ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH was 6.5 to 7.5 which were monitored

using a standardized procedures. The specimens were not fed a day prior to and during toxicity tests to reduce faecal and excess food contaminating the test solution.

2.4. Treatments

Experimental groups were divided as follows:

Group I: Control group (without toxicant)

Group II: SiO_2-NPs at 5 mg/ L for 24 h

Group III: SiO2-NPs at 5 mg/ L for 48 h

Group IV: SiO_2-NPs at 5 mg/ L for 96 h

Each group were maintained with 10 fishes. Fish mortality was observed throughout the experiment.

2.5. Histology of liver

Liver tissues were collected by sacrificing the fish. The tissue was fixed in 10 % buffered formalin for 24 hours. Tissue was dehydrated in ascending grades of alcohol and was cleared in xylene until they became translucent. Tissue was transferred to molten paraffin wax for 1 hour to remove xylene completely and then impregnated with wax. Then the blocks were cut in a rotary microtome to prepare sections of thickness 4 to 6 microns. The sections were stained with haematoxylin and eosin and mounted in DPx. The structural alterations were observed under light microscope in the sections of liver and were compared with those of control tissues. Photomicrographs were taken using Cannon shotcamera fitted to the Carl Zeiss Axioscope 2 PlusTrinocular Research Microscope.

3. RESULTS AND DISCUSSION:

The median lethal concentration of SiO_2 in zebra fish, *Danio rerio* has been reported as 50 mg/ L (Ramesh et al., 2013). The sub lethal concentration (5 mg/ L) in zebra fish was chosen in the present study to evaluate the histological changes in liver of *Oreochromis*. Histopathological studies areconsidered as a sensitive tool to detect chemical toxicity. Histopathology of control liver showed normal architecture having granular parenchymatoushepatocytes with clear cytoplasm and spherical nucleus (Figure. 1).

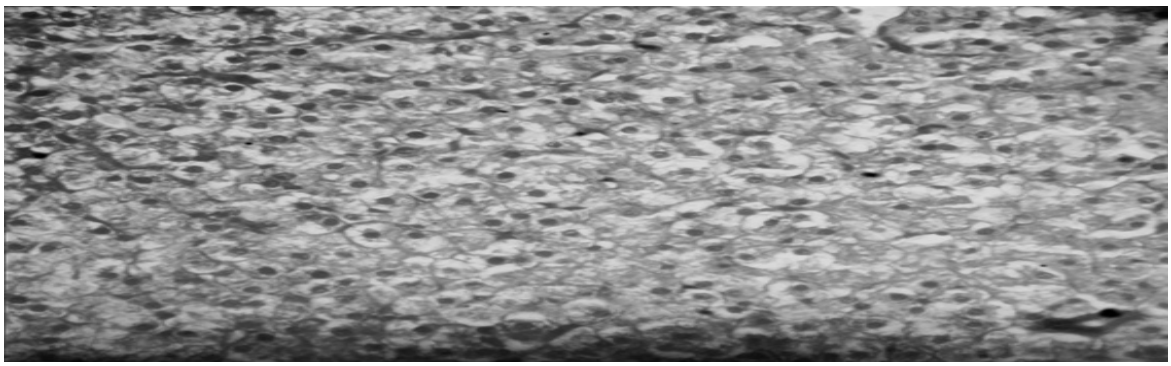

Figure. 1: Photomicrograph (40X magnification) of normal liver tissue showing granular parenchymatous hepatocytes with clear cytoplasm and spherical nucleus

Fish liver is regarded as a major site of storage, biotransformation and excretion of toxicants. Exposure to silica nanoparticles at 5 mg/ L for 24 h showed disorganized hepatic parenchyma when compared to control hepatocytes (Figure. 2).

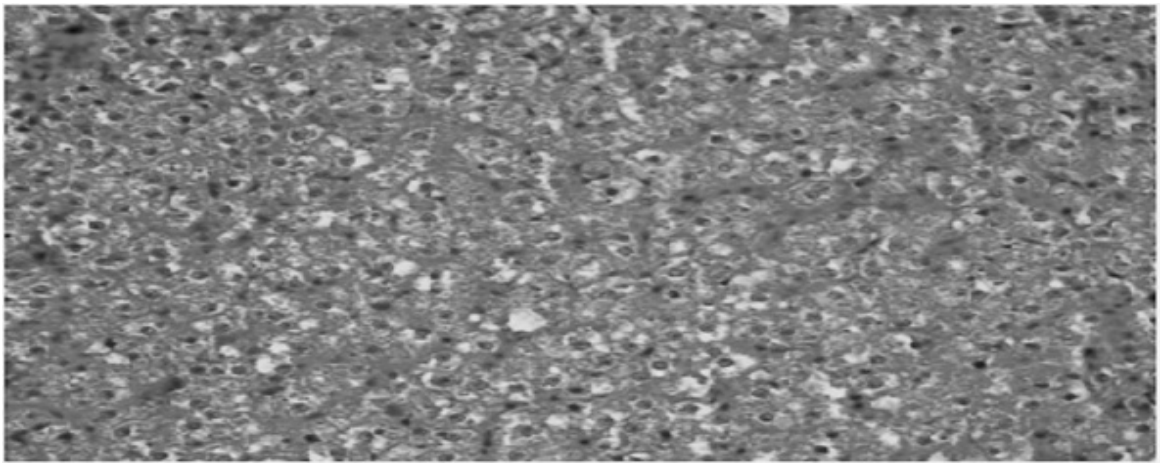

Figure. 2: Photomicrograph (40X magnification) showing disorganized hepatic parenchyma of fish exposed to SiO_2-NPs at 5 mg/ L concentration for 24 h

At 48 h of treatment vacuolization and enucleated hepatocytes were observed (Figure. 3) and at 96 h of nano-silicon dioxide exposure showed necrotic hepatocytes with leucocyte infiltration (Figures. 4 and 5). Exposure to toxicants in general, causes basic pathologies to fish liver as disorganisation of hepatic parenchyma cells, change in size and shape of nucleus, migration of nucleus and focal necrosis (Hibya, 1982). In the present study the disorganized parenchyma of liver tissue after 24 h of treatment may be due to the acute toxic effect of exposed silica nanoparticles. The increase in hepatic vacuolization after 48 hof SiO_2-NPs exposure may be due to the imbalance between the rate of synthesis of lipids and glycogen in vacuoles in the parenchymal cells and the rate of their release into the systemic circulation (Gingerich, 1982). Vacuole formation is also a defensive mechanism against toxicant exposure to prevent the substance from interfering with the biological activity of hepatocytes (Mollendorff, 1973). In the present study silica nanoparticles exposure at 48 h caused absence of hepatocyte nucleus and vacuolization in cytoplasm as a first pathological changes associated to the exposed nanoparticles. Similar observations have been reported when fish exposed to one of the estrogenic environmental contaminants, bisphenol A (Chitra and Maiby, 2014).

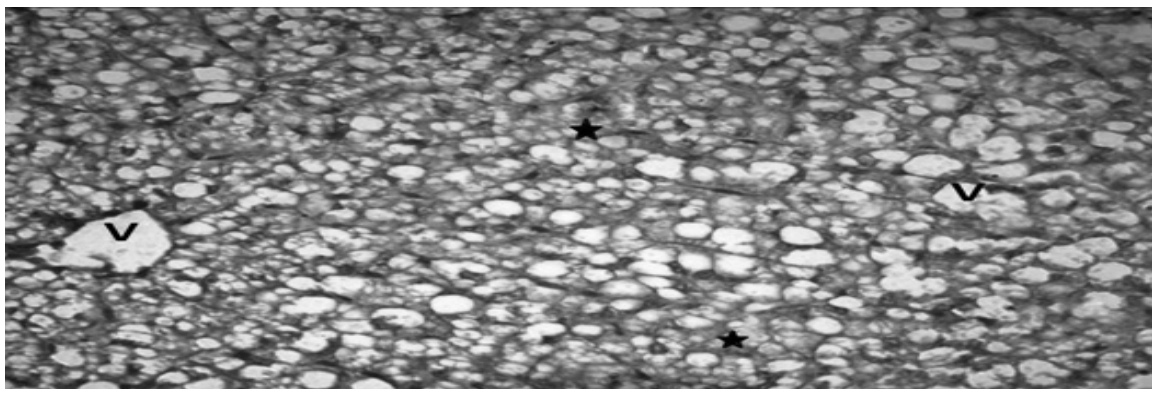

Figure. 3: Photomicrograph (40X magnification) showing vacuolization (V) and enucleated (*) hepatocytes in fish exposed to SiO_2-NPs at 5 mg/ L concentration for 48 h

Nano-silicon dioxide exposure for 96 h showed two main lesions as leucocyte infiltration and necrosis with elongated nucleus. Alteration in size and shape of nucleus have often been regarded as signs of increased metabolic activity either to biotransform or to excrete the exposed toxicants (Braunbeck et al., 1990).Hepatic necrosis are irreversible injury caused to liver as a result of SiO_2 exposure, which could be due to disturbed biochemical process as enzyme inhibition, failure on protein synthesis, carbohydrate metabolism, reactive oxygen species production, damages in cell membrane and failure of ATP synthesis (Mela et al., 2007; Vidya and Chitra, 2015). Leukocyte infiltration is another morphological disturbance found in liver of fish when exposed chronically to toxicant. The severity of hepatic lesions due to silicon dioxide nanoparticles exposure was observed to be increased in time-dependent manner.

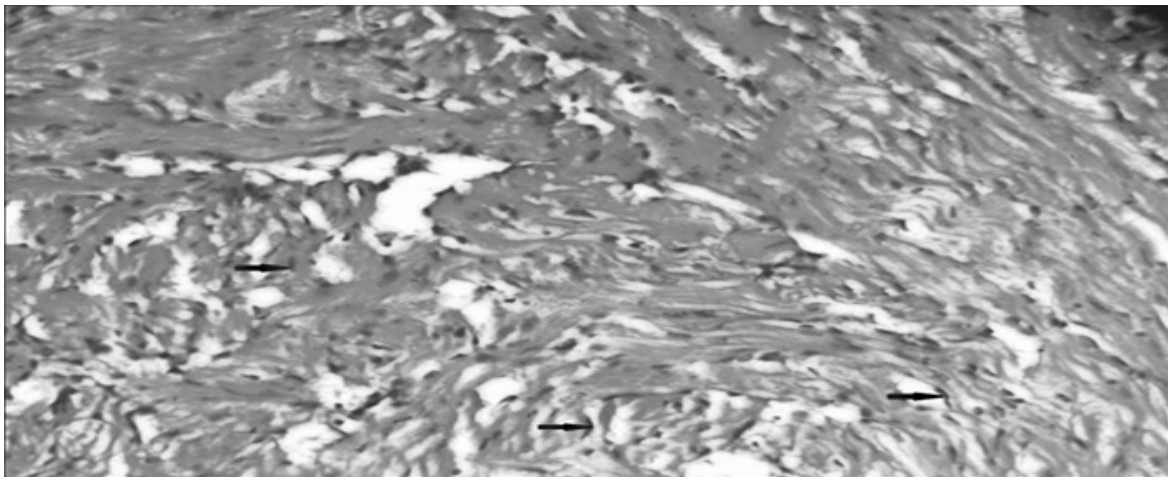

Figure. 4: Photomicrograph (40X magnification) showing hepatic necrosis and elongated nucleus (→) in fish exposed to SiO2-NPs at 5 mg/ L concentration for 96 h

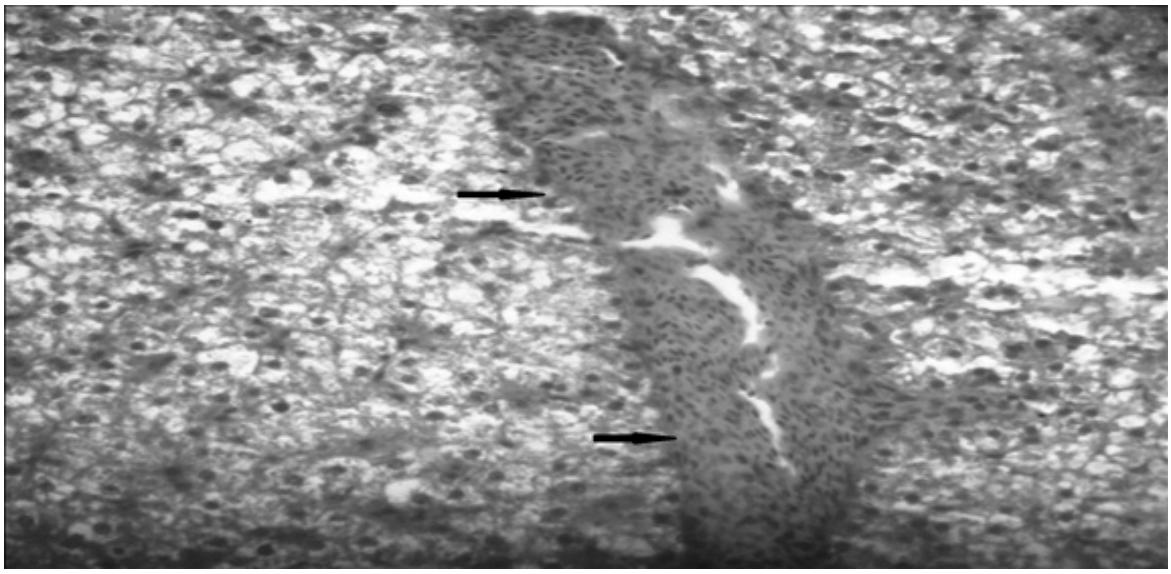

Figure. 5: Photomicrograph (40X magnification) showing leucocyte infiltration (→) in fish exposed to SiO_2-NPs at 5 mg/ L concentration for 96 h

4. CONCLUSION

The outcome of the present study is the modification in the architecture of liver directly affects the metabolism of fish consequently diminishing its life fitness. Therefore, from an ecological point of view silica nanoparticles exposure may be likely to affect the entire fish population and its food chain.

5. ACKNOWLEDGMENT

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CHAPTER - III

FLAME RETARDANTS – INCREASING PRESSURE ON THE GLOBAL ENVIRONMENT

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ABSTRACT

Environmental studies on flame retardants (FRs) took off in the late 1990s. Brominated FR (BFR) concentrations appeared to be high and increasing in many environmental matrices. BFRs, such as polybrominated diphenylethers (PBDEs), hexabromocyclododecane and tetrabromobisphenol-A, were used mainly in upholstery textile and electric instruments and electronics. Although organophosphorus-based flame retardants (PFRs) had already been used before, because of the concern on the BFRs, companies started to use more PFRs as well as other FRs (e.g. halogen-free, metal-based) as substitutes for BFRs. Apart from the chlorinated PFRs, their persistence is much lower than that of the BFRs, although at some hot spots with ongoing PFR emissions PFRs are nonetheless found in the environment. New data emphasize the health risks from contamination with FRs in the indoor environment. Worldwide, people make more frequent use of electronic equipment such as computers, and live in better insulated houses. FRs can be released from the equipment and furniture through evaporation (off-gassing), by wear and tear (small particles breaking off from foam, textile fibers, etc.) or direct transfer into dust. Consequently, the discussion on exposure of humans to FRs suddenly got a different character. Instead of dietary exposure, e.g. through fish or milk consumption, exposure through uptake via inhalation, dermal uptake or hand-mouth contact, the latter especially for young children, became a central theme. The situation indoors is even more complex as some of the FRs are also used as plasticizers in polymers or as additives in waxes and consequently contribute even more to the total indoor exposure of humans to chemicals. Chlorinated paraffins are an example to show that different applications of the same group of compounds can lead to high concentrations in indoor dust. This overview seeks to draw some tentative conclusions on the current environmental problems with FRs, addressing both for the legacy BFRs and the more recently emerging FRs, indoor and outdoor environment and exposure to e-waste.

1. INTRODUCTION

Triggered by publications on the occurrence and increasing concentrations of brominated flame retardants (BFRs) in human milk[1] and in sperm whales[2], many research groups started studies on BFRs in the 1990s and found substantial concentrations of mainly tetra- and pentabrominated diphenylethers (BDEs), both related to the use of PentaMix as FR in upholstery textile and electric instruments and electronics. These BDEs were found in riverine and marine sediments, invertebrates, fish and marine mammals. Soon, it appeared that decaBDE (BDE209) was applied in even higher volumes in textile and housing of electronics, and was present in sediments at even higher concentrations compared to the PentaMix related BDEs. A debate started about the bioaccumulative properties of decaBDE and its possible degradation to lower brominated BDE congeners[3,4]. In addition, two other BFRs were soon detected in the environment: hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A)[5,6]. HBCD appeared to be strongly bioaccumulative, while TBBP-A has a less lipophilic character. Ricklund et al.[7] discovered decabromodiphenylethane (DBDPE) in sediments. DBDPE was used as an alternative for decaBDE, and showed largely similar properties. Other BFRs were also found[8,9], although most of them appeared to be present in somewhat lower concentrations in the environment compared to the pentaBDE's, decaBDE and HBCD. Although organophosphorus-based flame retardants (PFRs) had already been used before, because of the concern about the BFRs, companies started to use more organophosphorus-based FRs as well as other FRs as alternatives for BFRs. The European research project ENFIRO (www.enfiro.eu) investigated 15 halogen-free (HF) FRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. It appeared that in many applications BFRs could be replaced by alternative FRs, such as those based on metals (e.g. zinc stannate or aluminum trihydrate) or phosphorus-based FRs. Although only a pilot project with a limited amount of time, the ENFIRO project was, able to recommend suitable alternatives for BFRs for which environmental damage was estimated to be marginal. These proved to be less toxic and also accumulated less in the food chain. For example, 3,4:5,6-dibenzo-2H-1,2-oxaphosphorin-2-oxide (DOPO) was found to be a valuable alternative for printed circuit boards. For epoxy resins melamine polyphosphate (MPP) in combination with aluminum diethylphosphinate (Alpi) are good alternatives. The substitution of BFRs is not a simple one-to-one replacement: the combination of FR, matrix and application is important. ENFIRO did not find alternatives for all polymer blends, but the project was a pilot and lasted for only three years.

The number of PFRs, including chlorinated PFRs detected in the environment for the first time during this project and by others, over a short period of time was overwhelming[10,11]. PFRs appeared to be present in many different types of equipment. Unless chlorinated, the persistence of the PFRs is much lower than that of the BFRs, although at some hot spots with ongoing PFR emissions PFRs were nonetheless found in the environment[12]. Meanwhile, a series of authors has pointed to the importance of the indoor environment. Worldwide, people spend more in fron tof the computer and television, and live in better insulated houses. FRs can be released from the equipment and furniture through evaporation (offgassing) or by wear and tear (small particles breaking off from foam, textile fibers, etc.). Consequently, the discussion on exposure of humans to FRs suddenly got a different character. Concentrations of persistent and bioaccumulative compounds in the environment often leads to dietary

exposure, e.g. through fish or milk consumption. However, the presence of chemicals in indoor dust and air leads to human exposure through uptake via inhalation or hand-mouth contact, the latter especially for young children. Here, persistence and accumulation in the food chain play a less important role. The situation indoors is even more complex as some of the FRs are also used as plasticizers in polymers or as additives in waxes and consequently contribute even more to the total indoor exposure of humans to chemicals.

2. **OUTDOOR ENVIRONMENT**

Following discussions between the bromine industry and European Commission representatives, a long-term monitoring program was carried out for decaBDE (BDE209) in the European environment. The program made use of sparrowhawk eggs (*Accipiter nisus*) from the UK and glaucous gull (*Larus hyperboreus*) eggs from Bear Island (Norway), sewage sludge and sediment samples from Western Europe. Increasing or decreasing trends are not visible in the BDE209 data for sparrowhawk (Figure. 1) and glaucous gull samples over the eight years of the monitoring program, with the exception of increased BDE209 levels in the glaucous gull eggs collected in 2012. A study on BDE209 in bird tissues sampled in China[13] reported a higher frequency of BDE209 detects (79.4% of samples) with average BDE209 levels (in ng/g lipid weight (lw)) well above detection limits: e.g. sparrowhawk muscle (192), liver (254), kidney (83 ng/g lw); and in common kestrel muscle (2,150), liver (2,870) and kidney (483 ng/g lw). The sparrowhawk egg data in the European monitoring study fall in the lower end of the concentration range found by Lindberg et al.[14] who reported BDE209 concentrations in wild peregrine falcon eggs ranging from <7 to as high as 430 ng/g lw. The ten sediment sampling locations range from very low ng/g organic carbon (OC) contamination levels, e.g. in Elbe, Ems, Seine and Outer Humber, to high µg/g OC, e.g. Western Scheldt, Liverpool Bay and River Mersey (Figure. 2). Apart from the decreasing values in the Western Scheldt sediment no further decreases in BDE209 concentrations were observed, neither in sediment nor in sewage sludge or birds' eggs. Western Scheldt sediment at the same sampling location contained ca. 200 ng/g BDE209 in 1995, which is two thirds of the BDE209 concentration in 2011[15]. At some locations an increase of BDE209 was observed, for example in River Mersey sediment. With regard to environmental levels of BDE209, the effects of the stewardship program of the bromine industry may have been limited to only a few locations. The BDE209 levels in UK sediment and sewage sludge are still very high. This could be indicative of the persistence of BDE209 and consequently, the limited degradation into lower brominated congeners in sediment although it cannot be excluded that ongoing BDE.

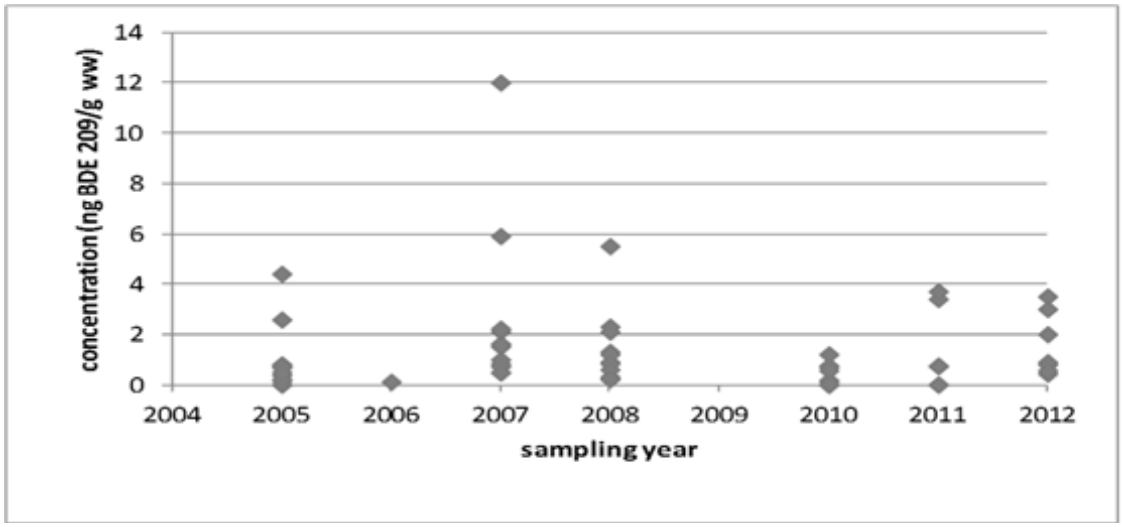

Figure.1: Overview of DecaBDE (BDE209) concentrations (ng/g wet weight) in individual sparrowhawk eggs from the UK collected between 2004 and 2012. (Note: the 8 non-detects to date are plotted as zero on this graph.)

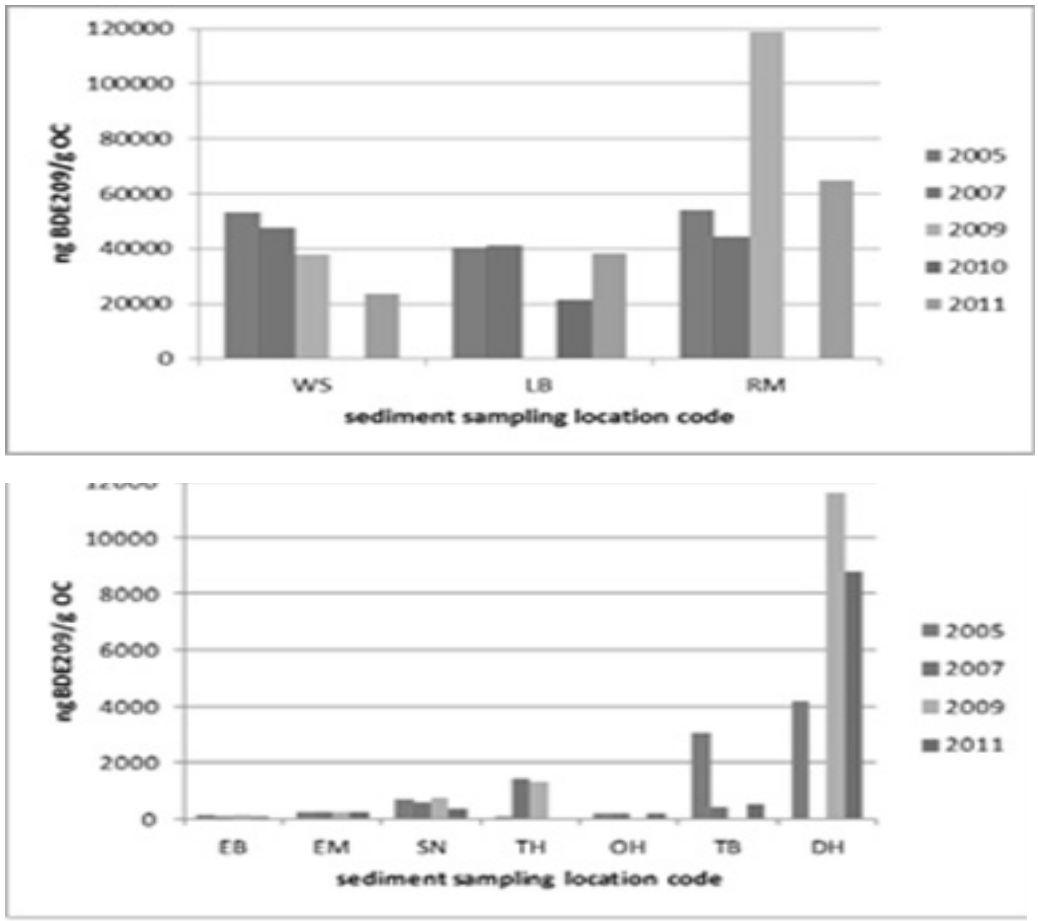

Figure.2: BDE209 concentrations in sediments, in ng/g

OC; EB: Elbe, EM: Ems, SN: Seine, TH: Thames, OH: Outer Humber, TB: Tees Bay, DH: Dublin Harbour, WS: Western Scheldt, LB: Liverpool Bay, RM: River Mersey. 209 emissions have played a role as well. NonaBDEs were not determined in sediment samples but they were found in some of the sparrowhawk egg samples. Abassi et al.[16] estimated that considering only the first use (no reuse and/or storage) of PBDE-containing products, approximately 60% of the US/Canadian stock of PBDEs in 2014 or ca. 70,000 tons, 95%of which is BDE209, will still be in use in 2020. Although numbers in Europe may be different, a similar outlook can be predicted. Given the persistence of BDE209 in sediments and the availability in products and future release into the environment, substantial decreases in BDE209 concentrations in sediments are not expected in the near future. This confirms the suggestion of Ross et al.[3] on the creation of large environmental reservoirs of BDE209. Although it is known that BDE209 can degrade when exposed to light[6], the actual degradation of BDE209 may be very slow because light penetration in water and sediment is normally very limited. Tokarz et al.[17] reported half-lives of between 6 and 50 years for reductive debromination of BDE209 in sediment. Consequently, at the locations studied lower brominated congeners may become available at relatively low levels, but over a very long period. Similarly, HBCD, applied in roof insulation polyurethane foam, will become available only at the end of product life, which is estimated at periods of ca.30-100 years. With that, both chemicals are clearly of concern for the next generation.

3. **A GROWING FAMILY**

As mentioned above, the family of FRs that have been found in the environment has grown substantially during the last two decades. The groups of HFFRs and PFRs are at least as large as the BFR group. In addition, there are many examples of combined applications of various FRs in the same product, often with one or more synergists[18,19]. The 'Future Market Insights' industry analysis report 2014-2020 confirms that flame retardants markets will continue to grow, particularly in the Asia-Pacific region, driven by growth in the construction and automotive industries, and by regulations on fire standards. All FRs have their own characteristics and toxicity, which makes their analysis, evaluation and risk assessment rather complex. The list of FRs identified in indoor air and dust, in homes, school, offices and hotels, but also in cars and airplanes is long and growing. Among many examples, Ballesteros-Gómez identified tris(2,4,6-tribromophenoxy) 1,3,5-triazine (TTBP-TAZ) in plastic electronic products and house dust, showing that also new BFRs are still entering the market[20]. Since the ban on the Penta and Octa BDE-mixes, the total BFR production has only grown. Leaching of two new FRs, used as alternatives for decaBDE, resorcinol bis(diphenylphosphate) (PBDPP) and bisphenol A bis(diphenylphosphate) (BPA-BDPP) from consumer products was shown by Kemmlein et al.[21] These were also reported by Brandsma et al. in dust in various EU countries, as well as in dust collected in cars[22]. A chlorinated PFR not previously recorded in the environment, namely 2,2-bis(chloromethyl) propane-1,3-diyl-tetrakis(2-chloroethyl)bis(phosphate), known commercially as V6, was identified in polyurethane foam from baby care products, in houses and cars[23]. Obviously, a large cocktail of substances is present in indoor situations, which is much more complex than expected. On top of the BFRs and PFRs, substantial concentrations of chlorinated paraffins (CPs) – up to almost 250 ng/g wet weight – have been reported in indoor fish from

the North Sea and Baltic Sea[24]. These levels are substantially higher than those reported for BFRs and PFRs in indoor air and dust. CPs not exclusively used as FRs – additional use is as plasticizer, as extreme pressure additives in metalworking fluids and as additives in paints[25] – but their production volume is very high. Production Figures of CPs are ca.1 million tons per year in China alone. These ongoing high production volumes – compared e.g. to the total global production of polychlorinated biphenyls (PCBs) ever of between 1 and 1.5 million tons – and their application as FR, and additives in plastics and paints, and their persistence may explain the significant levels found indoors[25]. Fridén et al. report that adult exposure to CPs was predominantly via inhalation, while dust ingestion was suggested to be more important for toddlers. The authors report that human exposure in Sweden to CPs from the indoor environment is not negligible[26]. In China an increase of CP concentrations is reported in the outdoor environment[27]. CPs are classified according to their carbon chain lengths. They are divided into short (C10-13), medium (C14-17) and long (C≥18) chained CPs. Short-chained CPs (SCCPs) have a relatively high bioaccumulation potential (BCF>5000 & TMF>1), and data suggest that SCCPs fulfil criteria under the United Nations Environmental Program (UNEP) Stockholm Convention for designation as a persistent organic pollutant (POP), including long range transport. Data on the bioaccumulation potential of medium-chained CPs (MCCPs) is less clear, although it is suspected that they have a bioaccumulation potential too. The bioaccumulation potential of longer chained CPs (LCCPs) is less likely. Further research is recommended to assess the bioaccumulation potential of MCCPs. Although studies mainly focused on SCCPs, limited data suggest that especially MCCPs are widely used and show higher environmental concentration levels than SCCPs. Another group of chlorinated FRs is that of the dechloranes. These compounds have been detected in substantial amounts in fish[28] and sediment[29] from the Great Lakes, Canada and in dolphins from the southern Atlantic Ocean[30]. These compounds are obviously bioaccumulating and persistent. In spite of all information on the environmental and health risks of chlorinated contaminants it is surprising that these compounds have got so little attention until now.

4. **INDOOR EXPOSURE**

The risk of dietary exposure seems still to the greatest for the legacy BFRs. The less persistent PFRs and HFFRs generally show a lower tendency to bioaccumulate. Some chlorinated PFRs were found in herring from the Western Scheldt, but that area is known to be a hot spot for these chemicals, so the continuous input of new PFRs supersedes metabolism and degradation[12]. Indoors, the situation is entirely different. Both legacy BFRs and many emerging HFFRs and PFRs have been identified in indoor air and dust. High levels of CPs – close to 1 g/kg – have been reported in indoor dust from Germany[31] have been reported. Also dechloranes have been found in indoor dust at levels up to 124 mg/kg[32]. Abdallah et al. concluded that compared to dietary and inhalation exposures, dust ingestion constitutes an important pathway of exposure to HBCD and TBBP-A for the UK population[33]. PFRs are rapidly metabolized in humans and many cannot be found as parent compounds in human tissues, milk or blood, although a number has been found in human milk from Sweden[34]. Searching for metabolites in urine is an option. Tris(o,o,o-cresyl)phosphate (TOCP or TMPP in the Bergman nomenclature system[35]) was suggested to be related to neurotoxic symptoms in airplane crew members (organophosphorus-induced delayed neuropathy)[36]. Its analysis in

the pilots is, however, extremely difficult, so reliable values are currently lacking. On top of everything else, information on toxicity is scarce for almost all compounds discussed here. The perpetual problem of using chemicals without testing them properly is valid more than ever here. Although analytical instrumentation now enables very sensitive detection at the picogram level, the presence of so many FRs is clearly of concern with regard to possible human health effects. An additional problem is in recycling of electronic instrumentation and flame retarded polymers. If flame retardants are not being removed from the materials before their application after recycling, they may unintentionally end for example up in children's toys, baby products or in other materials which do not need to be flame retarded at all[23]. In that way specific groups may be exposed unintentionally. Not only the toxicity of individual FRs needs to be addressed, but also mixture effects of FRs and multiple other chemicals present indoors such as plasticizers and components in waxes, and metabolites[19]. This is a huge task and will require collaboration by scientists, authorities and manufacturers. Meanwhile, reduction efforts are welcome, such as in California where nowadays FR-free furniture can be bought[37]. Most likely we can live with much lower amounts of FRs in many products. At the same time, we should not forget to protect ourselves properly to prevent horrible fire-death scenarios.

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EFFECT OF HEAVY METAL IN THERMAL EFFLUENTS ON LATA FISH (*Channa sp*)

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ABSTRACT

Now a days the global energy demand has increased with the growing urbanization and industrialization and these has been largely meet by fossil fuels. Coal meets 29.6% of global primary energy needs and its share in the world's electricity is about 42% (World coal association, 2011). For meeting this demand it causes air, water and land pollution and hamper the ecological balances. Our study is to investigate the effects of heavy metal contamination on aquatic fauna due to release of heavy metal from thermal power plant effluent (untreated) to the nearby reservior. The effluents contain heavy metals, *viz* Hg, Cr, As, Pb, Ni. In aquatic ecosystem heavy metals are considers as the most important pollutant and are extremely dangerous for the health of fish. Fishes are used as the bio-indicators, playing an important role in monitoring heavy metal pollution. Heavy metals are taken up by fishes either from ingestion of contaminated food via the alimentary tract or through the gills and skin and after absorption transported through blood stream to the organs and tissues where they accumulated [*J. Aquac Res Development Volume 6 Issue- 4. 1000328. Issn; 2155-9546 JARD*]. Review of study have been shown that order of accumulation of lead was gill> liver> muscle [*Agric. Bio. J.N.Am., 2012, 3 (12): %10-512*]. For the study of heavy metal contamination, thermal effluent water of Bandel Thermal Power Station (BTPS) was collected and lata fish (*Channa sp*) were cultured in this water for one week at different concentrations -9:1, 8:2, 1:1, pure BTPS water. The fishes were sacrificed and blood, liver, kidney, muscle, brain tissue, gill, scales were collected for following biochemical assay - total serum protein, alanine aminotransferase (AST), gama glutamyl transferase (GGT), lactate dehydrogenase (LDH), Lipid peroxidation (LPO), catalase activity and acetylcholinesterase activity. Blood slides were also prepared to study micronuclei formation. The ultimate result of this study will help us to find the toxicity level and to search remedy to prevent this contamination so that a healthy aquatic ecosystem can be maintained.

KEY WORDS: Thermal effluents, lata fish (*Channa sp*), heavy metal.

1. INTRODUCTION

Growing industrialization and urbanization is a good sign of development but these create a threaten condition for natural flora and fauna. To support the upcoming development, use of fossil fuel is gradually increased and emit their polluted discharge to the environment which make a great problem. Coal, maximally used fossil fuel, is a major problem to the aquatic animal mainly for the fish. The effluent water released from the thermal power is discharged into the nearby natural water source such as river, lake, sea etc. This thermal power plant effluent water contains heavy metal such as Hg, Cr, Pb, As, Ni etc. The non-biodegradable heavy metal can bio-accumulated in fish, directly from water or by ingestion of food (kumar and Mathur, 1991)[1]. This leads to a great concern through worldwide for the aquatic environment due to released of heavy metal. Some metal such as Cu and Zn are regared as trance metal and performed protective function in organisms. But heavy metal such as Cd, Pb, Ni and Hg are extremely toxic (Merian, 1991). Heavy metal accumulated in the kidney, liver, gills and heart and produce oxidative stress factor (Farombi, et.al,2007)[2] in organism.

The aim of the study is to assess how the presence of heavy metal in the thermal power effluent affects the different enzymatic parameter of lata fish and make a conclusion about the hazards and their possible remedy of these major problems.

2. MATERIALS AND METHODS

2.1. Experimental design

For the base line study thermal power plant effluent water was collected from Bandel Thermal Power Station (BTPS) in 7 days interval. Lata fish (*Channa sp.*) has been have chosen as experimental model. Total experiments were divided in 5 sets and each set was provided different concentration of BTPS water, one is control (Tap water); next 9:1 (Tap water: BTPS water); 8:2 (Tap water: BTPS water) 1:1 (Tap water: BTPS water) and lastly Pure BTPS water. Each set contains five fishes and the fishes were cultured for 7days in different concentration. Fish were fed common fish food ad libitum throught the entire experimental period. Then they were sacrificed and collected body tissues for doing the following biochemical assay.

2.2. Collection of tissue

After sacrifice of the fish, liver, kidney and muscle tissue were collected and kept separately in petridish (-80◦) till homogenization. A part of the tissue was diluted for quantitative estimation of protein and biochemical assay.

a) **Isolation of serum from blood**

Blood was drawn by ventricular puncture of etherized (approximately 1ml from each fish) by the routine procedure using sterile disposable syringe and needle. Blood was collected in 15ml centrifuge tube (Axygen scientific, Lot no. 061016058) without EDTA. Serum was obtained by centrifugation.

b) **Sample homogenization and centrifugation**

50mg tissue were homogemized in 2ml of Phosphate buffer(PBS) and the homogemized

tissues were spun in refrigerated centrifuged(REMI C 24model, India) at 5000rpm for 15 min at 4◦ C. After that the supernants were stored at -80◦ C for biochemical assay.

c) **Estimation of Total Protein**

For quantitative estimation of total protein the Lowry et al (1951) was used.

d) **Estimation of AST and ALT**

Estimation of AST (Aspertate transaminase) and ALT (Alanine Amino Transferase) the method of Bergmeyer and Brent (1974) was used.

e) **Estimation of Lipid Peroxidation (LPO)**

The spectrometric assay of Lipid peroidation (LPO) was performed following the protocol of Buege and Aust (1984) with some minor modification.

f) **Estimation of Total Thiol Content**

For the estimation of total thiol content the protocol of Sedlak and Lindsey (1968) was followed with minor modification.

g) **Estimation of Catalase Activity**

The quantitative measurement of Catalase activity was done by the method of Chance and Maehley (1955).

h) **Estimation of acetylcholine Esterase(AchE) activity**

For the estimation of acetylcholine esterase (AchE) activity was done by the method of Ellman G. L., Courtney K. D., Andres V. Jr., and Feath-erstone R. M. (1961).

i) **Physical Properties of water**

Physical properties of water was estimated following the method of APHA (2005).

j) **Heavy metaml estimation of sample water**

50ml of sample water was digested with Di-acid mixture (HNO3 : HClO4) on a hot plate (APHA, 1992, 1998,2005). The solution was filtered through Whatman No.42 filter paper and diluted to 50ml for analysis by Atomic Absorbtion Spectrophotometer (GBC 902, Australia).

3. RESULT AND DISCUSSION

Analysis of physical parameters of sample water revealed that pH value, Ca, Mg and chloride concentrations were within the permissible limits, but total alkalinity value was higher than permissible limit and the sample water (BTPS water) was highly alkaline. Fluoride value was also higher than recommended permissible limit. The Ca & Mg values were very low. The phosphate, Ammonia, total Iron, Cr, Cd & Pb were higher than the permissible limits.

Heavy metal contamination in aquatic environments is of critical concern due to the toxicity of metals and their accumulation in aquatic habitats. The heavy metals have a great ecological significance due to their toxicity and accumulation nature. World have a great concern to aquatic environment due to release of heavy metals. For the evaluation of health

in aquatic organisms fishes are widely used, these pollutants build up in the food chains which are responsible for adverse effects and death in the aquatic systems (Farkas, et. al., 20 02).

Table 1: Physical properties of BTPS water in relation to permissible limit:

Physical properties	**Actual amount in BTPS water (mg/L)**	**Permissible limit (mg/L)**
pH	8.41	8.5
Total Alkalinity	5200	200-600
Hardness	131	300-600
Calcium	36.8	75- 200
Magnesium	9.40	30-100
Chloride	21.33	250
Fluoride	2.0	1.0
Phosphate	0.1mg/L	0.1mg/L
Ammonia	0.5mg/L	>0.02mg/L
Total Iron	0.2mg/L	0.3-1.0mg/L
Chromium	0.15mg/L	0.05mg/L
Cadmium	0.10mg/L	0.01mg/L
Lead	0.25mg/L	0.1mg/L

Review of literature suggests that fishes living in the lower water layer and river bottom had higher metal concentration than in upper and middle layer. Benthic carnivorous and euryphagous fish had higher metal concentration than in upper and middle layer [Yi Yj, et al., 2012], Heavy metals have tendency to accumulate in liver and kidney tissue and lead to oxidative stress [BioMed Research International volume 2013].To assess the degree of hazards the following biochemical assays were preformed which may be beneficial for environment as well as for maintaining the ecological balance.

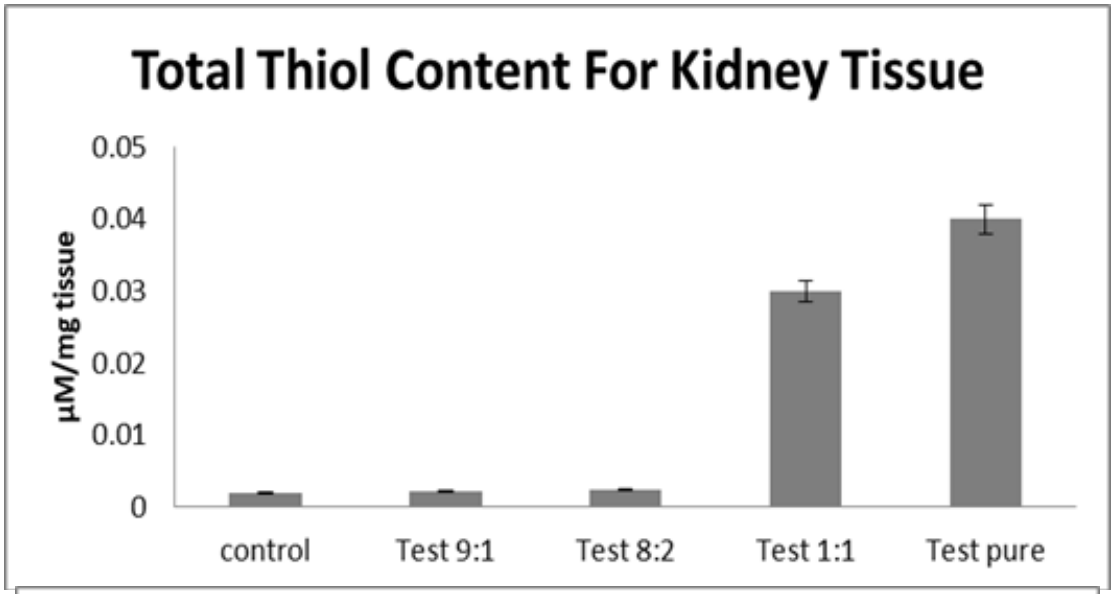
Table 2: Result of LPO activity *Channa* sp for 7days treatment.

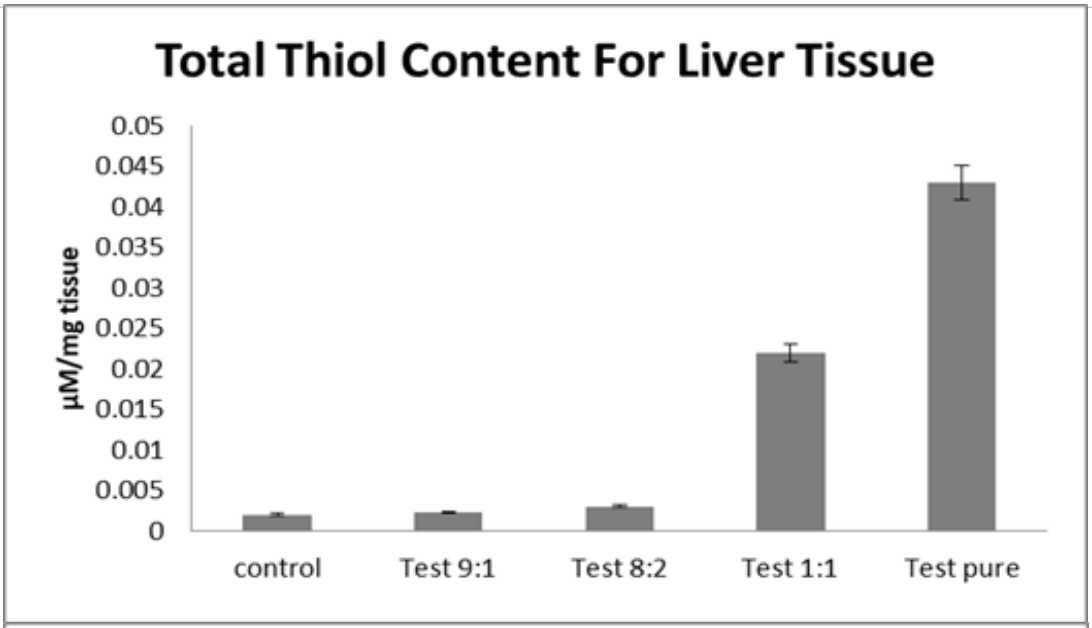
Sample water	Tissues					
	Liver	Sig.	Kidney	Sig.	Muscle	Sig.
Tap water (Control)	0.007±0.038		0.003±0.001		0.003±0.002	
Tap water: BTPS water (9:1)	0.348±038. 0	P<0.001	0.118±0.011	P<0.001	0.026±0.009	P<0.001
Tap water: BTPS water (8:2)	0.444±0.35	P<0.001	0.164±0.008	P<0.001	0.050±0.012	P<0.001
Tap water : BTPS water (1:1)	0.720±0.05	P<0.001	0.184±0.005	P<0.001	0.156±0.005	P<0.001
Pure BTPS water	0.734 0.021±	P<0.001	0.416±0.011	P<0.001	1.26±0.055	P<0.001

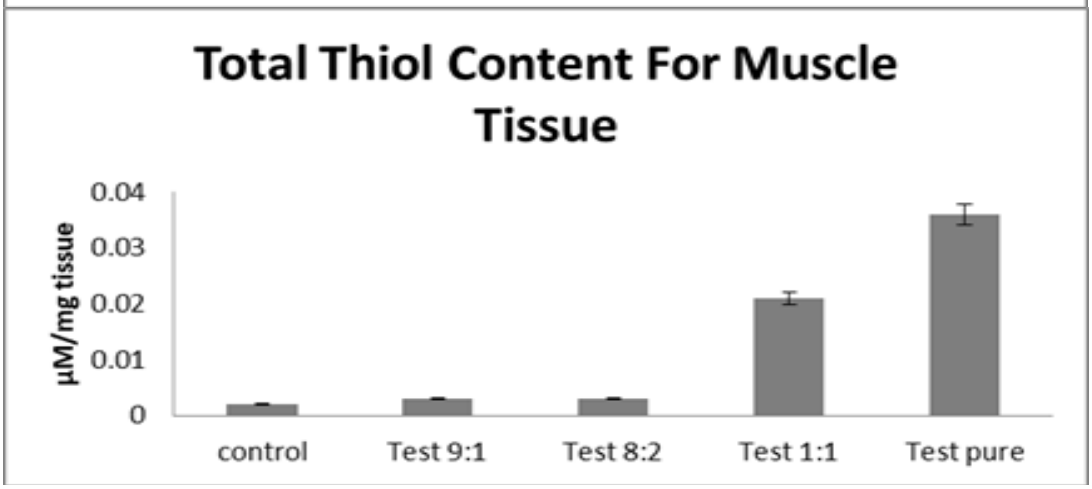
LPO: In case of Liver, Kidney and Muscle tissue lipid peroxidation (LPO) level is significantly increased in the test group of 9:1, 8:2, 1:1 and pure BTPS water compared to control group. The possible reason of the above finding is that, the presence of heavy metals (Pb, Cr, Cd) in the BTPS water may produce primary lipid peroxidation product- lipid hydroperoxides and some secondary lipid peroxidation product – MDA, 4-hydroxynonenal (4-HNE). Lipid hydroperoxides produce oxidative stress in tissues and single O_2 (molecular oxygen in its first excited singlet state - 1) which reacts with amino acid and protein. These may cause oxidation of side chain, backbone fragmentation, dimerization/aggregation, unfolding or conformational changes, enzymatic alterations in cellular handling and turnover of protein [Oxidative Medicine & Cellular Longevity; Vol.2014]. Initial reaction between MDA and free amino acids produce oxidative stress and MDA is an important contributor to DNA damage and mutation (point and frameshift), strand break, cell cycle arrest and induction of apoptosis.

Result of Total Thiole content *Channa* sp for 7days treatment.

Sample water	Tissues					
	Liver	Sig.	Kidney	Sig.	Muscle	Sig.
Tap water (Control)	0.002 0.00±		0.002±0.00		0.002 ± 0.00	
Tap water: BTPS water (9:1)	0.0025 0.00±	P<0.01	0.0025±0.00	P<0.01	0.003 ± 0.00	P<0.05
Tap water: BTPS water (8:2)	0.003 0.00±	P<0.001	0.0028±0.00	P<0.01	0.003 ±	P<0.01
Tap water : BTPS water (1:1)	0.022 0.011±	P<0.01	0.03±0.008	P<0.001	0.021 ± 0.01	P<0.01
Pure BTPS water	0.043 0.001±	P<0.001	0.04±0.004	P<0.001	0.036 ± 0.009	P<0.001


Total Thiol Content For Kidney Tissue
µM/mg tissue
0.05
0.04
0.03
0.02
0.01
0
control
Test 9:1
Test 8:2
Test 1:1
Test pure


Total Thiol Content For Liver Tissue
µM/mg tissue
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
control
Test 9:1
Test 8:2
Test 1:1
Test pure

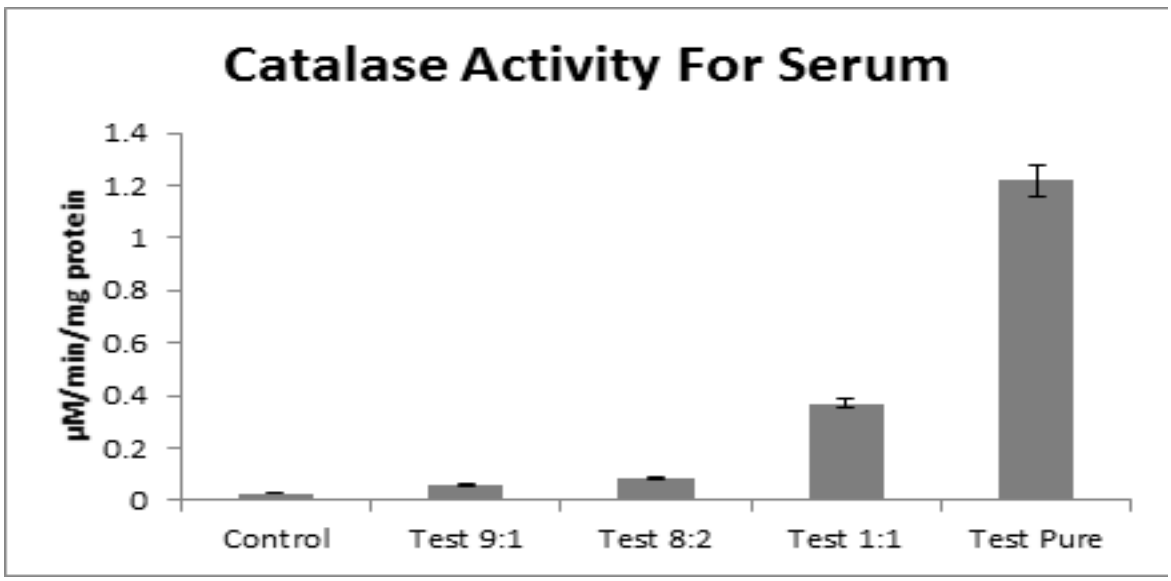

Total Thiol Content For Muscle Tissue
µM/mg tissue
0.04
0.03
0.02
0.01
0
control
Test 9:1
Test 8:2
Test 1:1
Test pure

Thiol : In case of Liver, Kidney and Muscle tissue total thiol content of 9:1, 8:2, 1:1 and pure test group is significantly increased compare to control group. At the same time muscle tissue is less affected compared to liver and kidney tissues.

The increase in total thiol content may be due to defense activity of thiol against free radicals. Review of study found that thiol share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level [Total Thiol; Biomedical importance and their alteration in various Disorders; Journal (on-line/ unpaginated);].

Result of Catalase activity from serum *Channa sp*.for 7days treatment.

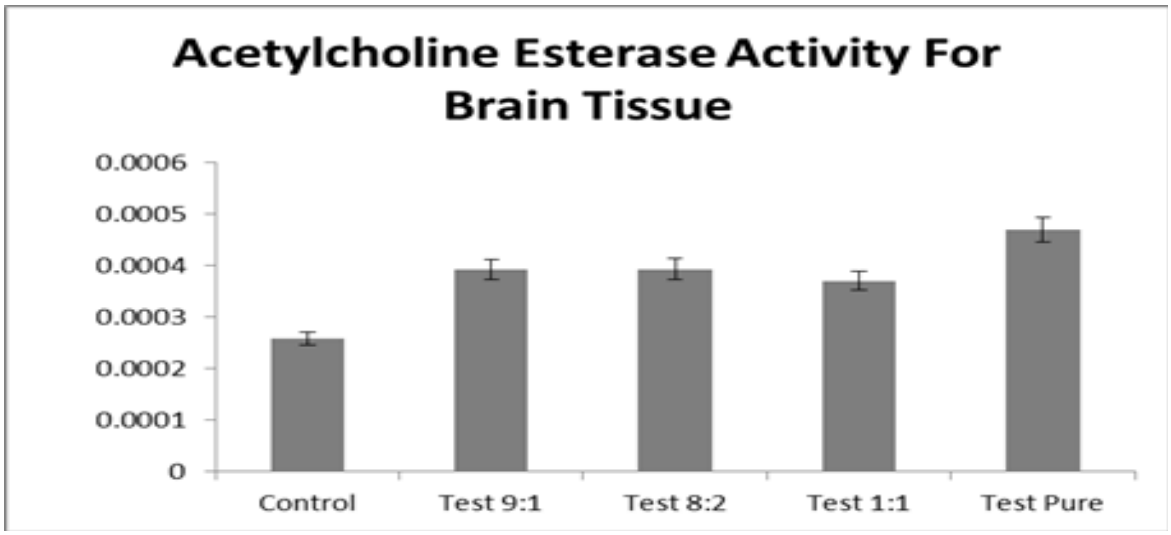
Sample water									
Tap water (Control)	Sig.	Tap water: BTPS water (9:1)	Sig.	Tap water: BTPS water (8:2)	Sig.	Tap water : BTPS water (1:1)	Sig.	Pure BTPS water	Sig.
0.026 0.006±	P	0.058 0.003±	P <.001	0.084 0.00±	P <.001	0.370 0.027±	P <.001	1.22 0.141±	P < .001



Catalase Activity: For serum the catalase activity is also significantly increased in 9:1, 8:2, 1:1 and pure test group compare to control group, due to defense the system from oxidative stress. The Catalase activity can be considered as a sensitive biomarker for bio monitoring the aquatic environment [Effect of Cadmium on catalase activity in four tissues of fresh water fish, Heteropneustes fossilis (Bloch)].

Result of Acetyl choline esterase (AchE) from Brain tissue *Channa sp* for 7days treatment.

Sample water									
Tap water	Sig.	Tap water: BTPS water (9:1)	Sig.	Tap water: BTPS water (8:2)	Sig.	Tap water : BTPS water (1:1)	Sig.	Pure BTPS water	Sig.
0.000258 ± 0.00		0.000392 ± 0.00	P <.001	0.000379 ±0.00	P < .001	0.00037 ± 0.00	P <.001	0.00047 ± 0.00	P < .001

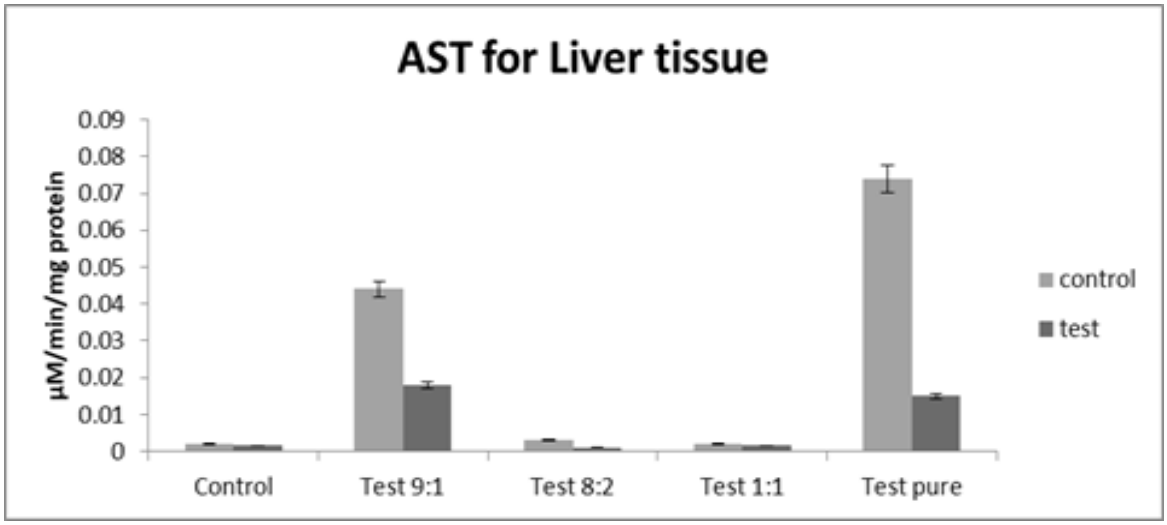


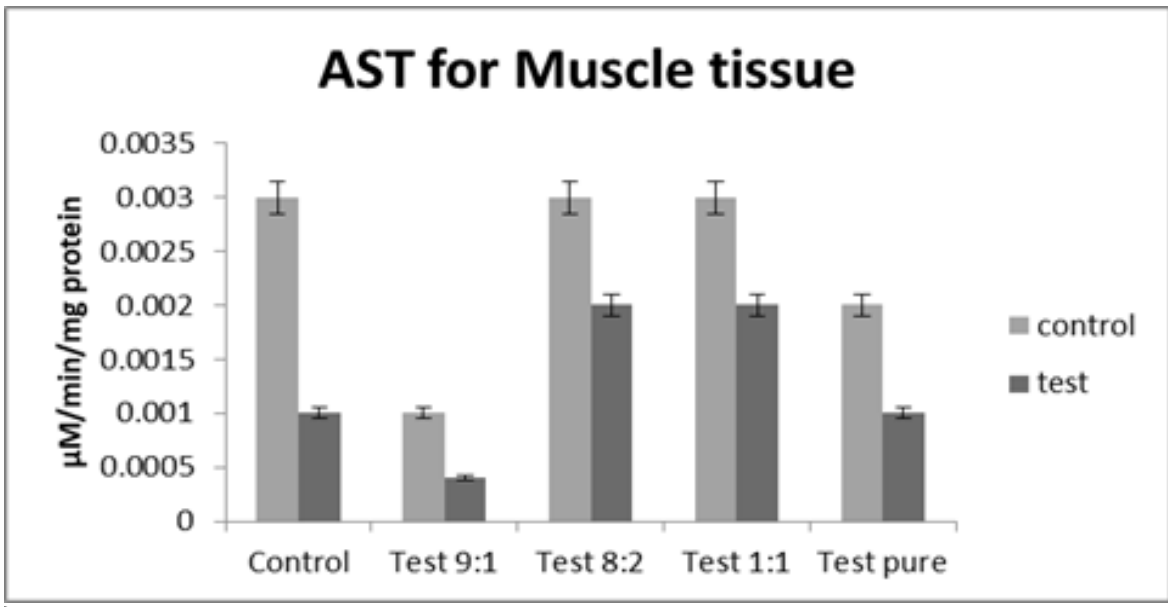
AchE activity: The brain AchE activity level shows a significant increased value of 9:1, 8:2, 1:1 and pure test group compare to control group.

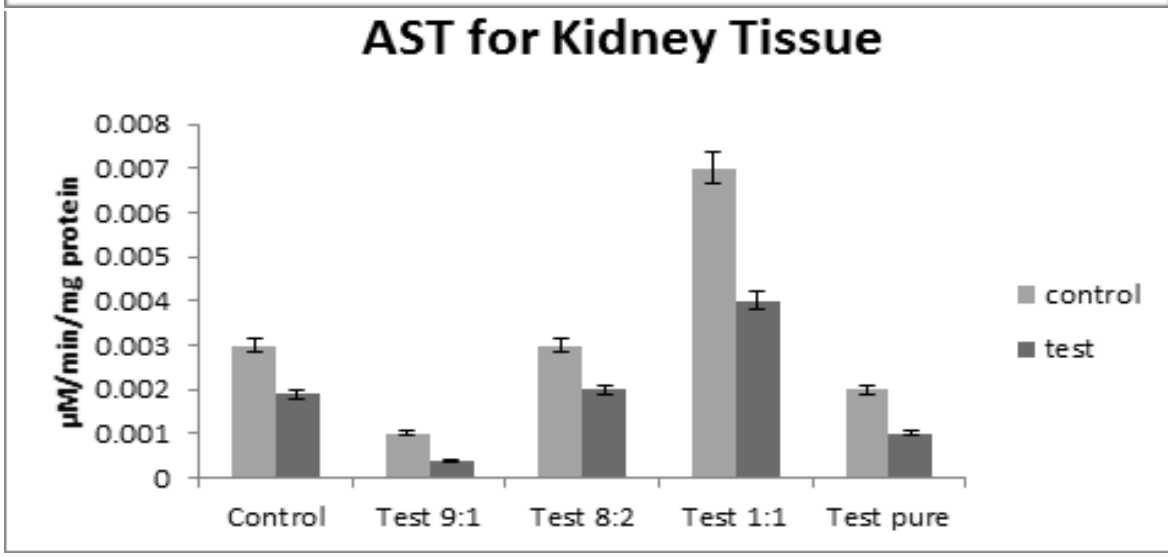
The significantly increased level of AchE may be due to catalytic activity of acetylcholine, the most excitatory neurotransmitter in the central nervous system. Literature study support that AchE activity may serve as enzyme biomarker of heavy metal neurotoxicity [D.Santos, et. Al. 2012]. The actual cause of elevated level of AchE needs further study at the enzymatic pathway.

Result of AST activity of *Channa sp*. for 7d treatment.

Tissue	Tap water (Control)	Sig.	Tap water: BTPS water(9:1)	Sig.	Tap water: BTPS water (8:2)	Sig.	Tap water: BTPS water (1:1)	Sig.	Pure BTPS water	
Liver	0.002 0.00± 0.0018 0.001±		0.004 ± 0.006 0.018 ± 0.002	P<0.001 P<0.001	.003 0.00± 0.001 0.00±	P<0.001 Non-sig	0.002 0.00± 0.002 0.004±	P<0.05 Non-sig	0.074 0.009± 0.015 0.004±	P<0.001 P<0.001
Kidney	0.003 0.001± 0.019 0.004±		0.001 0.00± 0.0004 0.00±	P<0.001 P<0.001	0.003 0.00± 0.002 0.00±	Non-sig P<0.001	0.007 0.001± 0.004 0.001±	P<0.001 P<0.001	0.002 0.00± 0.001 0.001±	P<0.001 P<0.001
Muscle	0.003 0.00± 0.001 0.01±		0.001 0.00± 0.0004	P<0.001 Non-sig	0.003 0.00± 0.002 0.00±	Non-Signf Non-Signf	0.003 0.001± 0.002 0.00±	P<0.05 Non-Sig	0.002 0.00± 0.001 0.00±	P<0.05 Non-Sig


AST for Liver tissue
µM/min/mg protein
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test

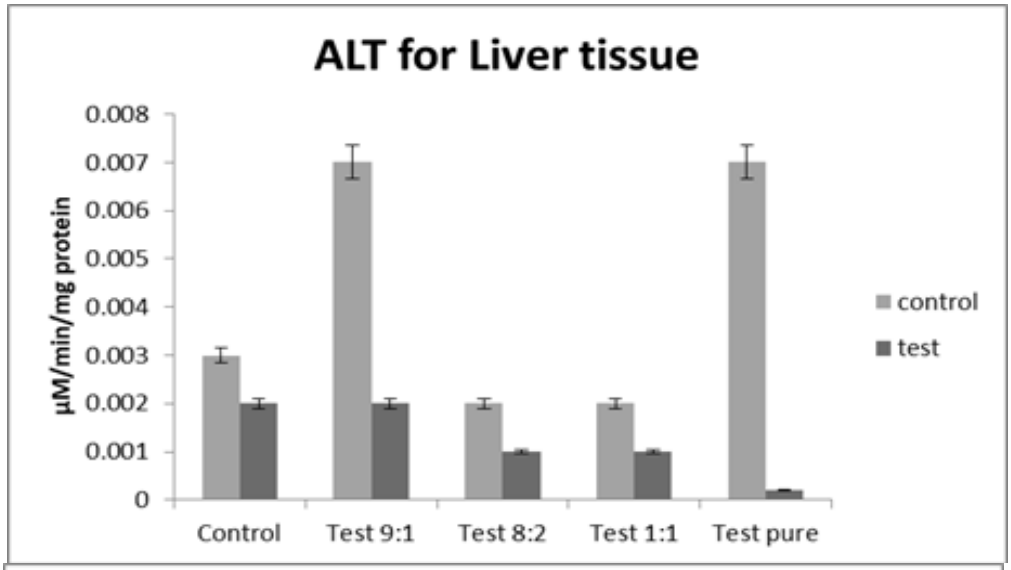

AST for Muscle tissue
µM/min/mg protein
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test

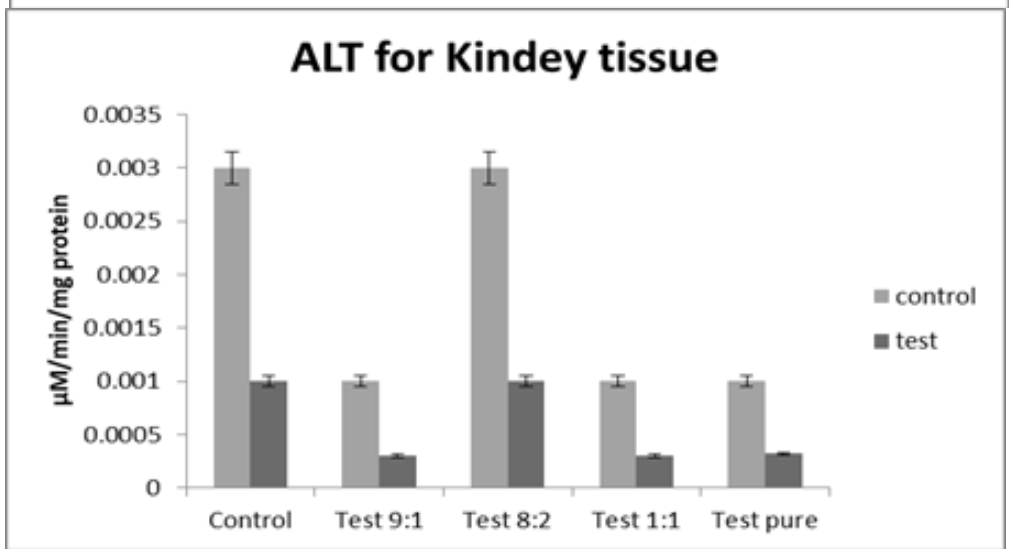

AST for Kidney Tissue
µM/min/mg protein
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test

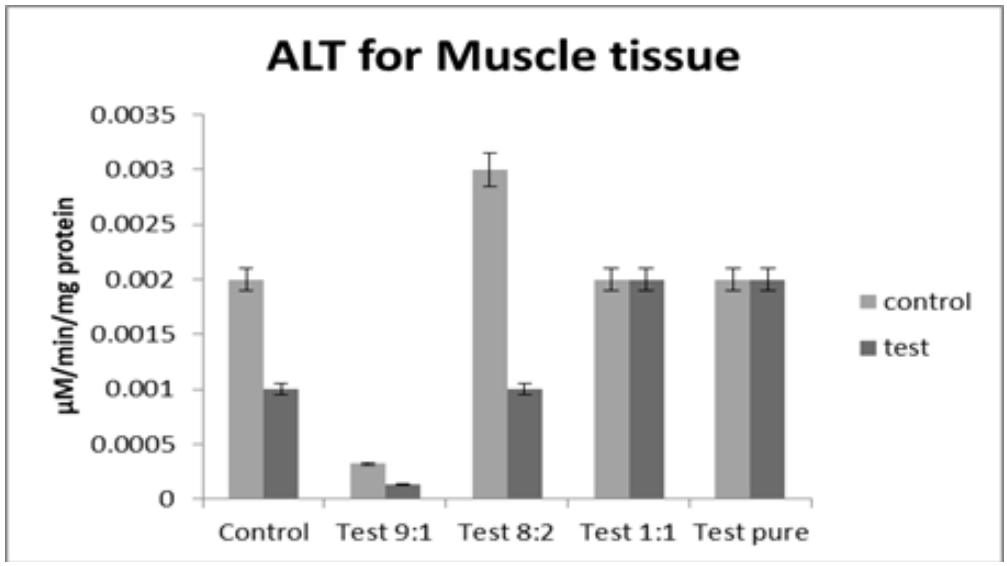
AST activity: In case of Liver AST level shows a significant increase in 9:1, 8:2, test group compare to control group in the control AST estimation. But in 1:1 and pure test group it shows significantly decreased level of AST. In case of Kidney it shows a significant increased level of AST activity in 1:1 test group and significantly decreased AST level in 9:1 and pure test group compare to control group in the control AST estimation. In Muscle tissue the AST level is significantly decreased in 9:1 and pure test group compare to control group in the control AST estimation. But in both in Muscle and Kidney tissue no significant change is found in 8:2 test group when compared with control group in control AST estimation.

Result of ALT activity of *Channa sp*.for 7days treatment.

Tissue	Tap water	Sig.	Tap water: BTPS water (9:1)	Sig.	Tap water: BTPS water (8:2)	Sig.	Tap water: BTPS water (1:1)	Sig.	Pure BTPS water	Sig.
Liver	0.003 0.00± 0.002 0.00±		0.007 001± 0.002 0.001±	P <0.001 Non-Sig	0.002 0.000± 0.001 0.00±	P<0.001 Non-Sig	0.002 0.00± 0.001 0.000	P<0.01 P<0.001	0.007 0.001± 0.0002 0.001±	P <0.001 P <0.001
Kidney	0.003 0.001± 0.001 0.000±		0.001 0.00± 0.0005 0.00±	P<0.01 P <0.001	0.003 ± 0.00 0.001 0.00±	Non-Sig Non-Sig	0.001 0.001± 0.0003 0.00±	P<0.001 P<0.001	0.001 0.00± 0.0003 0.00	P <0.05 P <0.01
Muscle	0.002 0.001± .001 0.00±		0.00032 0.00± 0.00013 0.00±	P <0.01 P <0.001	0.003 0.001± 0.001 0.00±	Non-Sig Non-Sig	0.002 0.001± 0.002 0.002±	Non-Sig Non-Sig	0.002 0.00± 0.002 0.00±	Non-Sig P <0.05


ALT for Liver tissue
µM/min/mg protein
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test


ALT for Kindey tissue
µM/min/mg protein
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test

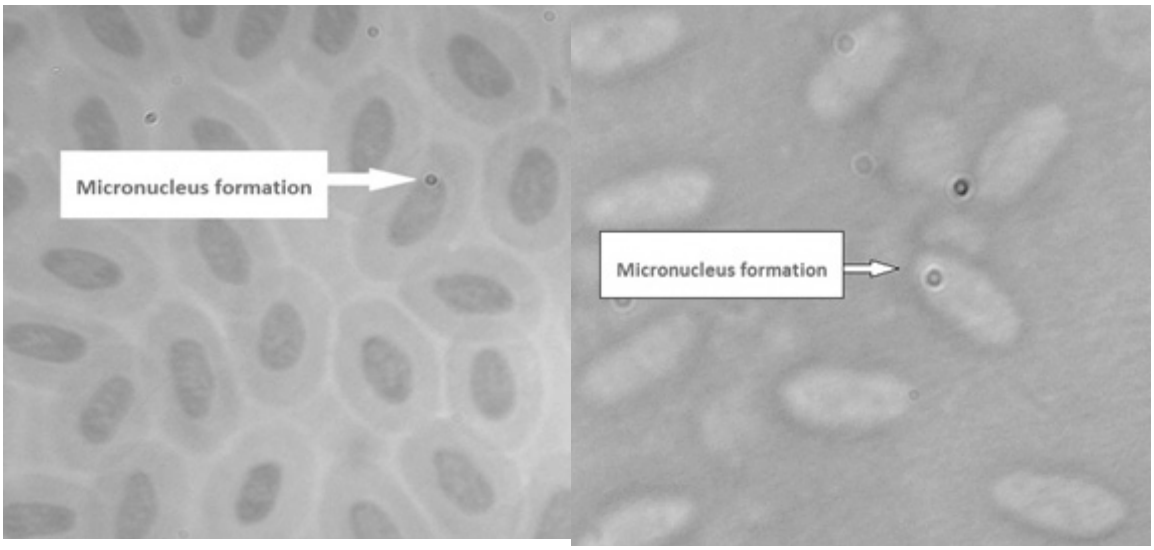

ALT for Muscle tissue
µM/min/mg protein
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
Control
Test 9:1
Test 8:2
Test 1:1
Test pure
control
test

ALT activity: In case of Liver , ALT activity is significantly increased in 9:1 and pure test group but significantly decreased in 8:2 and 1:1 test groups in compared to control group in control ALT estimation. But in case of Kidney Alt level is significantly decreased in 9:1, 1:1 and pure test group. But no significant change is found in case of 8:2 group in control ALT estimation. In Muscle tissue the ALT level is significantly decreased in 9:1 test group but no significant change was found in other test group in control ALT estimation.

The actual cause of reduced level of AST and ALT was not found. It may be due to increased activity of other enzyme assay like LPO, Catalase, Total Thiol. Further study will be needed for the establishment of actual cause.

3.1. Haematological study

Heavy metal have potentiality of doing DNA adducts, Chromosomal aberration, DNA strand break and micronuclei formation. It has been suggested that exposure of heavy metal produces electrophilic ions and radicals which interacting with nucleophilic sites of DNA and leading to breaks and other damage. [Evaluation of Micronucleus Test's Sensitivity in Fresh Water Fish Species, 2007]



4. CONCLUSION

From the above result analysis it can be concluded that industrial effluents needs more purification and proper elimination of waste materials especially heavy metals so that the aquatic life forms will be less harmed . It helps to maintain a healthy ecosystem. This is important to prevent the bioaccumulation of these heavy metals in the food chain and helps to prevent the possible physiological hazards in human.

5. ACKNOWLEDGEMENT

I am very thank full to my guide Dr. Sarmila Pal, Associate Professor of Hooghly Mohsin College at Zoology Dept. I'm also thankful to my friend Trina Sadhukhan for her great help. I like to express my heartiest thank to the Zoology Dept. of Hooghly Mohsin College for providing me the laboratory support.

We highly acknowledge for the support of chemical dept. of BTPS. I'm also thankful to Mr. A K Sasmal HOD chemical dept. Of BTPS.

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CHAPTER - IV

QUALITATIVE AND QUANTITATIVE MEASUREMENT OF BROMINATED FLAME RETARDANTS IN ELECTRONIC EQUIPMENT USING XRF AND LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

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ABSTRACT

In the present study, portable x-ray fluorescence (PXRF) was used as a screening tool to determine the presence and levels of bromine in selected electronic and electrical equipment. Bromine was detected in majority of the screened electronic products. Of these, keyboards had the highest concentrations which ranged from 174 to 4019 μg g^{-1}, while the lowest concentrations were found in cell phones, which ranged from ND to158 μg g^{-1}. For the gas chromatography-mass spectrometry analysis, dust samples were collected from selected electronic equipment and digested using ultrasonic bath. The digested samples were subjected to column clean up and, thereafter, injected into the gas chromatograph-mass spectrometer. Eight brominated diphenyl ether (BDE) congeners namely (BDE-28, -47, -99, -100, -154, -153, -128, and -183) and three novel brominated flame retardants (NBFRs) TBB, TBPH and TBPE were identified in the collected dust samples. The sum of PBDEs ranged from 1814.7 - 5970.6 ng g^{-1} with a mean of 3456.3 ng g^{-1}, while that of NBFRs ranged from <dl - 3376 ng g^{-1} with a mean of 1237.2 ng g^{-1} dry weight. The highest concentrations in electronic dust were observed for BDE-47 ranging from 1525.0 -3797 ng g^{-1} with a mean of 1442.6 ng g^{-1}. This is about 9 times greater than the mean of BDE-153 (154.0 ng g^{-1}) and six times more than the mean of BDE-28 (238.6 ng g^{-1}) and BDE-128 (219.8 ng g^{-1}). Penta-BDE represented the major congeners, in which BDE-47, BDE-99 and BDE-100 were detected in all dust samples analyzed. In the case of NBFRs, a contribution of approximately13% to 38% was observed .Of the sixteen dust samples analyzed, three were found to contain 2-ethylhexyl-2,3,4,5-Tetrabromobenzoate (TBB), bis (2-ethylhexyl) tetrabromophthalate (TBPH) and 1,2-bis(2,4,6-tribromophenoxy)ethane (TBPE). While keyboards were found to contain these compounds (NBFRs) below detection limits. Printer (a) and (V) monitor contained only TBB and TBPE giving the total of NBFs 1237.2 ng g^{-1} for printer (a) and 665.1 ng g^{-1} for (V) monitor, respectively. The detection of NBFRs in electronic dust may be due to their use as replacements for penta-, octa and deca-BDE. The presence of TBB and TBPH may have migrated through dust from the treated furniture which was stored at the same location with the screened electronics. As compared to other studies, the use of XRF in this study was useful as a screening tool for the identification of brominated flame retardants in electronic equipment.

1. INTRODUCTION

Studies have confirmed the presence of PBDEs in several indoor and outdoor air, house and office dust3 4 5 6, landfill leachates 7 8 and sediments and soil8'. It is, therefore, evident that PEDEs are present in a variety of environmental samples. Within the indoor environment, however, it is likely that exposure to PEDEs continues due to the presence of house and office equipment manufactured before the PEDEs was banned. According to Mcpherson et al (2004) about 40% of PEDEs are used in the outer casing of electronic equipment such as television, computers and printers and Deca-BDE been the mostly used BFR. The phasing-out of polybrominated diphenyl ethers has paved the way for the use of 'Novel' Brominated Flame Retardants (NBFRs) as their replacements. The NBFR formulations include, but limited to, 2-ethylhecyl-2,3,4,5-tetrabromobenzoate (TBB), bis(2-ethylhexyl-tetrabromophthalate (TBPH) and 1,2Bis(2, 4, 6- tribromophenoxy) ethane (TBPE). Penta-BDE being replaced with TBB and TBPH, while Octa-BDE and Deca-BDE replaced with TBPE and DBDPE respectively" Allen et al. (2008) 12 used X-ray fluorescence (XRF) to identifY products containing bromine (Br) as an indicator of PEDEs. A correlation between Br and PEDE concentrations measured using XRF and GC-MS was observed. Furthermore, an association between Br levels in products andPEDE concentrations in house dust were observed. Kajiwara et al. (2011)13 also used XRF to screen for FRs inselected electronic products. Using XRF, Webster et al. (2009)14 outlined the possible mechanisms of BFRs, particularly PEDEs migration from PEDE-containing products and their distribution in house dust as follows:(1) through volatilization from the polymer followed by air-dust partitioning, (2) abrasion of the polymer surface causing the release of FR-enriched particles or fibres, and (3) direct transfer of FRs from the FR containing polymer to dust. Volatilization is strongly suggested to be the main mechanism for the release of more volatile compounds whereas abrasion is considered more likely for less volatile compounds.The main goal of this study was, therefore, to understand which office products act as a source of BFRs to indoor office dust with respect to human exposure. In order to investigate this, a portable X-ray fluorescence (XRF) was used as a screening tool to determine the presence and levels of bromine and quantification ofPEDEs and 'novel' BFRs in selected electronic and electrical equipment within the office environment.

2. MATERIALS AND METHODS

XRF (Olympus Innov-X DELTA portable XRF analyzer) was first used to identify and measure bromine levels in computers, laptops, printers and cell phones. Prior to use, the portable XRF was calibrated on the Delta docking station (DDS) and by using the 316 stairtless steel coupon. Pure BDE standards (BDE-28, -47, -77,-99,-100, -118, -128, -183, -209 and 13C-BDE-77, -139, -209) and 2-ethylhexyl-2.3.4.5-tetrabromobenzoate(TBB), Bis(2-ethylhexyl) tetrabromophthalate (TBPH), 1,2-Bis(2,4,6-tribromophenoxy) ethane (TBPE), were purchased from Cambridge Isotope Laboratories Inc (Andover, USA). Sodium sulphate (Purity 99.9%), Silica gel (100-200 mesh), glass wool, pesticarb and HPLC grade organic solvents (dichloromethane, hexane, acetone and toluene) were purchased from Sigma Aldrich (Chemie GmbH, Steinheim, Germany). Dust samples were collected from the aforementioned office equipment using pre-weighed glass wool by swiping on the surfaces of the equipment. After collection, the samples were wrapped with aluminium foil and kept in the refridgeratoruntil extraction.

The extraction method used in this study was as described by Olukonle et al (2015). Briefly, about 100 mg of dust was weighed and extracted with 3 mL mixture of toluene: dichloromethane (1:1, v/v) for 15 min at 45'C using ultrasonic bath (Emalsonic S40H, Germany). Prior to extraction, dust samples were spiked with 3 f!L mixture of labelled 10 ng f!L·' BDE-77, -139 and -209 surrogate standards to monitor recoveries, and left to calibrate for 3 h. Extraction was repeated three times for each sample and the supernatants were transferred into clean tubes. Finally, the extracts were reduced under rotary evaporator and subjected to cleaning by column chromatography. The column was packed with 0.16 g silica gel, 0.06 g pesticarb, and finally with 0.5g sodium sulphate. Before introduction of the extract into the column, the packed column was eluted with 12 mL of toluene: dichloromethane (1:1, v/v). Thereafter, the purified extracts were concentrated under a stream of nitrogen gas to about 100 .LL, toped up with 150).LL of toluene and further reduced to about 160).LL. Prior to analysis, the reduced extracts were spiked with 10).LL of 2.0 ng L-1 of internal standard (BDE-118). One microlitre of the extract was injected into the GC-MS under optimized instrumental conditions. Analyses of PEDEs and NBFRs were performed using Shimadzu model 2010 plus gas chromatography coupled with a model QP 2010 ultra-mass spectrometer (Shimadzu, Japan) using electron ionization and injected automatically by Shimadzu AOC-20i auto sampler. Operation mode was in the selected ion-monitoring (SIM). A 15 m column; DB5 (0.25mm ID, 0.1).Lm dr) was used for separation. The oven temperature program set at 90"C (min), 30 "C min·' to 300 "C min- 1(5min) and 10 "C min·' to 310 'C min·' (JOmin). Helium (purity 99,999%) was used carrier gas and set at a constant flow of 1.5mL min- 1.The injector, transfer line, and ion source temperature were set at 290, 300 and 250 "C, respectively.

All laboratory glassware were washed with soap water, rinsed and soaked overnight in 10% (v/v) nitric acid aqueous solution and finally rinsed with de-ionized water. Silica, glass wool and sodium sulphate were baked at 450"C for 12 h to remove impurities. To avoid absorption of moisture, glass wool was wrapped with aluminum foil and kept in the desiccator. Silica gel and sodium sulphate were stored in separate glass jars, which were pre cleaned and rinsed with a mixture of hexane: acetone (2:1, v/v), then sealed to protect them from moisture absorption and contamination. Samples were collected and wrapped with aluminum foil, to protect them from light. Extraction was performed in the absence of light (electricity). Retention times of the unknowns were matched with those of individual standards and quantification was done by monitoring the molecular ions using external methods.

3. RESULTS AND DISCUSSION

Bromine was detected in majority of the screened electronic products. Of these, keyboards had the highest concentrations which ranged from 174-4019 µg g-1', while the lowest concentrations were found in cell phones, which ranged from ND-158 µg g-1 with a mean of 33.5 µg g-1. For the gas chromatography-mass spectrometry analysis, eight brominated diphenyl ether (BDE) congeners namely (BDE-28, -47, -99,-100, -154, -153, -128, and -183) and three novel brominated flame retardants (NBFRs) TBB, TBPH and TBPE were identified in the collected dust samples. The IPBDEs ranged from 1814.7-5970.6 ng g·' with a mean of3456.3 ng g-1, while that of NBFRs ranged from <dl - 3376 ng g-1 with a mean of 1237.2 ng g-1 dry weight. The highest concentrations in electronic dust were observed for BDE-47 ranging from 1525.0 -3797 ng g-1 with a mean of1442.6 ng g-1 This is about 9 times greater than the mean ofBDE-153 (154.0 ng g-1) and six times more

than the mean ofBDE-28 (238.6 ng g-1 and BDE-128 (219.8 ng g-1). Penta-BDE represented the major congeners, in which BDE-47, BDE-99 and BDE-100 were detected in all dust samples analyzed. In the case of NBFRs, a contribution of approximately l3 % to 38 % was observed. Of the sixteen dust samples analyzed, three were found to contain 2-ethylhexyl-2,3,4,5-Tetrabromobenzoate (TBB), bis (2-ethylhexyl) tetrabromophthalate (TBPH) and 1,2-bis(2,4,6-tribromophenoxy)ethane (TBPE). While keyboards were found to contain these compounds (NBFRs) below detection limits. Printer (a) and (V) monitor contained only TBB and TBPE giving the total of NBFs 1237.2 ng g·' for printer (a) and 665.1 ng g·' for (V) monitor, respectively. The detection of NBFRs in electronic dust may be due to their use as replacements for penta-, octa and deca-BDE. The presence of TBB and TBPH may have migrated through dust from the treated furniture which was stored at the same location with the screened electronics. With respect to keyboard, printers and monitors, the concentrations of Br determined using XRF exhibited the following trend: keyboard>printer>monitors. This trend was repeated in the GC-MS analysis of dust samples. With the novel BFRs, the concentration trend was: monitor>printer>keyboard. The observed trends suggest that the XRF can be used as a reliable screening tool for indirect measurement of PEDEs in dust samples.

4. ACKNOWLEDGEMENTS

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MEASUREMENT OF RADIOACTIVITY IN SHORE LIVING MOLLUSC AROUND NUCLEAR INSTALLATIONS

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ABSTRACT

Activities of ^{210}Po and ^{210}Pb in whole body tissue of mollusc (*Littorina sp*) collected along Kudankulam coast were studied. A significant variation in the accumulation was noted between species and between seasons ($p<0.05$). ^{210}Po and ^{210}Pb were found accumulated more in the digestive glands. The radionuclide concentration decreased with increasing size. The ^{210}Po:^{210}Pb activity ratio was greater than unity in all the tissues except the shell. The biological concentration factor for organs varied between ~10^3 and 10^4 for both the radionuclides. The total internal dose rate varied from 3.3 to 5.4 μGy/h. The calculated dose rate indicated that the molluscs were environmentally safe.

KEY WORDS: ^{210}P, ^{210}Pb, Radiotoxicity, Mmolluscs, Radiation dose, Eenvironmental safety.

1. INTRODUCTION

Marine invertebrate species, especially molluscs are considered useful bioindicators of radioactive contaminants, and sometimes the contaminant levels in these animals are directly proportional to the available levels in the environment. [1] Studies on metal accumulation in molluscs provide us with a good indicator of temporally and spatially average concentrations of bioavailable contaminants in aquatic ecosystems. [2] Mollusc species are widely distributed in inter tidal rocky shores and have been used for aquatic biomonitoring programmes and they concentrate radionuclides and metals [3].

Molluscs are primary consumers living in the intertidal region and play role in transfer of food chain. Bivalve molluscs have been recognized as first-order biological indicators of elemental contamination, since they ingest detritus which has a high degree of radionuclide association [3]. Apart from environmental issues, mussels are considered to be a nutritious and delicious food in developing countries [4]. They have high export value; therefore their consumption containing radioelements could lead to very high internal radiation dose [5-7].

The concentration of ^{210}Po ($t_{1/2}$ = 138.4 days) and ^{210}Pb ($t_{1/2}$ = 22 years) in marine ecosystem

has received much interest from the scientific community because of the high toxicity and radioactive dose compared to anthropogenic radionuclides [8]. These radionuclides get accumulated in the edible portions of marine organisms, and are considered to be the most important contributor of radiation dose received by humans via fish and shellfish consumption [9]. The ^{210}Po in marine organisms is generally derived from the food chain [10], and significant variations are noted in its accumulation in different species. Although sufficient data of ^{210}Po and ^{210}Pb have been generated in the marine environment of various regions, sufficient data are not available for different marine molluscs. Thus the objectives of this study were: (i) to find out the distribution of ^{210}Po and ^{210}Pb in the whole body and soft tissues of intertidal gastropod, mollusc *Littorina sp* and, and (ii) to establish the internal and external exposure to the species using ERICA Model. This study will provide significant baseline data for this region where a mega nuclear power hub is getting established (4 × 1000 MWe).

2. MATERIALS AND METHODS

2.1 Sample collection and Processing

The study was carried out along the coastal regions of Kudankulam (8˚11’34”N, 77˚42’25”E) located in the southeastern coast of peninsular India, in the distal end of the Gulf of Mannar Biosphere Reserve (Figure. 1). Kudankulam is very close to one of the major tourist destinations of south India – Kanniyakumari. The mollusc *Littorina sp* (n=300) was handpicked from the intertidal rocky zone along the shore using a metal scalpel. The collections were made at three seasons, pre-monsoon (July to September), monsoon (October to February) and post-monsoon (March to June), from July 2011 to June 2012. In the laboratory, the specimens were maintained in seawater for three days for depuration. After mortality, the samples were washed thoroughly with tap water to remove the external debris, sand and silt. The morphometry of the animal was measured with a caliper scale and weighed. Specimens from each species were separated into different groups based on their shell length. Certain specimens were randomly selected and analysed whole and the remaining dissected out to remove the tissues. Tissues of interest were pooled from all the specimens representing each species, weighed and dried in an oven at 105 °C. The dried samples were homogenized and packed in polythene bags. Seawater (~100 L) samples were collected from the coastline of the nuclear power project site and from nearby sites and also from inshore to derive the biological concentration factor (BCF) and exposure assessment. Bottom sediment samples (2–3 kg) were collected at the place of collection of molluscs with the help of a Van-Veen grab sampler.

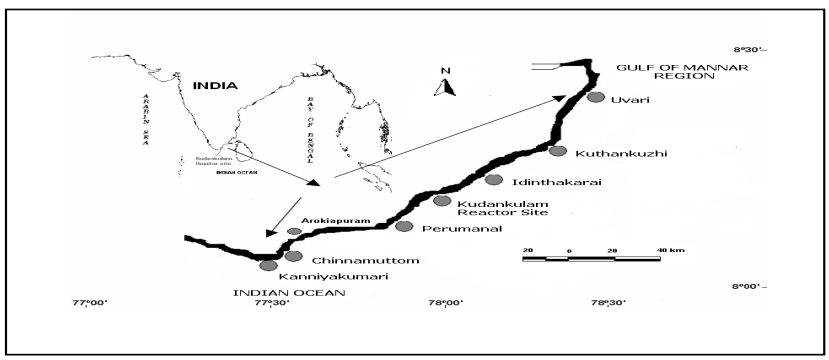

Figure 1: Map showing the study area

2.2 Determination of Radionuclides

The seawater sample, after collection, was filtered out using a 0.45-µm filter paper. The sample was made acidic to pH 1 using concentrated HCl and stirred for 3 hours along with ^{208}Po tracer (0.2 Bq). Fe carrier (500 mg) was added to this solution and once again stirred for another few hours. $Fe(OH)_3$ was precipitated at pH 9 by adding concentrated ammonia solution. The sample was kept overnight for settling; then the supernatant was decanted, and the precipitate collected after three washings and centrifuged. The final precipitate was digested using small amount of HNO3 and HCl mixture and after attaining incipient dryness, the residue was dissolved in 100 ml of 0.5 N HCl and filtered. To the filtered solution, 5 mg ascorbic acid, 5 ml of 20% hydroxylamine hydrochloride and 5 ml of 25% sodium citrate solution was added. The pH of the solution was adjusted to 1.5–2 with 1:5 ammonia. The solution was stirred on a hot plate by using a magnetic stirrer. A Perspex holder with a silver disk was immersed into the solution. The ^{210}Po deposition is continued for 6 h at a temperature 85–90°C, then disk is removed, washed with distilled water and acetone, dried and counted in an alpha counting system for 6000 sec (Nucleonix-make, efficiency 35% using ^{241}Am and ^{239}Pu standard, minimum detectable limit 0.02 Bq). A second deposition was carried out immediately in a new planchette to completely remove the polonium and tracer. The ^{210}Po deposition was made within 8 days from the time of sample collection and counted for its combined activity along with the uncertainty within next two days. Therefore, decay corrections were applied in the reported ^{210}Po values. The solution, after plating, was stored for 6 months to allow the ingrowth of ^{210}Po from its parent, ^{210}Pb. Subsequent determination of the ingrown ^{210}Po was carried out as described above [11]. The yield tracer ^{208}Po was added one day before the plating to achieve isotopic exchange. The ^{210}Po activity corresponds to that of its parent, ^{210}Pb, in the sample. ^{208}Po recovery was 94 ± 2% by this method. Quality was checked internally using the reference material, IAEA 315 within ± 1 SD of the reference value. Blank samples were analyzed for every 10 samples in terms of quality control as per USEPA [12]. The accuracy and precision of radiochemical determination of ^{210}Po and ^{210}Pb were estimated to be less than 8%.

5-10 g of each tissue sample was wet-digested using 70% concentrated HNO_3 followed by the addition of 40% H_2O_2 along with ^{208}Po tracer (0.2 Bq) [8]. This step was repeated three to four times until the colour of the residue changed from pale white to white. After complete digestion, nearing dryness, concentrated HCl was added to remove HNO_3, and the final residue was dissolved in 100 ml of 0.5 N HCl for the electrochemical deposition. The recovery of ^{210}Po was found to be 98 ± ^{210} ٪٢Pb was determined as explained previously.

The normality of the data set was checked using Lilliefors test (n≥50) and potential outliers, if any, were tested using Walsh's test (n>60) [12]. These tests were performed using ProUCL *v* 4.1. A mixed model 1 three-way analysis of variance (ANOVA) was used to test for group mean differences for each element by factors such as site (*A*), season (*B*) and whole body (*C*). Tukey's mean difference Bland-Altman test was the multiple comparison procedure used in conjunction with ANOVA to determine which mean values were significantly different from one another [13].

3. RESULTS AND DISCUSSION

On comparing the concentration of ^{210}Po and ^{210}Pb among various tissues, the hepatopancreas registered the maximum activity. The concentration was in the order: hepatopancreas > Intestinal tissues > soft tissue > shell. A significant allometric relationship was noted between the length and weight of all the species ($p < 0.05$, Figure. 2). The ^{210}Po and ^{210}Pb activities in the whole body of molluscs ranged from 20.4 ± 6.5 to 25.5 ± 9.9 Bq kg^{-1} and from 4.2 ± 1.8 to 5.2.7 ± 2.5 Bq kg^{-1}, respectively. The ^{210}Po was noted higher during monsoon (October to February) for all the species. Meanwhile, the accumulation pattern of ^{210}Pb showed non-uniformity between species and seasons (Figure. 3). Three-way ANOVA showed a significant variation in the concentration of ^{210}Po between season and whole body tissue. The results of the present study showed lower activity concentration on comparison with literature for different gastropods. The reason may be due to the animal's exposure duration to seawater, which is very low since they live most of the time in the rock–water interface and the entry of ^{210}Po might occur only through food. To study the influence of size on the whole body accumulation of ^{210}Po and ^{210}Pb, each species was categorized into different groups and the activities recorded. The activities were higher for the animals of the size group 0.4–0.9 cm and lower for the size group 1.9–2.2 cm. The observed information was concordant reported from Mandapam coast, Gulf of Mannar [14]. Previous research had also shown that radionuclide concentrations in mussels significantly decreased with mussel size, shell length or mussel weight [15-16].

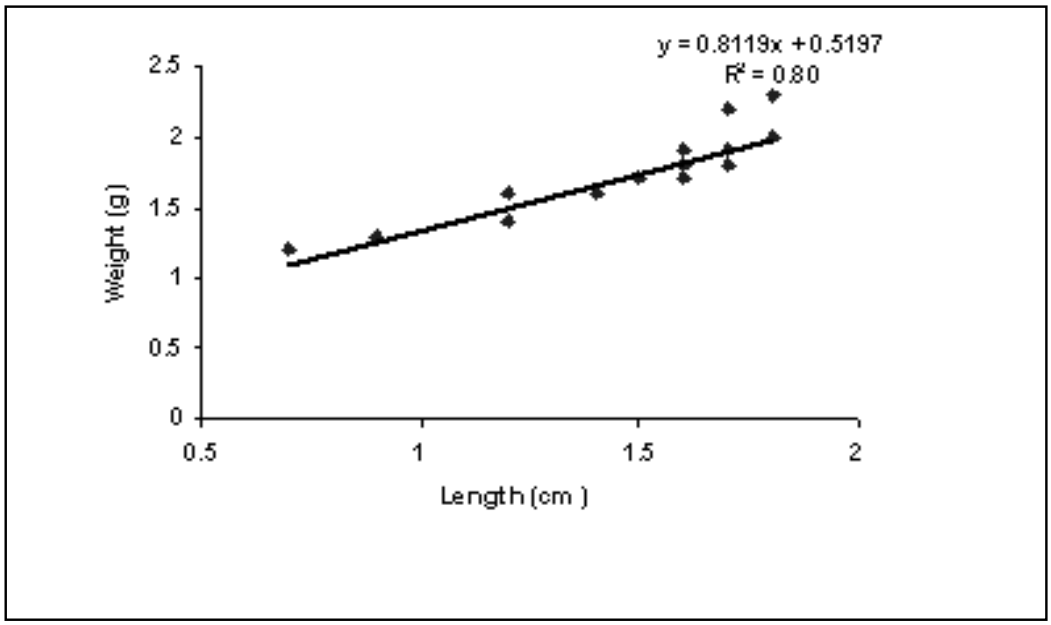

Figure. 2: Length-weight relationships obtained for *Littorina sp*

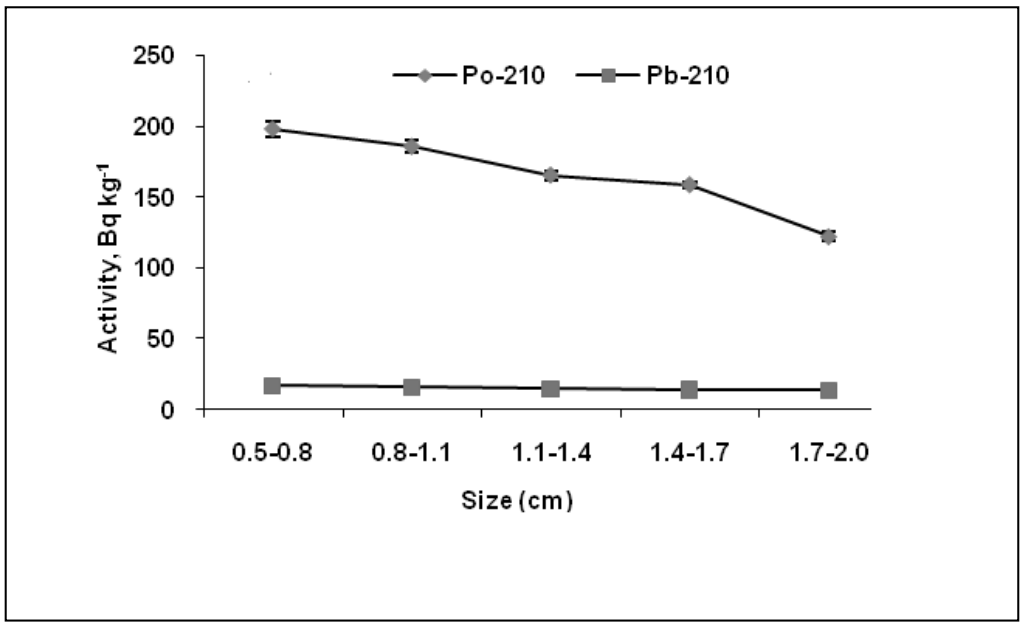

Figure. 3: Accumulation of ^{210}Po and ^{210}Pb in marine mollusc based on size

Table 1: ^{210}Po and ^{210}Pb (Bq kg^{-1}) in various tissues of Mollusc

Tissues	*C. radiate*	
	^{210}Po	^{210}Pb
Soft tissue (muscle)	22.3 ± 5.2	4.5 ± 1.9
Shell	14.6 ± 3.7	19.6 ± 3.5
Hepatopancreas	754.5 ± 15.9	47.8 ± 2.4
Gills	16.9 ± 1.5	5.9 ± 1.1
Intestinal parts	175.8 ± 6.5	6.9 ± 1.5

The highest ^{210}Po levels recorded in the hepatopancreas of molluscs may be due to the fact that this organ exhibits intracellular digestion by absorbing food from the stomach. The assimilation of radionuclides depends upon the digestive physiology of individual organisms [17]. The digestive gland of the mussel accounts for approximately 10% of the total soft tissue weight but contains between 15% and 36% of the ^{210}Po soft tissue inventory and this observation is concordant with that of Wildgust et al. [18]. These authors suggested that environmental fluctuations of ^{210}Po are better reflected in the digestive gland than the whole soft tissue because this organ is the major entry point of particle-bound ^{210}Po. On the other hand, the shell of molluscs tends to concentrate less ^{210}Po when compared with other organs is due to no secretary function other than protection. In the marine ecosystem,

the accumulation of ^{210}Po in the marine biota reflects the trophic position of the species in the food web rather than the geographic location, water depth, or other environmental parameters whereas, the behaviour of ^{210}Pb in the food chains seems to be slightly different. ^{210}Pb concentrations do not increase from phytoplankton to copepods as much as ^{210}Po, and ^{210}Pb transfer to planktivorous fish, such as sardines, is less efficient than ^{210}Po transfer. The enhancement of ^{210}Po concentrations is very pronounced not only in biota feeding upon bacteria and phytoplankton at the base of the food chain, such as the small zooplankton, but also in large organisms, such as mussels (observed in the present study also) and sardine fishes.

Using measured ^{210}Po and ^{210}Pb levels, dose assessment was performed using the Tier 2 ERICA Environment Dose Assessment tool 1.0 [19]. This model calculates internal dose rates to the molluscs from the uptake of radionuclides and external dose rates from irradiation by seawater and sediment. Input to the model includes activity concentrations of medium and biota, concentration factors, occupancy factors, dose conversion factors and dimension. All the species of molluscs were assumed to have 100% sediment surface occupancy as they live on rock surfaces and are always kept wet by spray. The partition coefficient of ^{210}Po and ^{210}Pb was assumed to be the same for rock as well as for coastal sediments.

The shapes of the organisms were assumed to be elliptical and the densities of the animals were assumed to be 1 g ml^{-1}. On the basis of our measurements, the weighted average dose rate from internal exposure of ^{210}Po and ^{210}Pb varied from 3.4 to 5.23 μGy h^{-1} and from 0.0021 to 0.0040 μGy h^{-1}, respectively. The expected risk quotient calculated for molluscs ranged from 0.33 to 0.54 and it is lesser than the screening value of 1. The molluscs are considered environmentally safe from the ionizing radiation exposure. The calcul;ated values are also lesser than the guideline value, such as the PNEDR (Predicted-No-Effect Dose Rate) i.e. the potential value below which it is accepted that a chronic dose has no effect at the population level, reported to be 10 μGy h^{-1} for aquatic ecosystems [20-21].Thus in the present study, the contribution of ^{210}Po only to the chronic irradiation may be nearly 50% of this value.

4. CONCLUSION

The present study revealed a significant amount ^{210}Po and ^{210}Pb in molluscs. Hepatopancreas was found to be the indicator tissue and it can be considered as a critical organ for ^{210}Po accumulation. A comprehensive picture on radiation exposure to marine mollucs and the simplest calculation methods were elaborated. Though certain marine mollucs are considered as “biodicators” the species considered in the present study would help the nuclear authorities for regular monitoring purpose in order to run the emerging Nuclear power plant ecofreindly.

5. ACKNOWLEDGMENTS

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PHYSIOCHEMICAL PROFILING OF ENVIRONMENTAL SAMPLES FROM GOLD MINING IMPACTED AREAS FOR TOTAL MERCURY DETERMINATION IN RANDFONTEIN, SOUTH AFRICA.

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ABSTRACT

Physiochemical characteristics of a typical mine impacted environment have a potential to influence the mobility, toxicity and bioavailability of mercury in the environment. Therefore, profiling chemical constituents of environmental samples is an important initial step towards accurately quantifying mercury concentrations in such environment. Soil, sediments and water samples obtained from gold mining environment affected by the legacy use of mercury amalgam were examined for possible anions, trace metals and pH using ion chromatograph (IC), flame atomic absorption spectrometry (FAAS) and pH meter, respectively. Elevated concentrations of sulphate and chloride were determined, for sulphate they ranged from 0.85-71.22 mg/kg, 4.85-295.32 mg/kg and 18.48-432.86 mg/l on soil, sediments and water, respectively. Chloride concentrations ranging from 2.06-3.89 mg/kg, 1.987-12.71 mg/kg and 43.19-132.09 mg/l were measured in soil, sediments and water, respectively. Similarly, elevated mean average concentrations of the following trace metals: Cd, Co, Cr, Cu, Ca, Ag, Al, Au, Fe, Mn, Zn, ranging from 4.42-152.19 mg/kg, 1.47-1249.49 mg/kg and 2.04-529.27 mg/l on soil, sediments and water were measured. The pH on soil, sediment and water samples ranged from 2.92-6.59, 3.62-6.59 and 2.49-8.17, respectively. Mercury concentrations increase with decreasing particle size and distance from the tailings dam, but increased with increasing sampling depth. The wide range of concentrations observed has the potential to influence the mobility, toxicity and bioavailability of mercury species, particularly in acidic environment such as gold mining affected area. From the current study, the physiochemical characteristics of the environment may have greatly influence the mobility, toxicity and bioavailability of mercury in the environment. Therefore, studies on mercury and its speciation products should endeavour to study physiochemical parameters in order to obtain a holistic view of the state of mercury and its speciation products in different environmental compartments.

KEY WORDS: physiochemical profiling, mercury, gold mining, legacy amalgam

1. **INTRODUCTION**

In view of the mercury poisoning incidences reported throughout the world, the United Nations Environment Programme (UNEP) and Governing Council (GC) initiated a global assessment of mercury in 2001 and this has precipitated the Minamata Convention on mercury. Since then, UNEP through its wings has undertaken to increase the understanding of different processes contributing to the release of mercury into the environment as these are critical to the development of relevant and cost-effective strategies towards the reduction of this global pollutant.

There are different sources of mercury that significantly contribute to global atmospheric pollution accounting for, approximately 1, 960 tonnes of mercury released into the environment in 2010 (UNEP 2013). Gold production from large and small scale mining in Africa for instance accounts for about 45% of the total mercury emissions in the world (UNEP 2013). In some gold mining regions, mercury exists as an associate of gold ores (Gustin et al. 2003; Kitula 2006). Coincidentally, mercury has also been extensively employed during the process of separating gold from its ores, where it forms a solid like compound with gold, known as "amalgam". During these mining activities, substantial amount of mercury were often released into the environment. Although majority of countries have abolished the use of amalgam process in gold processing activities, it is still being widely employed in some countries, particularly those where artisanal small scale gold mining is still being practised (Qui et al. 2012). Although mercury is a global pollutant of concern with varied sources, effects and pathways which have been studied and well documented (Makiese et al. 2013; Lecce et al. 2014), so far, inadequate studies have been conducted to understand the physical, biological and chemical factors that may influence the concentrations, distribution and toxicity of mercury in the environment, particularly in those found around gold mining affected areas where mercury amalgam was previously used.

Randfontein is one of the most impacted communities around gold mining areas in South Africa. This community is highly populated, especially with people belonging to the mid- to lower end of the country's socio-economic stratification. Presently, there is still inadequate information on physiochemical parameters of the environment, especially those that might influence the toxicity of mercury to exposed residents as well as the ecosystem. The physiochemical profiling of environmental samples is, however considered important in order to have a holistic view of the state of mercury and its speciation products in different environmental compartments since these can significantly influence the mobility, toxicity and bioavailability of mercury in the environment.

The study presents preliminary findings on the ongoing work towards the determination of mercury and its speciation products in environmental samples. The concentrations of selected anions, trace metals and the pH of soil, sediments and water samples collected from gold mining impacted areas in Randfontein were determined. Furthermore, the possible influence of various physical parameters on the concentrations of mercury, and in relation to particle size, sampling depth and distance from the tailings dam were also evaluated.

2. MATERIALS AND METHODS

2.1 Standards, reagents and apparatus

2.1.1 Standards and reagents

Standard solutions of Cd, Co, Cr, Cu, Ca, Ag, Al, Au, Fe, Mn, Zn standards for AAS supplied by FLUKA Analytical (Switzerland) and Pb standard supplied by MERCK (Germany) were used throughout the experiments. For anions multi-element standard VII (Merck, Germany) and certified multi-anions standard solution PRIMUS (Fluka, Switzerland) containing F^-, Cl^-, Br^-, NO_3^-, SO_4^-, PO_4^- were used to calibrate the instrument.

To digest the samples for trace metals determination, the following reagents: 70% nitric acid (HNO_3) , 37% hydrochloric acid (HCl) purchased from Sigma-Aldrich (Germany), and 30% hydrogen peroxide (H_2O_2) from SSM Instruments (Pty) Ltd (South Africa) were used. De-ionised water prepared in the laboratory using SG Series Compact purchased at Evoqua water technologies (United Kingdom) was used throughout the experiment.

2.1.2 Apparatus

All the glassware used was washed with a detergent soap, rinsed with water, soaked for overnight in 10% HNO_3, rinsed three times with de-ionised water and dried in a clean oven to eliminate contaminants (Losilao-Makisie, et al., 2012, USEPA, 1996).

2.2. Sample collection

The study area is situated in Randfontein located in south-western part of Johannesburg (30 Km West) close to Krugersdorp, in the province of Gauteng, South Africa. The geographic position of the study area is around S26° 12' 20.49" and E27° 47' 02.86" (Geographic coordinates, WGS84). For the purpose of these experiments, soil samples were obtained from tailings dam and surrounding environment where 12, 7, 7 samples for soil, water and sediments were collected, respectively. Soil was collected using acid-washed Ziploc bag and covered with black plastic bag as a commonly acceptable sampling procedure (USEPA, 1996, 2007) was implemented. Furthermore, soil was collected at different depths (<5cm and >10cm) and at different distance (30m and 60m) from the tailings dam. The samples were collected using polyethylene bottles which were previously soaked overnight in 10% HNO_3 and rinsed with de-ionised water three times. Soil and sediments were frozen while water sample were preserved with 1% $HNO_3$and refrigerated at 4^{o}C prior to further analysis.

2.3. Sample preparation and digestion

Soil and sediment sample were air dried for 24 h. The dried samples were gently macerated by hand to separate the lumps and sieved using stainless steel sieve of different sizes ranging from 45-630 µm. The fractions were subsequently subjected to particles size analysis. Samples of <45 µm were used for all other analysis of soil and sediments. For the determination of anions, USEPA method 300.0 was employed, where 1 g of soil and sediment sample was placed in a beaker and 100ml of de-ionised water was added and stirred with a magnetic stirrer for 15 min. The sample was thereafter filtered with a filter paper and finally with a 45 µm syringe filter. A similar filtration process was employed for the water samples. A portion of the unfiltered soil and sediment samples was used for the pH determination (Technical resource library, 2015).

To determine trace metals, acid digestion procedure for preparing soil samples for analysis of trace metals by FAAS outlined by USEPA (1996) was applied. About 1.5 g of soil and sediments was weighed and carefully transferred into a 250ml conical flask (and 100 ml was used for water samples). About 10ml of de-ionised water and HNO_3 were added and heated using a hot plate without boiling for 15 min. Sample was then allowed to cool before an additional 5ml of HNO_3 was added and heated initially for 30 min to achieve total decomposition and further heated for 2 h without boiling. The set-up was then allowed to cool down to room temperature. After cooling, 2 ml of de-ionised water and 3 ml of HCl was added. Thereafter, 1 ml (x3) of H_2O_2 was added consecutively to minimize the effervescence, and then heated for 2 hours without boiling and allowed to cool. After cooling, the sample was filtered into a 100 ml graduated volumetric flask and filled with de-ionised water to the graduation mark while rinsing the filter. The experiment was performed under the fume hood and samples were covered with a watch glass.

For total mercury analysis, acid digestion procedures for preparing solid, semisolid and water samples for analysis of mercury by CVAAS as outlined in USEPA methods 7471B and 245.1 were applied, respectively. Briefly, sieved and thoroughly homogenized samples without applying intense force to break large particles were used. About 1 g of soil was weighed and carefully transferred into a 250 ml round bottom flask (and 100 ml was used for water samples). Working under the fume hood and treating each sample individually, 10 ml of ultra-pure water was added. This was followed by the addition of 5 ml of aqua regia. The flasks were then sealed with a foil to prevent early loss of mercury vapour and heated for two minutes at 120^{o}C. The aliquots were allowed to cool down and 50ml of ultra-pure water was added. This was followed by the addition of 15ml of freshly prepared potassium permanganate solution. The set-up was left for 15 min to allow for complete oxidation of mercury. Specifically, for water samples, 8ml of potassium persulfate was added at this stage. Thereafter, the flasks were mounted with condensers and were sealed with foil to prevent any potential loss of mercury vapour. All the flasks were covered with glass wool within the heating chamber to ensure even distribution of the applied heat. The samples were then heated for 3 h, cooled and finally, 6 ml of sodium chloride-hydroxylamine hydrochloride was added to reduce excess potassium permanganate. The samples were then made up to the 250ml mark and kept in the refrigerator until instrumental analysis.

2.4. Quality assurance

Due to unavailability of certified reference materials for the measured elements of interest, a quality check was conducted by spiking a known amount of certified reference standard into a leached soil and ultra-pure water. An undigested 1 g of leached soil and 100 ml of ultra-pure water were spiked with 5 mg/l (anions), 1 mg/l (trace metal), 1 µl (mercury). From the measurements obtained, the percentage recoveries were calculated. The quality assurance measures used in this study include the analysis of blanks with each sample group. All the samples were also prepared in triplicate and from the triplicate measurement the mean as well as standard deviations were estimated. The instrument's detection limits were determined by analyses of low and high concentrations of the element of interest.

3. RESULTS AND DISCUSSION

3.1. The potential influence of anions

The mean concentrations of the selected anions are shown in Figure 1. In this case, sulphate had the highest concentrations ranging from 0.85-71.22 mg/kg, 4.85-295.32 mg/kg and 18.48 to 432.86 mg/kg for soil, sediment and water samples, respectively. Incidentally, the soil and sediment samples collected from the tailings dam generally had the highest concentrations. Interestingly, water samples did not show the same trend, although exhibited the higher sulphate concentrations than those obtained in soil and sediment samples. The sulphate levels obtained in the current study has a number of potential effects of the mobility, toxicity and bioavailability of mercury in the environment. First, mercury could bind with sulphur to form mercuric sulphide, which is a stable and less soluble compound. Consequently, mercury can be immobilized in the environment for an extended period if it binds to the compound under normal conditions. Second, the sulphate-rich mineral in tailing dams could support the growth and survival of sulphate-reducing bacteria, which play an important role during the methylation of mercury into methylmercury. Under anaerobic conditions, sulphate-reducing bacteria are the major groups involved in methylation of mercury species into methylmercury (Troung et al. 2013; Zheng et al. 2013). By so doing, the presence of sulphate could trigger the conversion of mercury from its less toxic forms into its most toxic form.

The measured chloride concentrations ranged from 2.06-3.89 mg/kg, 1.99-12.71 mg/kg and 43.19-132.09 mg/l for soil, sediment and water samples, respectively. Chloride is known to have several influences on the mobility, toxicity and bioavailability of mercury in the environment. For instance, the oxidation of mercury is highly dependent on the amount of chlorine present and less on the amount of other anions such as bromide and fluorine (Serre and Lee 2009). Furthermore, chloride concentration of about 20 mg/kg can influence the mobility of mercury because they have high affinity for mercury to form mercuric chloride. Mercuric chloride is highly soluble and can compete with hydroxide ions (OH^-) and other organic ligands for mercury binding (Xu et al. 2014). The solubility and affinity of mercury compounds determines their concentration and mobility in the environment. Mercuric salts such as mercury chloride, mercury (I) hydroxide and mercuric sulphide vary widely in solubility. For example, mercury chloride is readily soluble in water, while the mercuric sulphide is insoluble but soluble at pH less than 3.5 (USEPA 1997). Bromide and fluoride have also been shown to influence mercury speciation mainly because they act as ligands in complexation reactions (Ochoa-Gonzalez et al. 2013). In this study, the observed nitrate concentrations were relatively low and they ranged from 0.16-3.63 mg/kg, 0.4-76.57 mg/kg and 0.1-31.33 mg/l on soil, sediment and water, respectively. In contrast, nitrate concentrations in water were relatively lower than in sediments. Incidentally, a similar trend was observed on nitrite, where concentrations ranged from 0.006-0.013 mg/kg on soil, 0.01-76.57 mg/kg on sediments and below detection limit on water samples. Presently, it is not yet clear if both nitrate and nitrite have any direct effect or influence on the mobility, toxicity and bioavailability of mercury in the environment.

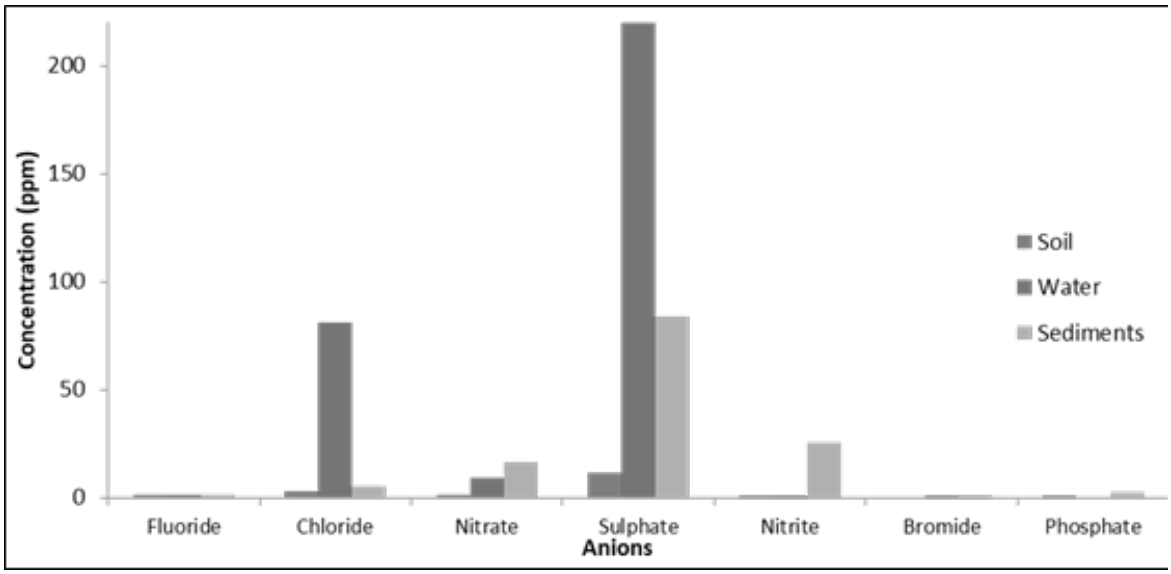

Figure.1: Overall mean average concentrations of anions in soil, water and sediment samples

3.2. The potential influence of trace metals

Elevated concentrations of trace metals have the potential to influence the mobility, toxicity and bioavailability of mercury in the environment. Figure 2 shows the measured concentrations of several trace metals in an environment that have been impacted by gold mining operations. Not so surprising, Iron (Fe) had the highest mean concentration possibly due to its natural abundance in the earth crust. The measured Fe concentrations ranged from 65.13-81.86 mg/kg, 46.95 to 1452.13 mg/kg and 0.53 to 373.27 mg/l for soil, sediment and water samples, respectively. The presence of Fe at elevated concentration is known to have a great influence on the concentration of mercury and its speciation products in the environment. The presence of Fe can adequately enhance the effectiveness of certain iron-reducing bacteria, because it can be oxidised to ferric (Fe(III)), which acts as a terminal electron acceptor that provides sufficient nutrients and energy to methylate mercury into methylmercury (Skyllberg 2010; Hines et al. 2012; Randall et al. 2013). Under anaerobic conditions, similar to sulphate-reducing bacteria, iron -reducing bacteria can be actively involved in the methylation of mercury species (Troung et al. 2013; Zheng et al. 2013).

Manganese (Mn) concentrations ranged from 6.1 to 259.74 mg/kg, 8.57 to 253.57 mg/kg and 0.53 to 372.27 mg/l for soil, sediment and water samples, respectively. Mercury might be sorbed to oxides (Mn, Fe) to form mercury oxides, which is a weak acid soluble compound. Trace metals such as lead (Pb), uranium (U), silver (Ag), zinc (Zn), cadmium (Cd), copper (Cu), manganese (Mn), amongst others, which are common in gold ores and legacy amalgam affected areas can also compete with mercury for complexation. Hence, they might subsequently influence the mobility, toxicity and bioavailability of mercury in the environment.

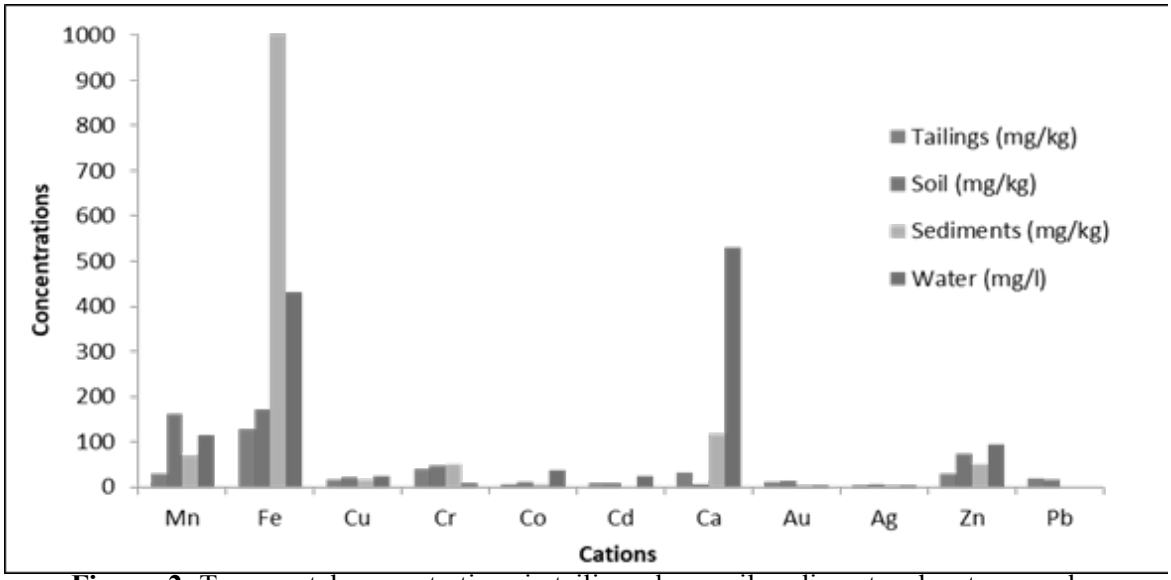

Figure. 2: Trace metal concentrations in tailings dam, soil, sediment and water samples

3.3. The influence of pH on mercury1

One of the major factors controlling the mobility, toxicity and bioavailability of mercury species in the environment is pH (Boszke et al. 2002 and 2003). Yang et al (2007) reported that soil pH values have been demonstrated to be the most important factor which can influence mercury speciation under various environmental conditions. Elevated pH increases the concentration of negatively charged hydroxyl ions (OH^-), which might attract and retain divalent mercury (Xu et al. 2014). Whereas, low pH is characterised by high positively charged hydrogen (H^+) ions which play a direct role in several reactions, hence reaction rate is pH dependent (Buss et al. 2005). For example, under neutral or alkaline conditions, the Fe^{3+} is precipitated as ferric oxide or oxyhydroxide. This reaction releases H^+ ions into the environmental solutions that further reduce other mercury containing compounds (Buss et al. 2005). Moreover, at low pH ranging from 1 to 3.5, stable compound such as mercuric sulphide are soluble and are dissociated. Hydrolysis of mercury increases with increasing pH, and mercuric (II) hydroxide ($Hg(HO)_2$) has been reported to be increasingly adsorbed by soil constituents from pH 5 to 9 (Farrah and Pickering 1978). Figure 3 shows the measured pH for soil, water and sediment samples collected from a gold mining impacted environment. The lowest pH was measured on tailings dam soil ranging from 2.92 to 3.97. The low pH on tailings dam is known to be due to the formation of acid mine drainage which is a major concern on abandoned gold mines. However, the pH obtained for soil from the surrounding community ranged between 4.63 and 6.59, and 3.56 to 8.17 for water samples, and 3.56 to 6.59 for sediment samples.

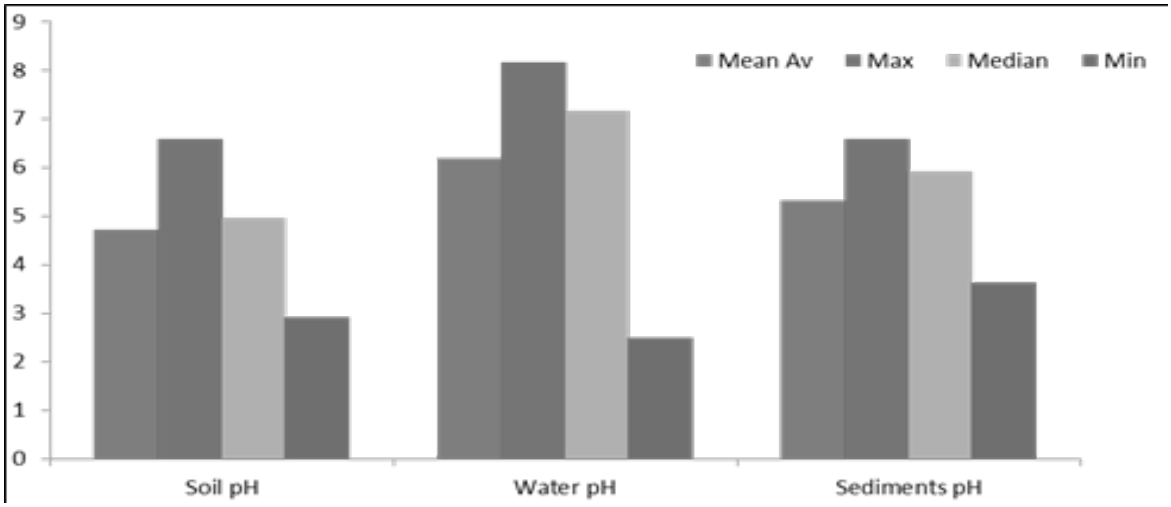

Figure. 3: Overall levels of pH obtained for soil, water and sediment samples

3.4. The influence of particle size

The possible influence of particle size on the levels of mercury in both soil and sediment were evaluated and the correlations between measured particle size and Hg concentrations are presented in Figure 4. It was observed that mercury concentrations generally decreased with increasing particle size. Consequently, fine particles could contain greater levels of mercury as observed in this study. Fine particles are easily eroded. Furthermore, suspended fine particles are easily inhaled by human and animals, especially those living in proximity to gold mine tailings dam. Since these fine particles are often laden with elevated levels of different mercury species, the possible inhalation of these particles may present an important exposure pathway to the toxic effects of mercury, especially amongst residents living in close proximity to the abandoned tailing dams. The observed total mercury concentrations ranged from 0.005-1.19 µg/g and 0.006-0.44 µg/g on soil and sediment samples, respectively. Finer particles are know to be easily eroded, and they can be transported over a long distance compared to larger particles, thus particle size have the potential to influence the mobility and distribution of mercury in the environment (Higueras et al. 2012; Hines et al. 2012).

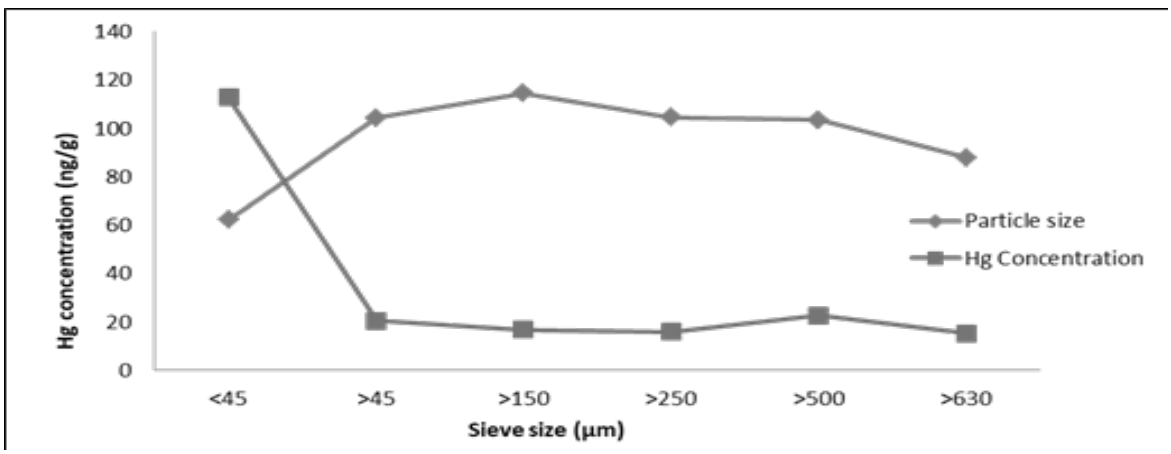

Figure 4: Mercury concentrations increases with decreasing particle size

3.5. Assessing the vertical distribution of mercury

To assess the effect of leaching on vertical distribution of mercury, samples were taken at different depths (surface <5 cm and below >10 cm), sieved by different aforementioned sizes and were analysed to determine their mercury concentrations. In this study, mercury concentrations were found to increase with increasing depth, thus great amounts of mercury are being leached into underlying soil, which may eventually get transported into the groundwater. In some soil profile, parent material and C-horizon contain a small amount of mercury than O- and A-horizons (Wang et al. 2012). However, studies have shown that soluble mercury generally increases with increasing depth and it can be leached into deeper layers mainly in the form of soluble organic and inorganic mercury (Wang et al. 2013; Teršičet al. 2014). Generally, large amounts of mercury can be leached until they reach the groundwater (Makiese et al. 2013), although this depends on the soil texture, type, constituents and available mercury species (Rodríguez et al. 2012; Xu et al. 2014).

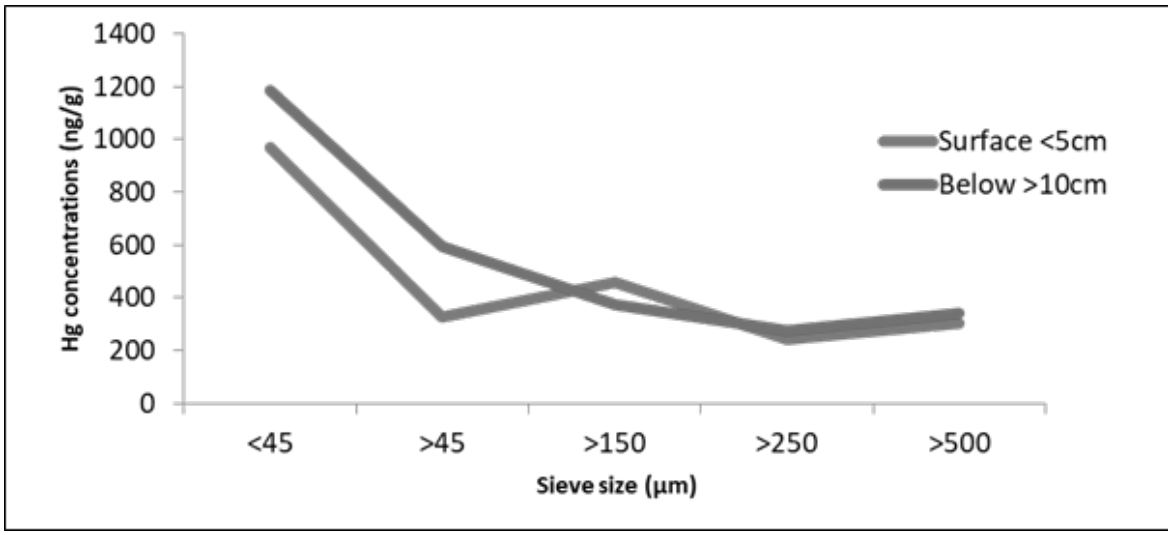

Figure 5: Mercury concentrations increase with increasing sampling depth

3.6 Assessing the influence of distance on total mercury concentrations

As shown in Figure 5, samples were taken at equidistance from the tailings dam towards different directions. On the western direction, concentrations of total mercury ranged from 0.60 µg/g at 30m (A) to 0.74 µg/g at 60m (B), and 0.64 µg/g at 30m (C) to 1.28 µg/g at 60 m (D) from the tailing dam towards the eastern direction. Generally, mercury concentrations increase with decreasing distance from the tailings dam, therefore tailings dam are potential sources of mercury in the environment. The concentrations were also found to decrease with increasing depth, hence underground water may be highly impacted by mercury from gold mine tailings dam over time.

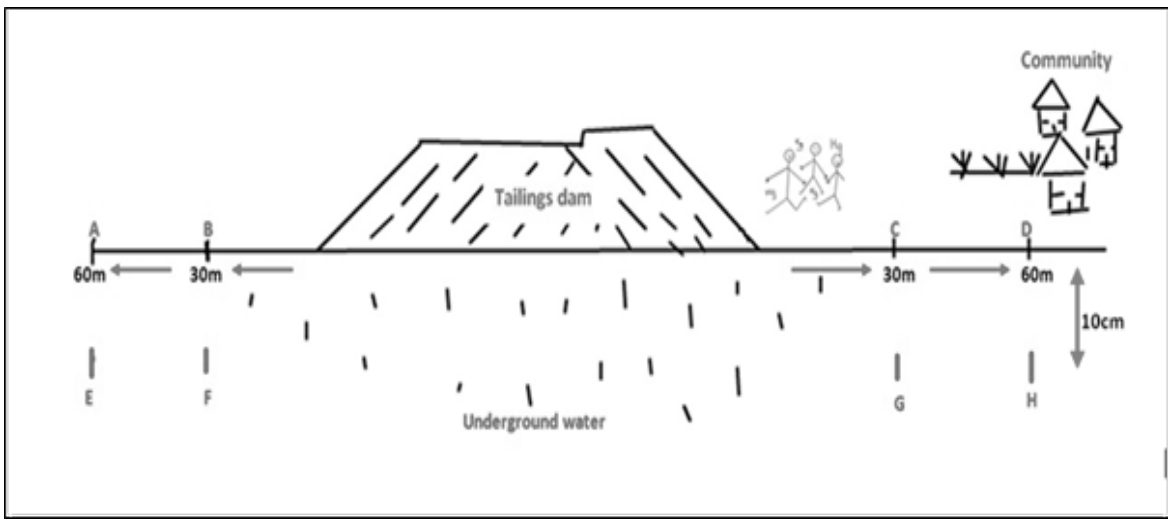

Figure 6: Mercury concentrations decrease with increasing distance from tailings dam and decrease with increasing depth

4. CONCLUSION AND RECOMMENDATION

A number of chemical parameters, including sulphate, chloride, iron and manganese were measured at high concentrations compared to other anions and trace metals, especially on samples from the tailings dam. Tailings dams were created as a result of mining operation and these were identified to be an important source of certain anions and trace metals, some of which are known to be harmful to human and the environment. Essentially, the chemical constituents do have the potential to influence mercury mobility, toxicity and bioavailability of mercury in a number of ways. Furthermore, some physical parameters such as particle size, sampling depth and distance from tailings dam could also influence the concentrations and distribution of mercury in the environment. It was observed that mercury concentrations increase with increasing depth and but decrease with increasing distance from the tailings dam. Higher concentrations of mercury were also observed in finer particle size (<45 mic), thus communities leaving in proximity to tailings dams are particularly more vulnerable to toxic effects associated with mercury species. It is recommended that studies on mercury and its speciation products should incorporate the characterization of physical, chemical and biological parameters in order to obtain a holistic view of the state of mercury and its speciation products in different environmental compartments.

5. ACKNOWLEDGEMENTS

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SYNTHESIS AND CHARACTERIZATION OF 2-HYDROXYACETOPHENONETHIO -SEMICARBAZONE NICKEL (II) COMPLEX AS INOPHORE FOR THIOCYNATE-SELECTIVE ELECTRODE

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ABSTRACT

In the field of Electrochemistry, potentiometery provides different analytical techniques to measure the voltametric outputs. The potentiometry equipped with Ion selective electrode (ISEs) more precisely used to measure different pH values by investigators so far. With the increase in population by which increase in pollution suggest us to use the ISEs as an important tool in the treatment of wastes and determination of blood electrolytes, have a market size comparable to that of glass electrodes. The lowest count to detect and the discrimination of interfering ions (the selectivity coefficients) have been improved in many cases by a factor of up to 10^6 and 10^{10}, respectively.

The main aspect of my presentation is synthesis of the ionophore, characterization of ligand (ionophores) synthesized, membrane preparation, fabrication and optimization of the membrane, determination of electrode response or membrane potential, effect of pH and response time, selectivity study and practical application of ion selective electrodes. The proposed electrode demonstrated higher selectivity for nitrate ion with improved performance as compared to other carriers reported in past. The electrode shows Nernstian slope of -57.8 ±0.4 mV decade^{-1} with improved linear range of 1×10^{-1} to 1×10^{-7} M, with a comparatively lower detection limit in the pH range of 3-10, giving a relatively fast response within 10 second and reasonable reproducibility. The selectivity coefficient was calculated using matched potential method. The electrode worked well for nearly 45 days.

1. INTRODUCTION

Thiocyanate (SCN^-) is a sulfur containing compound. It can be produced naturally either as either a non-functional detoxification product of cyanide ingestion or a defense compound against microbial infection. Thiocyanate was used in a number of industrial processes, such

as photofinishing, herbicide and insecticide. It was also used as dyeing agent, acrylic fiber production, manufacture of thiourea, electroplating and as corrosion inhibitiors [1]. The main source of thiocyanate is plants, biotic and abiotic decomposition of organic matter, and in vivo detoxification of cyanide. Thiocyanate ion is also present in humans as a result of the digestion of some vegetables, and as a metabolic product of compounds in tobacco smokes containing cyanide [2,3]. Due to this the concentra-tion level of thiocyanate is considered to be a good probe to distinguish between smokers and nonsmokers. It has been found that there is a correlation among the blood cyanide, the plasma thiocyanate, and the salivary thiocyanate [4].

Keeping in view the wide range of applications and the associated hazardous effects, monitoring of thiocyanate ions at trace levels is of utmost importance and requires immediate attention. In the litera-ture, there are a number of reports regarding estimation of thiocyanate ions in diverse matrices employing a variety of classical and instrumental methods like spectrophotometry [5], colorimetric analysis [6], chromatography [7], polarography [8], capillary zone electrophore-sis [9]and amperometry[10]. Unfortunately, trace level estimation of thiocyanate ion employing these methods gets seriously hampered due to involvement of complex, tedious, and time consuming methods of measurements along with the requirement of sophisticated and costly instrumentation. A simple and convenient method is thereby re-quired for the trace level estimation of thiocyanate ion which possesses properties like high sensitivity, selectivity, and fast response time. Recently, the trend of employing ion selective electrodes for the poten-tiometric estimation of a wide variety of analyte ions has increased and a number of ion selective electrodes for the selective cation and anion determination have been reported [11–20]. Potentiometric estimation using ion-selective electrodes (ISEs) offers unique advantages over the aforementioned methods in terms of some unique features like operational simplicity, easy sample preparation procedures, fast re-sponse time, wide linear concentration range, relatively better value of lower detection limit and enhanced selectivity for primary analyte ion in the presence of secondary ions. Some other important features of this technique include its non-destructive nature and inertness to the contamination, change in color, and turbidity of analyte ion solution. All these characteristic features make it a suitable and reliable tool in the hand of analytical chemists that can be employed for the estimation of analyte ions in samples of industrial and environmental origin.

The design of anion-sensitive membrane electrodes has been of great importance in the field of ion-selective electrodes (ISEs). The free energy contributions of the transfer of the anion from the aqueous solution to the membrane phase and the specific interaction of the anion with the receptor are fundamental factors for the selectivity of certain anion in ISEs.

The first factor that displays classical Hofmeister behavior for anion-sensitive membrane electrodes based on ion exchangers such as lipophilic quaternary ammonium or phosphonium salts is controlled by the free energy of hydration of ions involved [21,22]. Several polymeric liquid membranes potentiometric electrodes have been developed in the past [23,24]. Most of the thiocyanate-ion selective electrodes are based on vitamin B_{12} derivative [25], Rh(III) complex [26], metalloporphyrine [27], organomercury compound [28], Schiff base complexes of metal ions [29–32], phthalocyanines [33], imidepyridine [34], organozinc complex [35], zeolite [36] and nickel macrocyclic complex [37]. In all of these cases, the ligation of the prima-ry anion to the central metal ion is responsible for the observed selectiv-ity. Most of the thiocyanate potentiometric electrodes have a serious interfering affect of other anions, such as I^-, ClO^-_3, Cl^-, Br^- and IO^-_4.

Schiff's base metal complexes were studied extensively because of their attractive chemical and physical properties and their wide range of applications in numerous scientific areas [14]. The aim of the present work was to synthesized and characterized a Schiff base metal complex [2-hydroxyacetophenonethiosemicarbazone] nickel(II) and to develop a sensitive, accurate and selective electrode for thiocyanate ion determination. To this purpose, the modifications of the membrane composition were studied and their potential responses towards the thiocyanate ion were tested. Then the basic analytical parameters (Nernstian response, detection limit, solvent effect, pH dependence of potential, dynamic response time, selectivity, lifetime) of the construct-ed ion selective electrode was determined.

A great advantage of the proposed electrode is its simple and cheap construction. In addition, this kind of electrode is very comfortable to use. The proposed electrode was satisfactorily applied to the determination of thiocyanate ions in biological (urine and saliva) and environmental samples.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Reagent grade 2-hydroxyacetophenone, semicarbazide hydrochloride, plasticizersdi-butyl phthalate (DBP), dioctyladipate (DOA), di-octyl phthalate (DOP), 2-nitrophenyl octylether (NPOE), cation additive trioctylmethyl ammonium chloride (TOMAC), tetrahydrofuran (THF) and $AgNO_3$ were purchased from Merck and used as received. Poly (vinyl chloride) with high relative molecular weight was purchased from Fluka. A 0.1 M stock solution of thiocyanate was prepared by dissolving an appropriate and accurate amount of KSCN (Merck). A 1.0×10^{-8} to 1.0×10^{-1} M solution of thiocyanate was prepared daily by sequential dilution of the appropriate stock solution with doubly distilled water.

Sodium and potassium salts of anions (all from Merck) were of the highest purity available. All other reagents used were of analytical reagent grade and doubly distilled water was used throughout.

2.2. Synthesis of [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel (II) complex (ionophore)

The ligand 2-hydroxyacetophenonethiosemicarbazone was synthesized as reported earlier [38]. 2-hydroxyacetophenonethiosemicarbazone (2 mmol) and an ethanolic solution (20 mL) of nickel nitrate (1 mmol) were mixed together under constant stirring. This reaction mixture was refluxed at 75–80 °C for ~ 3 h. On keeping the resulting mixture overnight at 0 °C, the light green colored product of Ni(II) com-plex was separated out, which was filtered, washed with cold ethanol and dried under vacuum over P_4O_{10} (Figure. 1). $NiC_{18}H_{22}N_8O_{10}$: C, 37.98; H, 3.86; N, 19.19; Ni, 10.32. Found: C, 37.92; H, 3.81; N, 19.09; Ni, 10.28. Yield 68%, m.p. 247 °C.

2.3. Electrode preparation

The general procedure was used to prepare the electrode as reported earlier [11, 12, 20]. A thiocyanate selective polymeric membrane electrode was prepared by dissolving the mixture of fixed amount of ionophore, PVC powder, plasticizer and cation additive in the w/w ratio of 4% ionophore, 31% PVC, 63% NPOE and 2% TOMAC in 5 mL of THF. The resulting

mixture was evaporated slowly at ambient temperature until an oily concentrated mixture was obtained. A Pyrex tube (3-mm i.d. on top) was dipped into the mixture for about 10 s, so that a non-transparent membrane of about 0.3 mm thickness was formed. The tube was then pulled up from the mixture and kept at room temperature for about 6 h. The electrode was finally conditioned for 24 h by soaking in a 1.0×10^{-2} M KSCN solution before use.

2.4. EMF measurements

All electromotive force (emf) measurements were carried out at 25 ± 1 °C with a digital potentiometer (Model 5652 A, ECIL, India). Saturated calomel electrode (SCE) was used as a reference electrode. The electrochemical system is presented as follows:

Hg=Hg_2Cl_2 j KCl ð satd:Þj internal solution 1:0 10^{-2}M KSCN jj PVC membrane jj test solution j Hg=Hg_2Cl_2 jKCl ðsatd:Þ

3. PHYSICAL MEASUREMENTS

The stoichiometric analyses were carried out on a Carlo-Erba 1106 analyzer. ESI (Electrospray ionization) mass spectrum was recorded on a model Q Star XL LCMS–MS system. NMR (Nuclear magnetic resonance) spectrum was recorded with a model Bruker Advance DPX-300 spectrometer operating at 300 MHz using DMSO-d_6 as a solvent and TMS as an internal standard. IR spectra were recorded as KBr pellets in the region 4000–200 cm^{-1} on a FT-IR (Fourier Transform Infra-Red) spectrum BX-II spectrophotometer. The electronic spectrum was recorded on Shimadzu UV mini-1240 spectrophotometer. The molar conductance of complex was measured in DMSO at room temperature on an ELICO (CM 82 T) conductivity bridge. The magnetic susceptibility was measured at room temperature on a Gouy balance using $CuSO_4.5H_2O$ as calibrant. XRD (X-ray powder diffraction) was recorded on a Rigaku Dmax X-ray diffractometer with CuKa ra-diation (k = 1.5418 Å).

4. RESULTS AND DISCUSSION

4.1. Chemistry

The synthesized Schiff base ligand, 2-hydroxyacetophenonethiosemicarbazone coordinates to Ni(II) metal ion through nitrogen and oxygen. The molar conductance of the complex was measured immediately after the preparation of the solutions, corresponding to non-electrolyte behavior. However, on keeping the solutions for long time, DMSO replaces the anion and the complex give molar conductance corresponding to 1:2 electrolyte [39].

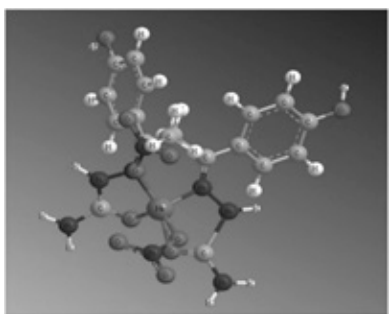

Figure 1: Structure of [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel(II) [Ni(L) $(NO_3)_2$] (Ionophore)

4.1.1. IR spectra

The IR spectrum of ligand (Figure. 2) shows two bands at 1685 and 1582 cm^{-1} which may be assigned to the ν(CO)and ν(CN) stretching vibrations, respectively [40–43]. On complex formation, the bathochromic shift of these bands indicates that the carbonyl and azomethine groups are coordinated to metal ion through oxygen and nitrogen atoms, respectively. This indicates that the ligand acts as bidentate coordinates through N, O donor atoms. The IR spectrum of Ni(II) complex (Figure. 3a and b) displays the new bands at 397 and 517 cm^{-1} which may be attributed ν(M–O) and ν(M–N) stretching vi-brations [44–46], respectively. IR spectrum of complex also displays the bands 1382 (ν_5), 1287 (ν_1) and 1165 cm^{-1} (ν_2) due to bonded anions.

The value of Δν ($\nu_5 - \nu_1$), i.e. 95 cm^{-1} suggests the monodentate coor-dination of NO^-_3 ions.

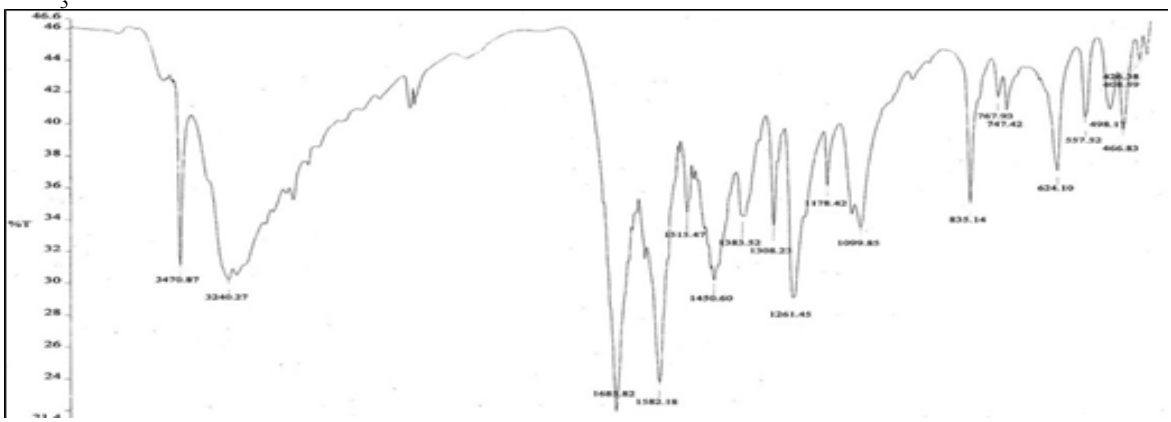

Figure 2: FT-IR [2-hydroxyacetophenonethiosemicarbazone nitrato]nickel(II) complex

4.1.2. Mass spectra

The electronic impact mass spectrum of the ligand (Figure. 4) and its [2-hydroxyacetophenonethiosemicarbazone nitrato]nickel(II) complex (Figure. 5) gives a final peak at 193 and 568.6 amu respectively which con-firms the proposed formula ($C_9H_{11}N_3O_2$ and $NiC_{18}H_{22}N_8O_{10}$, calculated atomic mass 193 and 568.7 amu)

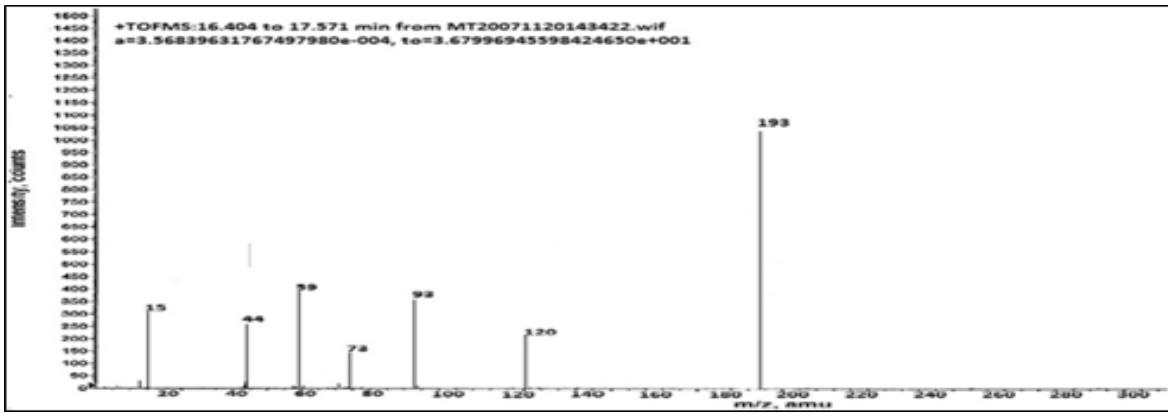

Figure 4: Mass Spectra of ligand

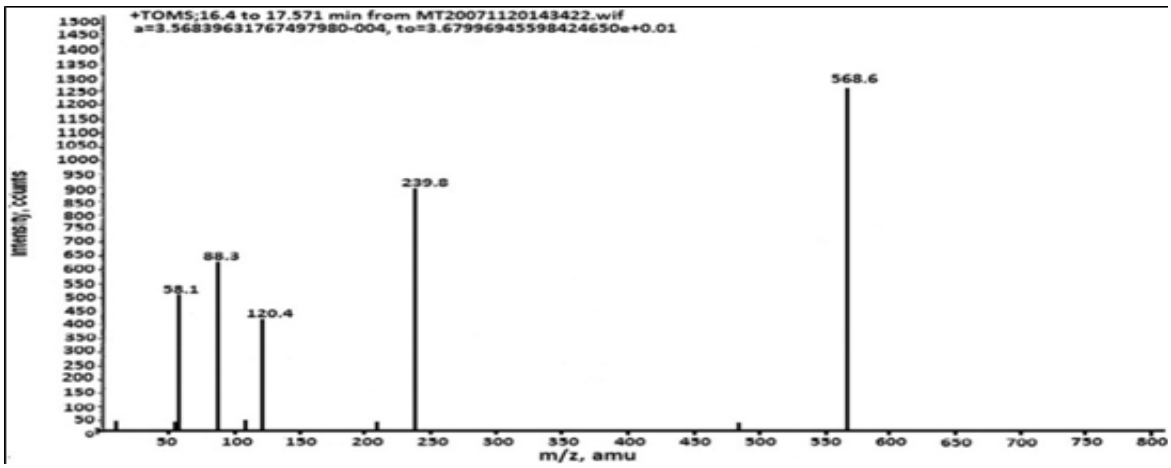

Figure 5: mass spectra of [2-hydroxy-acetophenonethiosemicarbazone nitrato] nickel (II) complex

4.1.3. Magnetic susceptibility and electronic spectrum

The magnetic moment observed for the [2-hydroxy-acetophenonethiosemicarbazone nitrato] nickel (II) complex is 2.96 BM which is consistent with the octahedral stereochemistry of the complex.

The electronic spectrum of the complex was recorded by using DMSO as a solvent. The electronic spectrum of [2-hydroxy-acetophenonethiosemicarbazone nitrato] nickel (II) complex displays three d–d absorption bands at 10,810 cm^{-1}, 18,621 cm^{-1} and 23,980 cm^{-1}. The ground state of nickel(II) in an octahedral coordination is $^3A_{2g}$. Thus, these bands may be assigned to three spin-allowed transitions: $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$, respectively. The position of bands indicates that the complex has six coordinated octahedral geometry [43].

4.1.4. Ligand field parameters

The ligand field parameters like Racah inter-electronic repulsion parameter B, ligand field splitting stabilization energy 10 Dq, covalency factor β and ligand field stabilization energy (LFSE) have been calculat-ed for the Ni(II) complex. The values of B (complex) was 678.06 cm^{-1} calculated by 15B (complex) = $\nu_1 + \nu_2 - 3\nu_1$ and 10Dq = ν_1. The Nephelauxetic parameter β was readily obtained by using the relation β = B(complex)/B(free ion), where B(free ion) for Ni(II) is 1041 cm^{-1} [47–49]. The value of β is 0.65, indicating the covalent character in metal ligand "σ" bond. Ligand field stabilization energy (LFSE) was calculated with formulae 12Dq/83.6 is 155.16 kJ mol^{-1}.

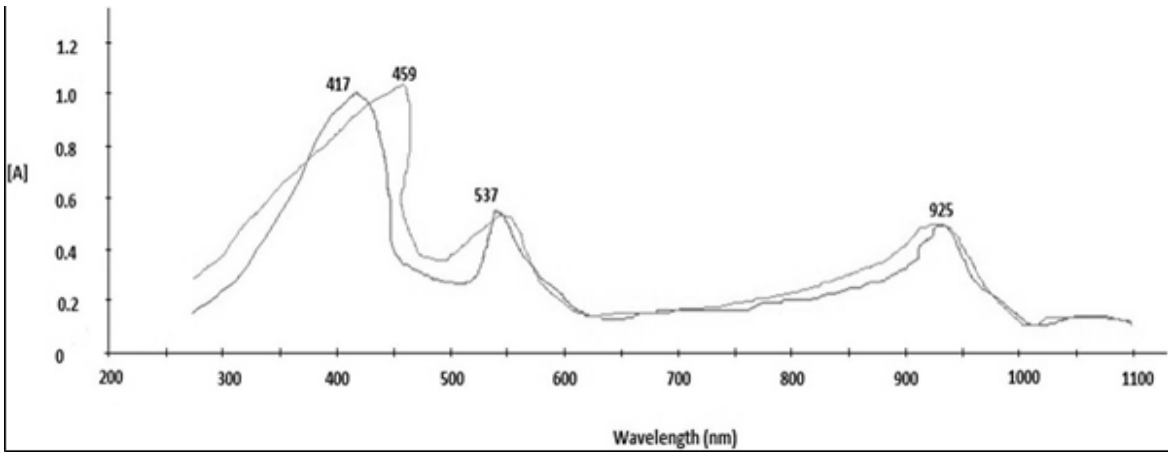

Figure 6: UV–vis absorption spectra of ionophore (A) and treated with KSCN solution (B).

4.1.5. X-ray diffraction study

The XRD pattern of the metal complex (Figure. 6) shows well defined crystalline peaks indicating that the sample was crystalline in phase [50,51]. The metal complex shows sharp crystalline XRD patterns, which differ considerably from that of the ligand. The appearance of crystallinity in the metal-Schiff base complex is due to the inherent crystalline nature of the metallic compound. The grain size of the metal Schiff base complex, d_{XRD} was calculated using Scherre's formula [52] by measuring the full width at half maximum of the XRD peaks. (Figure.7.)

$$\overline{0:9\lambda}\ ^{dXRD¼}\beta\eth cos\theta Þ$$

where 'λ' is the wavelength, 'β' is the full width at half maximum and 'θ' is the peak angle. The Ni(II) complex (ionophore) has the average crystallite size of 53 nm, suggesting the nanocrystalline.

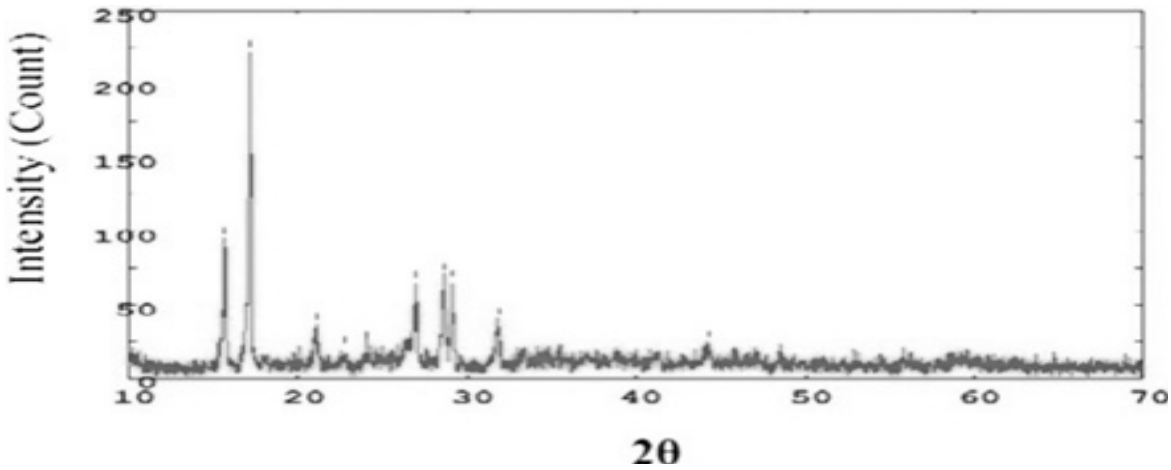

Figure 7: XRD pattern of [2-hydroxy-acetophenonethiosemicarbazone nitrato] nickel (II) complex

4.2. Preliminary potentiometry studies

From Figure 8, the selectivity sequence of the electrode based on Ni(II) complex for anions differs from the Hofmeister selectivity pattern of the classical liquid ion-exchange type ionophores. In the case of ionophores based on different metal ion complexes [27, 28], in ad-dition to the electrostatic interaction between the central metal ionand analyte anion, there is a coordination action between both species involved. Thus, the selectivity sequence is dominated by both electrostatic and coordination forces and it is expected that both the nature of the central metal ion and the coordination ligand properties play important roles in determining the selectivity of the ionophore towards a specific anion. In order to investigate the interactions between thiocyanate ions and the central metals, UV/vis spectra of the DMSO solutions containing the carriers are compared with that of the same solutions treated with (1.0×10^{-4} M) KSCN solution for 2 h (Figure. 8). The spectra of Ni (II) complex, show an absorption band at 354 nm in the DMSO solutions containing the carriers, while the spectrum of DMSO with the addition of an equimolar amount of KSCN absorbs at 365 nm, respectively.

The observed spectral shifts, together with the substantial increase in absorbance, after contact of the carrier solution with SCN^-, revealed a specific interaction between the ionophore in the membrane and thiocyanate ions. Moreover, the influences of other anions on the spectrum of the nickel (II) complex were also investigated, and almost no detectable spectral changes were observed.

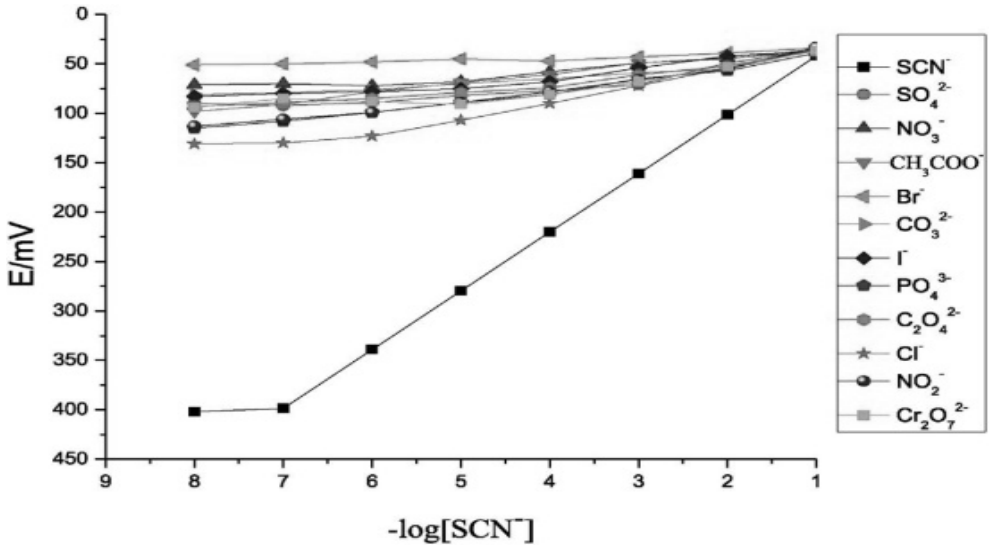

Figure 8: Potential responses of ion-selective membrane electrode based on [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel (II) for various anions.

4.3. Potential response

In a preliminary experiment we checked the suitability of [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel (II) complex as ion carrier in the preparation of the PVC membrane ion selective electrode for a wide variety of inorganic and organic anions including CH_3COO^-, $SO^2{}_4{}^-$, $Cr_2O^2{}_7{}^-$, $NO^-{}_3$, $NO^-{}_2$, $C_2O^2{}_4$, SCN^-, $PO^3{}_4{}^-$, Cl^-, I^-, Br^-, and $CO^2{}_3{}^-$over the concentration range of 1.0×10^{-8} to1.0

$\times 10^{-1}$ M. The results (Figure. 8) reveal that polymeric membrane electrode based on [2-hydroxyacetophenonethiosemicarbazone nitrato]nickel(II) complex as an ionophore exhibits superior response for thiocyanate ions in compari-son to other anions tested for the development of ion selective elec-trodes. The optimized thiocyanate selective polymeric membrane electrode (E-9) exhibits Nernstian slope (−59.4 ± 0.2 mV/decade of activity) for thiocyanate ions over a wide concentration range of 1.0×10^{-7} to1.0×10^{-1} M.

The performance characteristics of an ion selective electrode like potentiometric response, working concentration range, sensitivity, selectivity, and lower detection limit depends significantly on the membrane composition employed [53–55].

Ionophore content, nature and amount of plasticizers and amount of ionic additive are some of the basic parameters that affect the mem-brane composition and ultimately affect its performance characteristics (Table 1). The effect of variation in ionophore content on the response characteristics of the proposed thiocyanate selective polymeric membrane electrode was examined by preparing membranes (E:1–11) with varying amounts of ionophore. It is clear from the results (Table 1) that 4.0 mg of the ionophore is the optimum amount required for the proper functioning of the proposed thiocyanate selective elec-trode (E-9).

Another important ingredient of PVC membrane electrode based ion selective electrode (ISE) is the lipophilic cationic additive which is necessary to introduce permselectivity in the membrane. Response characteristics of an ISE are also strongly influenced by its presence [56]. In order to investigate the influence of lipophilic cationic additive on the response behavior of the proposed thiocyanate selective electrode, different membranes (E: 1–11) were prepared employing varying contents of TOMAC as anionic additive. Results revealed that electrode (E: 9) containing 2.0 mg of TOMAC exhibits superior response characteristics in terms of appreciable sensitivity and selectivity for thiocyanate ions.

Literature reports suggested that the nature of plasticizer influences the state of ionophore in the PVC matrix besides affecting the dielectric constant of the polymeric membrane [24, 57, 58]. Plasticizers enhance the flexibility and softness of membrane to secure the mobility of membrane. The effect of the plasticizers on thiocyanate selective electrode was investigated by using four different plasticizers (DBP, DOA, DOP and NPOE) with different polarities. The thiocyanate selective electrodes (E: 1–11) prepared by incorporating DBP, DOA, DOP and NPOE as plasticizers exhibited sub-Nernstian slope (−47.1, −49.5, −46.7, −43.6, −41.2, −39.6 and −38.2 mV/decade respectively). Among four plasticizers examined, 2-nitrophenyl octylether (NPOE) (E:9) resulted in the best sensitivity and linear range it helps to better extraction of polar ion Ni(II) with high hydration energy from aqueous solution to organic layer of the membrane. The detection limit, defined by the crossing of the two extrapolated linear segments of the calibration curve [57] (Figure. 9), was 8.6×10^{-8} M. The standard deviation of 10 replicate measurements is ±0.2 mV.

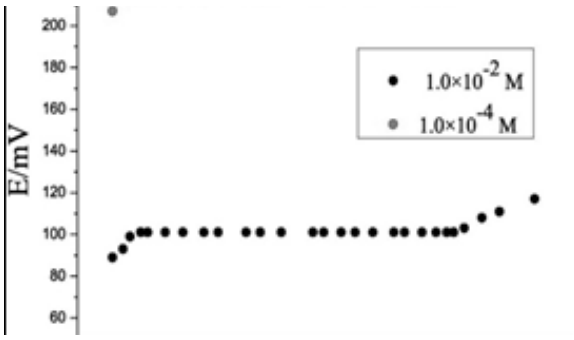

Figure 9

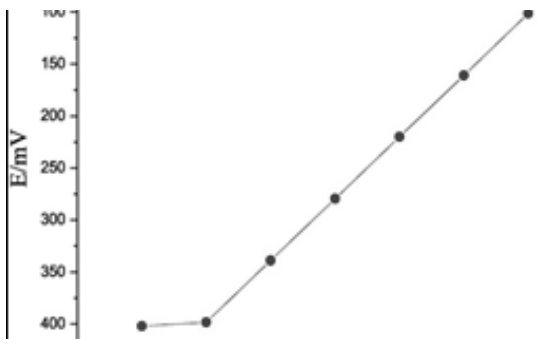

Figure 10

4.4. Effect of internal solution

The influence of the concentration of the internal solution on the potential response of the polymeric membrane electrode was studied. Three internal solutions with the concentration of KSCN, 1.0×10^{-3}, 1.0×10^{-2} and 1.0×10^{-1} M were examined with optimized membrane composition. The different internal solution concentrations changed the intercept of the resulting calibration plots. It has been noticed that a considerable effect on the linear range and detection limit cannot be seen in this case. However, a solution of 1.0×10^{-2} M KSCN in the internal solution showed a smooth Nernstian function of the polymeric membrane system and therefore, selected as concentration of internal solution.

4.5. pH and solvent effect on potential response

The effect of pH of the test solution on the response of the membrane electrode was examined at two fixed concentrations (1.0×10^{-2} and 1.0×10^{-4} M). As illustrated in Figure. 10, the potentials remained constant from pH 1.8 to 10.7. The observed potential drift at lower pH values is most probably due to the increase of the Cl^- ion concentration and simultaneous response of the electrode to thiocyanate and chloride ions [20]. At higher pH values the drift could be due to the interference of OH^- ions on the complex formation between thiocyanate ion and ionophores [19].

The utility of the electrode was also investigated in partially non-aqueous medium by using methanol–water, ethanol–water and acetone–water mixtures. The electrode works satisfactorily in mixtures having up to 20% (v/v) non-aqueous content without showing any con-siderable change in working concentration range and slope values.

4.6. Dynamic response time and life time

The dynamic response time of an ISE is an important parameter that must be considered if the electrode is going to have any type of practical utility. In this study, the practical dynamic response time has been recorded (for electrode no. 9) by immediate changing solutions with different SCN^- concentrations each having a ten-fold increase in concentration from 1.0×10^{-6} to 1.0×10^{-2} M. The actual potential versus time traces is shown in Figure. 11. As it is seen, the electrode reached the equilibrium response in a very short time of about 6s.

On the other hand, in order to evaluate the reversibility of the proposed electrode, a similar procedure in the opposite direction was adopted. The measurements were performed in the sequence of high-to-low from 1.0×10^{-2} to 1.0×10^{-6} M sample concentration. The results showed that the potentiometric responses of the electrode were reversible, although

the time required reaching equilibrium values (30 s) was longer than that for the low-to-high sample concentrations.

Lifetime studies were carried out based on monitoring the change in the slope and detection limit of the electrode with time. The results of the studies revealed a very slight gradual decrease in the slope (−59.4 mV/decade) after 3 months. After 3 months, changes in the slope from 59.4 to 53.6 mV/decade of activity were observed. During this period, the electrode was used for 2 h a day and 3 days a week. During nonusage, the electrodes were stored under dry conditions in an opaque closed vessel and before use were reequilibrated by dipping into 1.0×10^{-2} M $KSCN^-$ solution for 2 h.

4.7. Selectivity

Selectivity behaviour is obviously one of the most important characteristics of an ion selective electrode that is relative electrode response for the primary anion over other anions present in the solution, which is expressed in terms of potentiometric selectivity coefficient $K^{pot}_{A,B}$, which describes the preference of the membrane for an interfering ion as compared to SCN^-. The selectivity coefficient values were calculated by Matched Potential Method (MPM) [59, 60].

According to the MPM, a specified activity (concentration) of primary ions (A) is added to a reference solution and the potential is measured. In a separate experiment, interfering ions (B) are successively added to an identical reference solution, until the measured potential matches the one obtained before by adding primary ions. The MPM selectivity coefficient, $K^{pot}_{A,B}$ is then given by the resulting primary ion to interfering ion activity (concentration) ratio,

$K^{pot}_{A,B} =$	$A=a_B$
where	$A = (a'_A - a_A)$, a_A is the initial primary ion activity and a'_A

The concentration of SCN^- used as primary ion in this study was 1.0×10^{-2} M. The experimental conditions employed and the resulting values are given in Table 2 indicating they would not significantly disturb the functioning of the SCN^- electrode.

It has been shown that the anion selectivity behavior in ISEs exhibits anti-Hofmeister potentiometric patterns. The high potentiometry selectivity for thiocyanate ion must be related to the unique interaction between [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel (II) complex and thiocyanate ion. As seen from Table 3, the following results can be obtained for pro-posed ISE:

(1) The interfering effect of the ions is in the following order:

$SCN^-NI^-NSO^2_4{}^-NNO^-_2NCl^-NC_2O^2_4{}^-NNO^-_3NCH_3COO^-NPO^3_4{}^-NBr^-NCO^2_3{}^-\ NCr_2O^2_7{}^-$

(2) The selectivity of thiocyanate ion is high with respect to the other anions such as perchlorate, iodide and nitrate.

Thus, E: 9 was compared with some reported SCN^- selective electrode (Table 4). It is seen that the selectivity, working concentration range and pH range of the proposed electrode towards thiocyanate is better as compared to reported electrodes [26, 35–37].

4.8. Analytical applications

4.8.1. Potentiometric titration

The proposed thiocyanate-ion selective electrode was used as an in-dicator electrode in the potentiometric titration of SCN^- with $AgNO_3$ solution. A 7.0 mL of 5.0×10^{-3} M solution of KSCN was titrated with 1.0×10^{-2} M $AgNO_3$ at pH 5.0. As Figure. 12 observed in about 3.5 mL of the $AgNO_3$ is required to titrate thiocyanate solution and the potential of the electrode is increased upon addition of the $AgNO_3$ solution. From this Figure it is clear that the concentration of thiocyanate ion in solution can be accurately determined with this electrode.

4.8.2. Determination of thiocyanate ion in biological samples

The present PVC membrane electrode was also successfully applied for the determination of thiocyanate ions in biological (urine sample) solutions of smoker and non-smoker persons. Urine samples were diluted 1:10 (or 1:20) and adjusted to pH 5.0 with H_3PO_4 and KOH solutions. The calibration plots were used to determine the concentration of thiocyanate in these samples. A colorimetric procedure was used as the reference method [2]. The results shown as in Table 5 indicate good agreement with the results obtained from the colorimetric method.

4.8.3. Determination of thiocyanate ion in environmental samples

The proposed thiocyanate-selective electrode was also applied to the determination of thiocyanate ions in the river and tap water samples. The recovery of thiocyanate content of the samples was deter-mined using the proposed potentiometric method in the standard addition technique. The results (Table 6) indicated good recoveries for all samples. Thus, the suggested method is suitable for the determina-tion of thiocyanate in real.

5. CONCLUSION

On the basis of results discussed in this paper, the Schiff base ligand 2-hydroxyacetophenonethiosemicarbazone coordinates to Ni(II) as bidentate. [2-hydroxyacetophenonethiosemicarbazone nitrato] nickel(II) complex $[Ni(L)(NO_3)_2]$ (ionophore) was found to have octahedral geometry. Analytical data correspond to the monomeric composition of the complex. Ni(II) complex of Schiff base was used as an ionophore to fabricate a PVC-based ISE electrode. The ionophore undoubtedly rep-resents one of the most convenient materials for the preparation of thiocyanate ion selective electrode. The proposed electrode has wider working concentration range (1.0×10^{-7} to 1.0×10^{-1} M, and low re-sponse time (6 s). A comparison of the proposed electrode with report-ed electrodes (Table 1) shows that the proposed electrode is better than most reported sensors in terms of working concentration range, detection limit and response time. High sensitivity, stability, selectivity and low detection limit (8.6×10^{-8} M) make this electrode suitable for measuring the concentration of thiocyanate in a wide variety of samples without the need for pretreatment steps and without significant inter-actions from other anionic species present in the samples.

Table 1: Optimization and different composition of membranes (w%).

E. No.	PVC	Plasticizer		Ionophore	TOMAC	Linear range (M)	Slope (mVdecade^{-1})	Response time (s)
1	31	61	(DBP)	7.0	1.0	1.0×10^{-6}–1.0×10^{-3}	−47.1 ± 0.5	27
2	31	62	(DBP)	3.0	4.0	1.0×10^{-6}–1.0×10^{-3}	−49.5 ± 0.4	32
3	30	60	(DBP)	5.0	5.0	1.0×10^{-6}–1.0×10^{-3}	−46.7 ± 0.3	29
4	29	60	(DOA)	5.5	5.5	1.0×10^{-5}–1.0×10^{-2}	−43.6 ± 0.4	28
5	28	61	(DOA)	6.5	4.5	1.0×10^{-5}–1.0×10^{-2}	−41.2 ± 0.2	22
6	30	60	(DOA)	4.0	6.0	1.0×10^{-5}–1.0×10^{-2}	−39.6 ± 0.6	24
7	31	63	(DOP)	5.0	1.0	1.0×10^{-6}–1.0×10^{-2}	−36.5 ± 0.4	21
8	32	61	(DOP)	1.5	5.5	1.0×10^{-6}–1.0×10^{-3}	−38.2 ± 0.6	22
9	31	63	(NPOE)	4.0	2.0	1.0×10^{-7}–1.0×10^{-1}	−59.4 ± 0.2	6
10	32	59	(NPOE)	3.5	5.5	1.0×10^{-7}–1.0×10^{-2}	−42.5 ± 0.6	10
11	31	61	(NPOE)	5.0	3.0	1.0×10^{-6}–1.0×10^{-2}	−42.5 ± 0.6	15

Bold data indicates significant the main membrane composition that is used top prepare a thiocyanate selective electrode (proposed electrode).

Table 2: Characteristics of optimized thiocyanate-selective electrode.

Properties of the electrode	Values
Type of electrode	Polymeric membrane thiocyanate-selective
	electrode (PME)
Optimum composition w%	31:63:4:2 (PVC:NPOE:I:TOMAC)
Conditioning time and concentration	24 h, 1.0×10^{-2} M
Linear working range	1.0×10^{-7} to 1.0×10^{-1} M
Slope	-59.4 ± 0.2 mV/decade
pH	1.8–10.7
Detection limit	8.6×10^{-8} M
Lifetime	3 months
Response time	6 s
Storage	Buffer pH 5.5 with KSCN

Table 3: Potential selectivity coefficients of various interfering anions.

Interfering anions	Selectivity coefficient ($K_{A,B}^{pot}$)
SO_4^{2-}	−1.9
NO_3^-	−2.8
$Cr_2O_7^{2-}$	−4.4
PO_4^{3-}	−3.6
CO_3^{2-}	−4.2
Br^-	−3.8
$C_2O_4^{2-}$	−2.6
CH_3COO^-	−2.9
Cl^-	−2.4
I^-	−1.1
NO_2^-	−2.1

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BIOCHEMICAL ASSAY OF EMBRYO TOXICITY ON PHORATE EXPOSED MICE AND ITS REMOVAL BY VITAMIN C

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ABSTRACT

Phorate a organophosphate pesticide commonly use to control pests like various leaf feeding insects, mites and soil insects terrestrial crops such as beans, cotton, hops, peanuts, potatoes, sorghum, soyabean, sugar beats, sugarcane etc . Extensive use of pesticides in the agricultural field increased the possibility exposure even to a low level. The commercial use of pesticide also affects the non target organisms like fish, birds and mammals. Our study is to investigate the toxicity appearing in embryos during early and late pregnancy of female mice. Three group of female mice were taken: control, phorate (phorate was administered intra-peritonially at a concentration of 1mg/kg body weight), Phorate+vit C (also administered intra-peritonially at a concentration of 1mg/kg body weight). All sets of pregnant mice studied as 7 days, and 14 days exposure of phorate. After sacrificed them, following events were found: death of embryo on premature and mature state, unnatural fat deposition on mother's uterus but no malformed embryo was found. To establish a possible cause of the experimental finding following biochemical assays were performed on liver, kidney and brain tissue both on mother and embryo. The biochemical assays were estimation of total serum protein, alanine aminotransferase (AST), gama glutamyl transferase (GGT), lactate dehydrogenase (LDH), Lipid peroxidation (LPO), catalase activity and acetylcholinesterase activity. The above assays were also performed on Phorate + vit C exposed mice to study the ameliorating effect. In conclusion these experimental inference may be beneficial for the other non-target creatures that are crucially important for maintaining the natural balance of flora and fauna as well as for the human beings in future.

KEY WORDS: Embryological toxicity, Phorate, Vitamin C.

1. INTRODUCTION

Phorate,chemical name of Phosphorothioic acid , O- dietmyl S (ethsyl thio) methyl ester, an organophosphate, systemic and broad spectrum insecticide inhibit actylcholinesterase (AchE) activityby phosphorylating the serine hydroxyl group inthe substrate binding

domain, which causes acetylcholine and induces neurotoxicity *(Fulton and Key, 2001)* . Photare has been classified as a class I, high risk toxic OP compound with and LD_{50} of 1.1 t0 3.7 mg/kg bw for rats *(http://extoxnet.orst.edu/pips/Phorate.htm)*. Phorate is used against sucking and chewing insects, leaf hopper, mites, some nematodes, rootworms in agricultural field to protect the crops*(Phorate 171-205 JMPR 2004)*. Through the food chain it reaches to some non target organism other than pests and causes harmful hazards. Not only that phorate also has a harmful effect on embryo. Maternal and embryo toxicity was found at dietary doses of 0.5mg/kg/day fed to rats *(Michael A. Kamrin, John H. Montgamery 1999)*. R eview of study suggests substantial phorate induced generation of intracellular ROS is atributed to mitochondrial dysfunction and leads to oxidative stress [*Q.Saquib et. al.*]. The aim of the study is to evaluate the embryo toxicity due to expouser of phorate and their recovery by vit C.

2. EXPERIMENTAL DESIGN

A total number of 15 healthy Swiss albino mice (*Mus musculus*) weighing between 20 and 22 gms were randomly selected for experimental purpose and were subdivided into three groups, each group comprising five mice. One group for control which was provided normal diet ad libitum. The next group was provided with phorate (1mg/kg. body wt.)and the third group was provided with phorate and vit C (500mg/kg. body wt.) for recovery. All groups were provided the treatment for seven days and after that they were sacrificed and blood, liver and brain tissue were collected.

3. MATERIALS AND METHODS

a) Collection of tissue

After sacrifice of the fish, liver,kidney and muscle tissue were collected and kept separately in petridish (-80 °) till homogenization. A part of the tissue was diluted for quantitative estimation of protein and biochemical assay.

b) Isolation of serum from blood

Blood was drawn by ventricular puncture of etherized (approximately 1ml from each mice) by the routine procedure using sterile disposable synringe and needle. Blood was collected in 15ml centrifuge tube (Axygen scientific, Lot no. 061016058) without EDTA. Serum was obtained by centrifugation.

c) Sample homogenization and centrifugation

50mg tissue were homogemized in 2ml of Phosphate buffer(PBS) and the homogemized tissues were spun in refrigerated centrifuged(REMI C 24model, India) at 5000rpm for 15 min at 4° C. After that the supernants were stored at -80° C for biochemical assay.

d) Estimation of total protein

For quantitative estimation of total protein the Lowry *et al* (1951) was used.

e) Estimation of AST and ALT

Estimation of AST (Aspertate transaminase) and ALT (Alanine Amino Transferase) the

method of Bergmeyer and Brent (1974) was used.

f) **Estimation of Lipid Peroxidation (LPO)**

The spectrometric assy of Lipid peroidation (LPO) was performed following the protocol of Buege and Aust (1984) with some minor modification.

g) **Estimationof total thiol content**

For the estimation of total thiol content the protocol of Sedlak and Lindsey (1968) was followed with minor modification.

h) **Estimation of catalase activity**

The quantitative measurement of Catalase activity was done by the method of Chance and Maehly (1955)

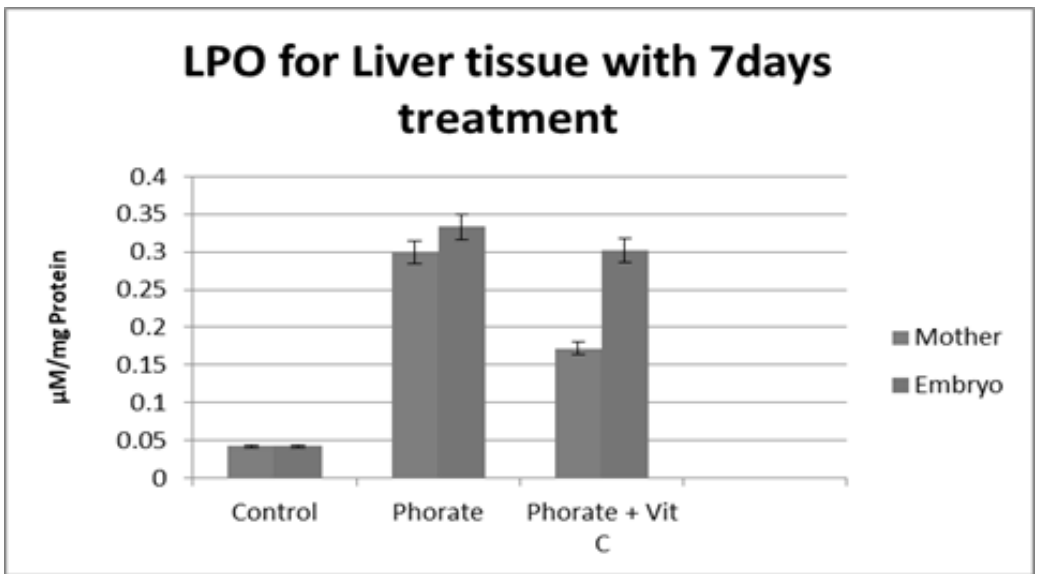
i) **Estimation of acetylcholine Esterase(AchE) activity**

For the estimation of acetylcholine esterase (AchE) activity was done by the method of Ellman G. L., Courtney K. D., Andres V. Jr., and Feath-erstone R. M. (1961), A new and rapid colorimetricdetermination of acetylcholinesterase activity. Bio-chem. Pharmacol.7,88Ð95.

4. RESULT AND DISCUSSION

Result analysis o f LPO for 7ds. Treatment of Phorate to Pregnant mother and their embryos

Sample	Tissues				
	Liver				
	Cont.	Treat.	Sig.	Recovery	
Mother	0.0418±0.005	0.300±0.041	P<0.001	0.172±0.000	P<0.001
Embryo	0.0418±0.005	0.333± 0.006	P<0.001	0.302±0.008	P<0.001

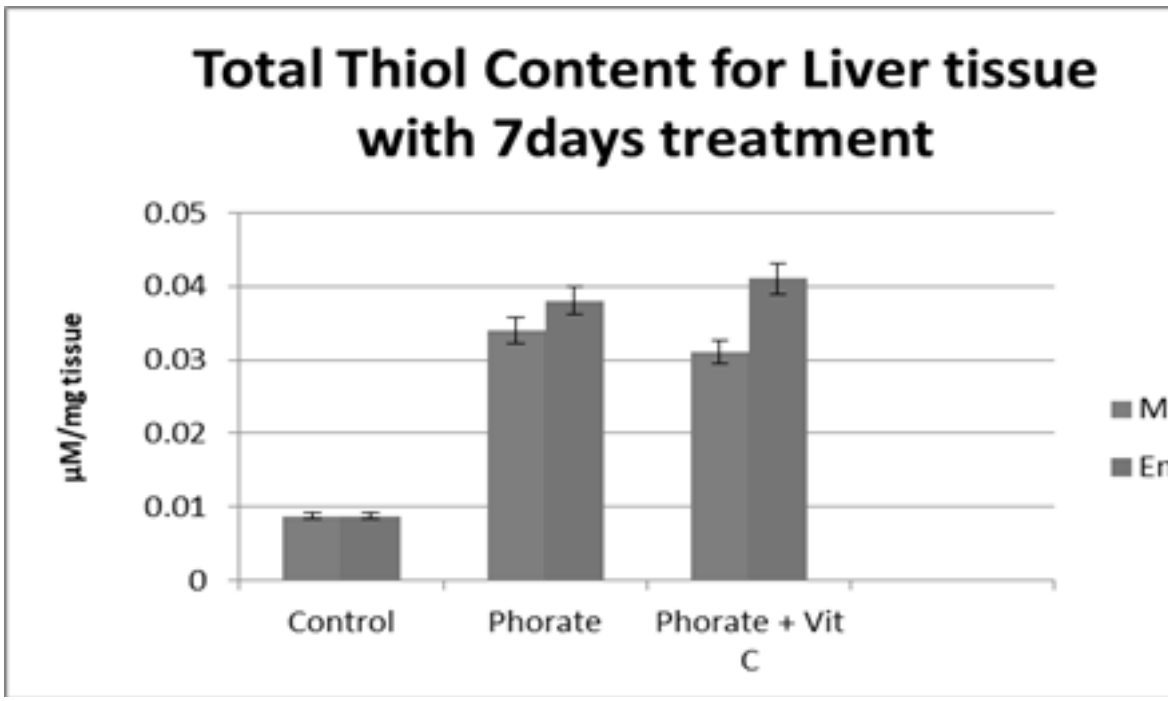


The LPO level was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother and embryo tissue in the recovery phase which were treated with both phorate and vit C.

The increased level of LPO both in mother and embryo after phorate treatment for 7 days may due to elevation of MDA level which is responsible for the generation of free radical, to protect the tissue from oxidative damage. Bagchi et. al. (1995) have shown that different classes of pesticides induce the production of reactive oxygen species (ROS) and tissue damage. Other reports indicate that the enzyme activities associated with antioxidant defence mechanisms are altered by insecticides both in vivo and in vitro (*patel and Chakarbarti 1982;*). The decreased level of LPO shows by the treatment of vit C in mother and embryo tissue. From the above finding it can be said that the anti-oxidant property of vit C protect mother and most importantly the embryo hepatic tissue from the oxidative damage.

Result analysis of Total Thiole content for 7ds. Treatment of Phorate to Pregnant mother and their embryos

Sample	Tissues				
	Liver				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0087 0.000±	0.034±0.005	P<0.001	0.031±0.000	P<0.001
Embryo	0.0087 0.001±	0.038±0.001	P<0.01	0.041±0.000	P<0.001

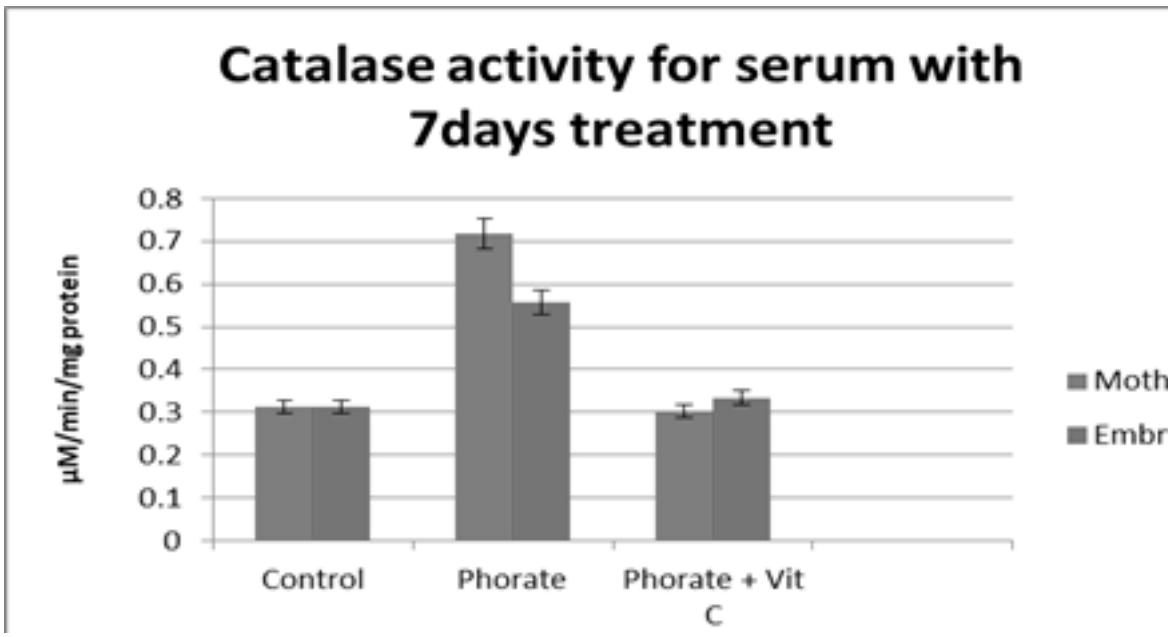


The total thiol content was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother tissue in the recovery phase which were treated with both phorate and vit C but in embryo the total thiol content was increased in the recovery phase means vit C can't provide protection.

The increase in total thiol content both in mother and embryo may be due to defense activity of thiol against free radicals. Review of study found that thiol share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level *[Journal (on-line/ unpaginated); ID code 6664]*. But the vit C protection significantly recover the free radical damage due to its antioxidant property in mother. But in case of embryo Vit C could not protect the embryo from these oxidative damage. It may be due to short term expoueser of Vit C on embryo.

Result analysis of Catalase activity of serum for 7ds. Treatment of Phorate to Pregnant mother and their embryos

Sample	Tissues				
	Serum				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.313±0.001	0.717±0.011	P<0.001	0.302±0.002	P<0.01
Embryo	0.313±0.001	0.558±0.038	P<0.001	0.334±0.001	P<0.001



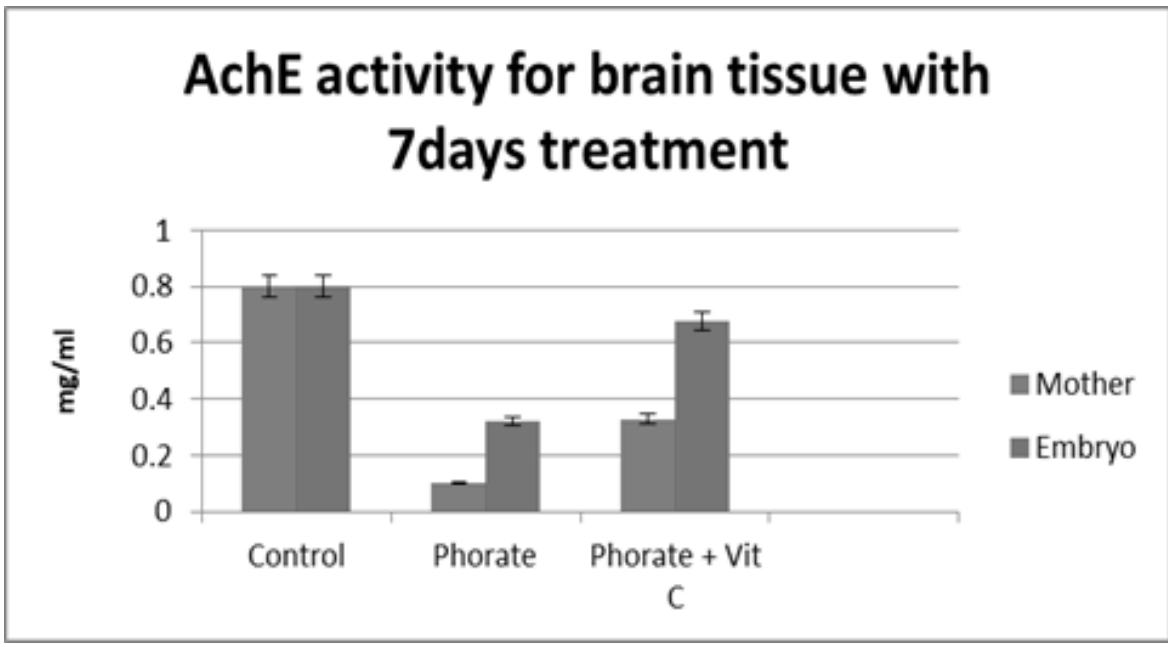
The catalase activity was significantly increased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother and embryo tissue in the recovery phase which was treated with both phorate and vit C.

The Catalase activity can be considered as a sensitive biomarker for bio monitoring the environment. The catalase enzyme assay result revealed that embryos need more protection from pesticide effects .It may be considered that vit.C was unable to cross the placental

barrier.

Result analysis of Brain Acetyl cholinesterase (AchE) activity for 7ds. Treatment of Phorate to Pregnant mother and their embryos

Sample	Tissues				
	Brain				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.800±0.001	0.102±0.010	P<0.001	0.329±0.005	P<0.001
Embryo	0.800±0.001	0.320±0.082	P<0.001	0.676±0.047	P<0.05

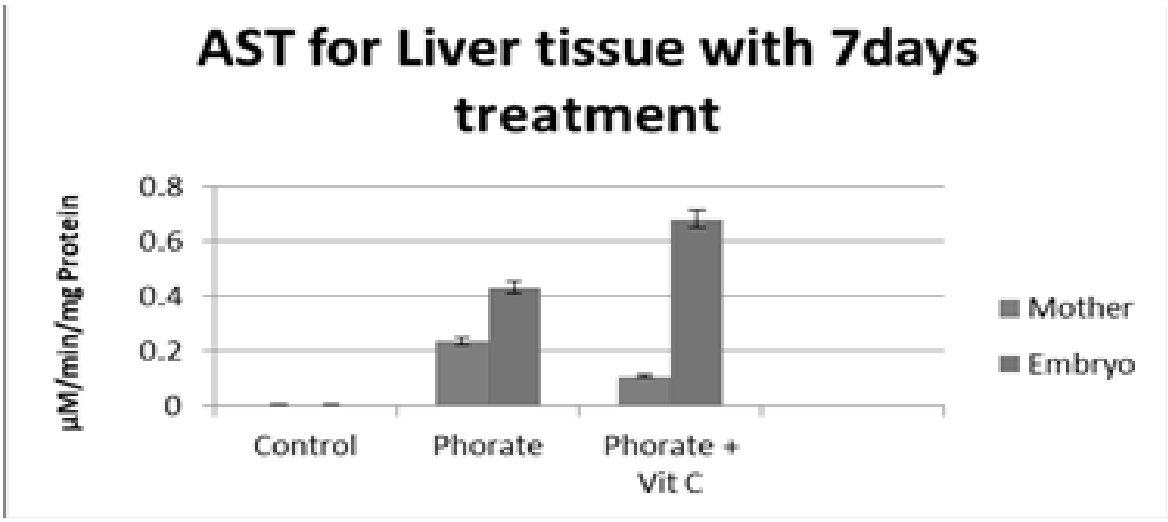


The AchE activity in brain tissue was significantly decreased in both mother and embryo, treated with Phorate in compare to control but decreased significantly in mother and embryo tissue in the recovery phase which were treated with both phorate and vit C.

The significantly decreased level of AchE may be due to inhibition of catalytic activity of acetylcholine, the most excitatory neurotransmitter in the central nervous system (brain). The possible reason may be due to oxidative stress upon neurotransmitter enzyme. The toxic effect of phorate was recovered or ameliorated by conjoint treatment of Vit.C with phorate, So that the cholinesterase enzyme activity increased both in mother and embryos. Specially in embryos the result of phorate + vit.C was near about to control result which may confirm that Vit.C has an antioxidant property against phorate treatment.

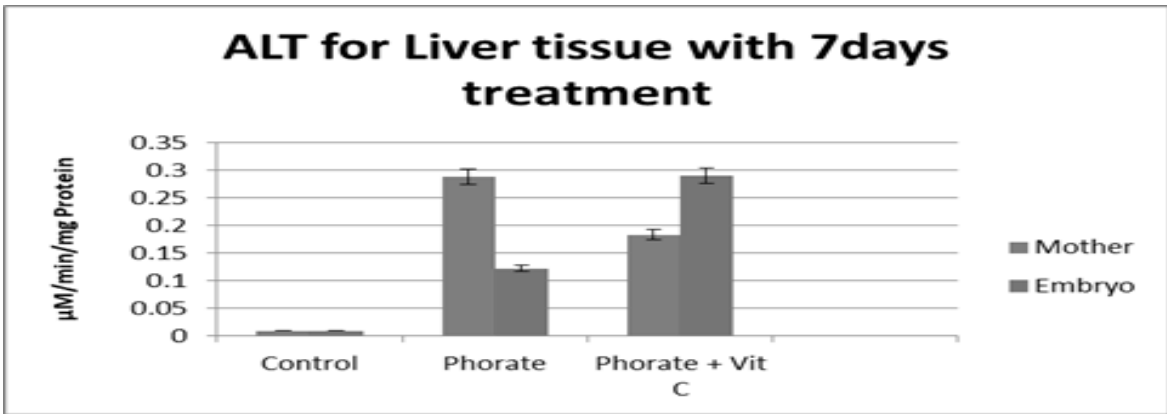
Result analysis of AST for 7ds. Treatment of Phorate to Pregnant mother and their embryos.

Sample	Tissues				
	Liver				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0041 0.001±	0.236±0.038	P<0.001	0.108±0.000	P<0.001
Embryo	0.0041 0.001±	0.432±0.041	P<0.001	0.680±0.007	P<0.001



Result analysis of ALT for 7ds. Treatment of Phorate to Pregnant mother and their embryos.

Sample	Liver Tissues				
	Cont.	Treat.	Sig.	Recovery	Sig.
Mother	0.0086±0.001	0.288±0.023	P<0.001	0.183±0.000	P<0.001
Embryo	0.0086±0.001	0.122±0.018	P<0.001	0.290±0.003	P<0.001



Both the AST and ALT level were significantly increased in both mother and embryo, treated with Phorate in compare to control but significantly decreased AST and ALT level were shown in mother tissue in recovery with vit C. But in case of embryo vit C could not recover the ASl and ALT level as its value was increased.

For detecting hepatic damage, liver AST and ALT was done and it shows a significant increased in phorate treated mother and embryo tissue. But by the treatment of vit C which is an anti-oxidant, reduces the AST level in mother and embryo which suggests its hepatoprotective activity (Toxicology report, 2015). In case of hepatic ALT level, it significantly reduced in mother liver tissue but doesn't affect the embryo. It may due to the single dose of vit C cannot protect the hepatic damage and needs more expouser of vit C.

5. **CONCLUSION**

The organo- pesticide phorate is an organic compound, unusual for the living body system used to eradicate the pest organisms. It is very much harmful for the living body system revealed by the unusually elevated level of enzyme activities for LPO, Thiol, Catalase, Acetly cholinesterase (AchE), AST & ALT.The application of vit.C may act as an ameliorating agent having anti-oxident property. This is true in case of direct treatment for pregnant mice (mother). But this defensive activity is not truely followed for the embryos as revealed by the higher level of enzyme activities in LPO, Thiol, AchE, AST & ALT and slightly increased level of catalase activity. From the overall results and discussions it is revealed that vit. C can act as an ameliorating agent against direct treatment of pesticide but in the embryos the administered dose of vit.C had little effect revealed by higher enzyme activities. So more investigation is required for the embryo-toxicity. We are investigating whether the placenta act as a barrier for vit.C.

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CHAPTER - V

INDOOR AIR QUALITY: A GLOBAL ENVIRONMENTAL PROBLEM

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ABSTRACT

Indoor Air Quality (IAQ) refers to the air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants. Understanding and controlling common pollutants indoors can help reduce your risk of indoor health concerns. Health effects from indoor air pollutants may be experienced soon after exposure or, possibly, years later.

The aim of this study is to present several general parameters of this subject, such as the causes of indoor air pollution, the factors that influence IAQ, the health effects and the actions which could prevent or minimize the effects.

1. INTRODUCTION

The major area of public concern and government policy, in terms of the impact of air pollution on human health, continues to be outdoor air. However, over the last two decades, indoor air quality (IAQ) has caused increasing concern due to the adverse effects that it may have on human health. The term "indoors" is used in relative literature to refer to a variety of environments, including homes, workplaces, and buildings used as offices or for recreational purposes. In addition, a number of studies have been carried out to measure various compounds inside vehicles during commuting activities. Most people in the developed world spend up to 90% of their time in an indoor environment and up to 60% of the workforce work in an office. (Tsakas & Siskos, 2011; Tsakas & Siskos, 2010; McCurdy et al., 2000; Ashford & Caldart, 2008; Andersson & Klevard Setterwall, 1996) Decreased ventilation rates for energy conservation, along with increased use of synthetic materials in buildings, have resulted in increased health complaints from building occupants (Siskos, 2003). Many indoor pollutants are either known, or suspected to be, allergens, carcinogens, neurotoxins, immunotoxins or irritants, while all may contribute to sick building syndrome (SBS). The set of health symptoms associated with SBS includes nasal, ocular and generalised diseases. According to various studies performed in public buildings by the National Institute of Occupational Safety and Health (Soldatos et al., 2003), the three most significant symptoms that were experienced in more than 70% of the buildings are dry

eyes, dry throat and headaches.

The IAQ and the presence of air pollutants in indoor environment is a worldwide issue, since many governments and environmental institutes have faced this serious phenomenon. Starting in the 1990s in Japan, tightly sealed buildings with low ventilation rates have been constructed. This, combined with the use of some new types of building materials has often resulted in IAQ problems. Many inhabitants suffering with SBS and multiple chemical sensitivity (MCS), have been reported (McCurdy et al., 2000; Zhang & Niu, 2004). As a result the Japanese Ministry of Health, Labour and Welfare have introduced indoor air guidelines for a range of VOCs including HCHO based on hazard assessments (Shinohara et al., 2009).

The importance of IAQ has also been recognised in Europe and has been identified as an important element within the European Collaborative Action (ECA) (ECA, 1998) and the European Environment and Health Action Plan (Dimitroulopoulou et al., 2006). In America, the State of California has adopted an active programme for the last two decades aiming to the reduction of indoor air pollution, which has led to a range of policy instruments (Waldman & Jenkins, 2004). Over recent years, important steps have been made towards setting IAQ standards and guidelines in the UK (Dimitroulopoulou et al., 2006) and recently, the UK Department of Health Committee on the Medical Effects of Air Pollutants (Short, 2001), launched a guidance document on the effects of indoor air pollutants. In many developing countries, exposure to indoor air pollution causes a major health burden (Committee on the Medical Effects of Air Pollutants, 2004). Increased concern regarding indoor air quality especially in the last two decades has led to a number of studies and meetings on the subject.

For example, in Greece many researchers have conducted significant studies about IAQ issue (Siskos & co-workers, 2001, 2003, 2005, 2010; Helmis & co-workers, 2007, 2009; Santamouris et al., 2001). With increasing concern in relation to health effects, in recent years the problem has come into sharper focus. Additional new sources of contaminants are being introduced, which haven't been measured before. In several countries, studies have been undertaken, in some cases involving comprehensive investigations of the factors governing air quality, so that effective control measures ranging from the setting of minimum ventilation standards, to controlling, or even banning, certain products such as urea-formaldehyde foam insulation or unvented paraffin or gas heaters. It is nevertheless recognized that some of the responsibility for maintaining acceptable and healthy indoor air quality will continue to rest with building owners and occupants of buildings.

Some studies have revealed a variety of contaminants of indoor air including odorous, non-odorous gases and vapours, and particles, and although there were suggestions that some of these contaminants could be responsible for health effects, proving causal relationships is exceedingly difficult even where elevated levels of potentially toxic substances exist (World Health Organization [WHO], 1989; Perry & Kirk, 1986; WHO, 1986; Priorities for Indoor Air Research and Action, 1991).

Finally, connections between climate change and indoor climate are strong but not generally recognized. Climate change impacts local and regional atmospheric conditions including air quality and thermal conditions. Building climate control must respond to local

climate and air quality to protect human health and support buildings' functional uses. Good indoor climate protects humans against local air pollution and the severe consequences of climate change. Technologies to control indoor climate requiring fossil fuel energy increase pollutant emissions including greenhouse gases; thus, indoor climate control impacts indoor and ambient air quality as well as global climate. Protecting humans and other living systems from the impacts of climate change involves adaptation and mitigation of the local and regional effects. Understanding and considering impacts of indoor climate control on regional air quality and global climate can reduce the negative impacts of building technology on building occupants as well as the entire global environment (Levin, 2008).

2. CATEGORIES AND SOURCES OF INDOOR AIR POLLUTANTS

There are many indoor air contaminants, which can be separated based on their effects on human health, the frequency of their appearance, their usual concentration levels, their sources etc. This chapter is focused, primarily, on those species common to indoor and outdoor air environments and those who are measured more often in indoor environments.

2.1 Radon

The main source of indoor radon is its immediate parent radium-226 in the ground of the site and in the building materials (Nero, 1988, 1989). Outdoor air also contributes to the radon concentration indoors, via the ventilation air. Tap-water and the domestic gas supply are usually radon sources of minor importance, with a few exceptions. In most situations it appears that elevated indoor radon levels originate from radon in the underlying rocks and soils (Castren et al., 1985). This radon may enter living spaces in dwellings by diffusion or pressure driven flow if suitable pathways between the soil and living spaces are present. It should be noted, however, that in a minority of cases elevated indoor radon levels may arise due to the use of building materials containing high levels of radium-226. Examples of such materials, used in some buildings, are by-product gypsum, alum shale and volcanic tuffs.

The United Nation Scientific Committee on the Effects of Atomic Radiations (UNSCEAR) has made a very simple model to try to estimate the relative contribution of these sources: for a "typical" house, with a radon concentration of 50 Bq/m3 at ground floor, the contributions of soil, building materials and outdoor air are, respectively, 60%, 20% and 20%, while for the upper floors in high rise buildings, where the radon concentration-is estimated to be "typically" 20 Bq/m3, these values become 0%, 50% and 50% (UNSCEAR, 1993).

2.1.1 Soil

For those who live close to the ground, e.g. in detached houses or on the ground floor of apartment buildings without cellars, the most important radon source is radium in the ground.

The radium concentration in soil usually lies in the range 10 Bq/kg to 50 Bq/kg, but it can reach values of hundreds Bq/kg, with an estimated average of 40 Bq/kg (UNSCEAR, 1993). Typical radon concentrations in soil gas range from 10000 Bq/m3 into 50000 Bq/m3. The potential for radon entry from the ground depends mainly on the activity level of radium-226 in the subsoil and its permeability with regard to air flow. Example of terrains

with a high radon potential are alum shales, some granites and volcanic rocks, due to high concentrations of radium-226 and the presence of eskers (gravel, sand and rounded stone deposited from subglacial streams during the ice ages), all these being characterised by high permeability. The ground could also be contaminated with waste tailings from uranium or phosphate mining operations with enhanced activity levels (Tyson et al., 1993).

The ingress of radon from the soil is predominantly one of pressure-driven flow, with diffusion playing a minor role (de Meijer et al., 1992). The magnitude of the inflow varies with several parameters, the most important being the air pressure difference between soil air and indoor air, the tightness of the surfaces in contact with the soil on the site, and the radon exhalation rate of the underlying soil. If there is no airtight layer between the basement and the ground, the underpressure indoors causes radon to be drawn in from the ground under the building. Underpressure occurs in most houses if either the adjustment of inlet and outlet of air in forced ventilation systems or the outdoor air supply for vented combustion appliances is inappropriate. The underpressure may be considerable for all types of ventilation systems when the inlet air is restricted too much. The tightness of the structures has to do with e building regulations and techniques and is very dependent on cracks, openings and joints. Structures are hardly ever so airtight that radon inflow is completely prevented. For example, to get a radon daughter concentration of less than 100 Bq/m3 EER in a house with a volume of 500 m3 and a ventilation rate of 0.5 air changes per hour, not more than 1 m3 per hour must be allowed to leak into the house if the radon gas concentration in soil air is about 50000 Bq/m3. Such values are quite typical.

2.1.2 Building materials

Building materials are generally the second main source of radon indoors, while in the Seventies they were considered the principal one (UNSCEAR, 1977; Meyer et al., 1986). Radon exhalation from building materials depends not only on the radium concentration, but also on factors such as the fraction of radon produced through material release, the porosity of the material and the surface preparation and finish of the walls. In general, no action needs to be taken concerning traditional building materials. Typical values for radium and thorium content in building materials are 50 Bq/kg or less (Nuclear Energy Agency Organisation for Economic Co-operation and Development-NENOECD, 1979). Building materials containing by-product gypsum (UNSCEAR, 1982) and concrete containing alum shale (Swedjemark & Mjones, 1984) may have much higher radium concentrations. The activity concentrations in brick and concrete may also be high if the raw materials have been taken from locations with high levels of natural radioactivity. Examples of such natural materials, used in some buildings, are volcanic tuffs and pozzolana (Sciocchetti et al., 1983; Campos Venuti et al., 1984; Battaglia et al., 1990), where radium and thorium content can reach some hundreds of Bqlkg. Other measurements of radioactivity content and exhalation of building materials are reported in NENOECD (1979).

Building materials are the main sources of radon-220 (also called “thoron”) in indoor air. Due to its short half life (55s), thoron originating in soil in effect is usually prevented from entering buildings and therefore makes negligible contribution to indoor thoron levels. For this reason and due to the greater difficulties of measurement, thoron concentration measurements are very much fewer than those for radon. Although the indoor thoron concentrations are usually low (Cliff, 1992; UNSCEAR, 1993), in some cases the doses due

to this isotope and its daughters are significant and comparable to those due to radon- 222 (Sciocchetti et al., 1983, 1992; Guo et al., 1992; Bochicchio et al., 1993; Doi & Kobayashi, 1994).

2.1.3 Outdoor air

Outdoor air usually acts as a diluting factor, due to its normally low radon concentration, but in some cases, as in high rise apartments built with materials having very low radium content, it can act as a real source. The radon concentration in outdoor air is mainly related to atmospheric pressure, and (in case of non-perturbative weather) it shows a typical oscillating time pattern, with higher values during the night.

Until a few years ago the average level of radon gas concentrations in the atmosphere at ground level was, in most cases, assumed to be of the order of few Bq/m3 -e.g. in the range of 4 to 15 Bq/m3 in USA (Gesell, 1983), but more recent measurements seem to indicate higher values, reaching some tens of Bq/m3 (Hopper et al., 1991; Robé et al., 1992; Bochicchio et al., 1993; Deyuan, 1993; Grasty 1994; Price et al., 1994). Quite high radon concentrations in the outdoor air have been reported near substantial radon sources, such as mine tailings (Tyson et al., 1993), or in the case of particular weather conditions, such as thermal inversion or very low precipitation (Grasty, 1994).

Ambient air over oceans has very low values (~ 0.1 Bq/m3) of radon concentrations, due to the minimum presence of radium in the sea water and the high solubility of radon in water at low temperatures. Therefore radon concentration in outdoor air of islands and coastal regions is generally lower than in continental countries, e.g. United Kingdom and Japan have an average outdoor air value of ~4 Bq/m3.

Taking into account recent measurements, the mean value of outdoor radon concentrations adopted by UNSCEAR in its last report has been changed from 5 to 10 Bq/m3 for continental areas and somewhat less in coastal regions (UNSCEAR, 1993).

2.1.4 Tap water

In wells drilled in rock the radon concentrations of water may be high. When such water is used in the household, radon can be partially released into the indoor air, causing an increase in the average radon concentrations. In a few regions, such as Finland and Maine (USA), the tap water from wells drilled in rock has been shown to contribute significantly to radon concentrations indoors. Radon concentrations in tap-water from deep wells can range from 100 kBq/m3 to 100 MBq/m3 (UNSCEAR, 1988). The indoor radon concentrations in these regions may already be high due to high rates of radon entry from the ground. The world average radon concentration in all types of water supplies is assumed to be 10 kBq/m3 (UNSCEAR, 1993).

2.1.5 Domestic gas

In some regions, natural gas used for cooking and heating contains elevated concentrations of radon, which is released on combustion. Normally this source is insignificant, and can be monitored at transmission and distribution points. Typically the radon level in natural gas is about 1000 Bq/m3. Natural gas, as it is usually supplied, contains gas from a number of wells and fields and thus can vary over time, depending on the proportions supplied by different sources (UNSCEAR, 1993).

2.2 Oxides of Nitrogen

2.2.1 NOx

A large number of studies of NO and NO_2 have been carried out in many different indoor air environments (Finlayson-Pitts, 1999; Pitts et al., 1985). Because of air exchange, indoor levels are generally higher when outdoor levels increase (Hoek et al., 1989; Rowe et al., 1991; Hisham & Grosjean, 1991; Spengler et al., 1994; Weschler et al., 1994; Baek et al., 1997). However, enhanced indoor levels can be found when combustion sources are present. These include gas stoves, paraffin heaters, water heaters, and cigarette smoke (Wade et al., 1975; Marbury et al., 1988; Ryan et al., 1988; Petreas et al., 1988; Hoek et al., 1989; Pitts et al., 1989; Spengler et al., 1994; Levy et al., 1998). While combustion generates primarily NO, the focus indoors has been on NO_2 because of its health impact. Again, the use of gas stoves was highly correlated with indoor NO_2, with an indoor/outdoor concentration ratio of 1.19 for homes with a gas range compared to 0.69 for those without a gas stove. The ratio was even higher for homes with a paraffin space heater, 2.3 compared to 0.85 without such a heater (Levy et al., 1998). Both the indoor and outdoor concentrations of NO_2 were higher in cities where at least 75% of the homes had gas stoves; for example, the mean outdoor NO2 concentration in such gas-intensive cities was 38 ± 20 ppb, compared to 14 ± 6 ppb in cities where fewer than 25% of the households had gas stoves installed. High concentrations of NO_2 have also been measured in indoor skating rinks where the use of ice resurfacing machines powered by propane, gasoline, or diesel fuel results in significant emissions (e.g., Brauer & Spengler, 1994; Brauer et al., 1997; Pennanen et al., 1997). Mean concentrations of NO_2 of ~200 ppb have been reported, with some rinks having concentrations up to 3 ppm! The indoor-to-outdoor ratios of the arithmetic mean concentrations varied from about 1 to 41, with an overall mean of 20.

In the absence of such sources of NOx, indoor and outdoor concentrations are quite similar (Weschler et al., 1994), since removal of NO and NO_2 indoors, e.g., on surfaces, is relatively slow. However, as it has been discussed shortly, although the surface reaction of NO_2 is relatively slow, it is still of interest since it generates nitrous acid (HONO). Different surfaces found inside homes have been found to have different removal rates for NO_2. In short, there is a variety of evidence that there are higher levels of NO_2 indoors when combustion sources are present and that the concentrations generated indoors can be quite substantial in some circumstances. One word of caution is in order, however, particularly in regards to earlier measurements of NO_2.

2.2.2 HONO and HNO_3

HONO is formed by the reaction of NO2 with water on surfaces. The reaction is usually represented as

$$2NO_2 + H_2O \rightarrow HONO + HNO_3 \quad (1)$$

Although the detailed mechanism is not known; gaseous HNO_3 is not generated in equivalent amounts, something which has been attributed to its remaining being adsorbed on the surface. This overall reaction occurs on a variety of surfaces in the laboratory and hence might be expected to also occur on surfaces in other environments, such as homes. This, indeed, is the case. (Pitts et al., 1985) first used differential optical absorption spectrometry (DOAS) to establish unequivocally that NO_2 injected into a mobile home forms HONO.

Interestingly, the dependence of the rate of HONO generation on the NO_2 concentration was similar to that measured in laboratory systems, consistent with production in, or on, a thin film of water adsorbed on surfaces. A number of studies have confirmed that the behavior is similar to that in laboratory systems; i.e., the rate of production of HONO increases with NO_2 and with relative humidity. Indoor levels of HONO as high as 8 ppb as a 24-h average and 40 ppb as a 6-h average have been reported in normal, in-use buildings and homes (Febo & Perrino, 1991; Spengler et al., 1993; Weschler et al., 1994). The ratio of HONO to NO_2 indoors can be quite large, up to ~0.15 (e.g., Febo & Perrino, 1991; Brauer et al., 1990, 1993; Spengler et al., 1993). This can be compared to typical values of a few percent outdoors. High levels of HONO (up to ~ 30 ppb) have also been measured in automobiles in use in polluted urban areas, and again, the ratio of HONO to NO2 was quite large, ~0.4, compared to 0.02-0.03 measured outdoors in the same study (Febo & Perrino, 1995). The generation of NO was attributed by Spicer and co-workers to a reaction of gaseous NO_2 with adsorbed HONO:

$$NO_2(g) + HONO(ad) \rightarrow H^+ + NO^-_3 + NO_3(g) \quad (2)$$

The same process was hypothesised to explain some time periods in a commercial office building when indoor NO actually exceeded outdoor NO (Weschler et al., 1994). As is the case in laboratory systems, equivalent amounts of HNO_3 are not observed as might be expected from the stoichiometry of reaction (1), likely due to HNO_3 remaining on the surface after formation and/or being taken up by surfaces. The accumulation of nitrate on indoor surfaces in a commercial building has been reported by Weschler and Shields (1996) and attributed to the formation and uptake of HNO_3 via reactions of NO_3 and/or oxidation of nitrite (i.e., adsorbed HONO) in an aqueous surface film. Subsequently, it was shown that HONO is also directly emitted by gas stoves (Pitts et al., 1989). In a house used for investigating indoor air pollution that had natural gas fueled appliances (a convective heater, a radiant heater, and a range with four burners), both the surface reaction of NO_2 and the direct combustion emissions contributed significantly to the measured indoor HONO. When an appliance was operational, the contribution of direct emissions was the more important source (Spicer et al., 1993). In short, the "dark reaction" of NO_2 with water on surfaces is ubiquitous and occurs not only in laboratory systems but also indoors. The combination of this heterogeneous reaction with combustion sources of HONO can produce significant concentrations of HONO indoors. As a result, there is a concern regarding the health impacts of nitrous acid, not only because it is an inhalable nitrite but also because it is likely the airborne acid present in the highest concentrations indoors.

2.3 Carbon monoxide and sulfur dioxide

As for NOx, combustion sources such as gas stoves and paraffin heaters can be significant sources of indoor CO. The ratio of indoor to outdoor concentrations of CO in homes using gas stoves has been measured to be 1.2-3.8 (Wade *et al.,* 1975), with the highest ratios found close to the source. Similarly, higher CO levels indoors compared to outdoors have been reported for restaurants in Korea, with those using charcoal burners as well as gas giving much higher concentrations (Baek *et al.,* 1997). In buildings where motor vehicle exhaust can be entrained from outdoors or attached parking garages, elevated indoor CO levels may also result (Hodgson *et al.,* 1991). On the other hand, in homes and offices where there was no direct indoor source of CO, the indoor to- outdoor ratio was about one, and sometimes

less. For example, in Riyadh, Saudi Arabia, CO concentrations were measured indoors and outdoors; the indoor to- outdoor ratio varied from 0 to 2, but was typically below one (Rowe *et al.,* 1989).

There have been a number of measurements of CO in the "indoor environment" of automobiles. Given that cars are major CO sources in urban areas, one might expect higher concentrations of CO during commutes and this is indeed the case. Typical CO concentrations of ~9-56 ppm have been measured inside automobiles during commutes in major urban areas (Flachsbart *et al.,* 1987; Koushki *et al.,* 1992; Ott *et al.,* 1994, 1995; Dor *et al.,* 1995; Fernandez-Bremauntz & Ashmore, 1995). This can be compared to peak outdoor levels of ~ 10 ppm in highly polluted urban areas. Thus, a significant enhancement of CO inside automobiles during commutes is common. For example, Chan *et al.* (1991) report a ratio of the in-vehicle CO concentration to that outdoors of ~ 4.5 in Raleigh, North Carolina. As is the case for CO, SO_2 levels indoors and outdoors tend to be similar if there are no combustion sources indoors.

2.4. Volatile Organic Compounds (VOCs)

Volatile organic compounds (VOC) are ubiquitous components not only of ambient air but also of indoor air environments, including offices, commercial and retail buildings, and homes (Shah & Singh, 1988; Finlayson-Pitts, 1999). There are three sources/categories for VOC: (1) entrainment of air from outside the building, (2) emissions from building materials, and (3) human activities inside buildings. As might be expected, given the nature of the sources, a very large variety of organic compounds have been identified and measured indoors (e.g., Brown *et al.,* 1994; Crump, 1995; Kostiainen, 1995). These numbers in the hundreds of different compounds, with the particular species and their concentrations depending on the particular sources present as well as the air exchange rates. Some of the compounds associated with the three sources: entrainment from outdoors, emissions from building materials, and anthropogenic activities - are now briefly reviewed.

Entrainment of air from outdoor sources: Entrainment of outdoor air through ventilation systems brings with it the species found in ambient air. Some of them, such as HNO_3, can be removed on surfaces such as those in air conditioning systems, and hence the indoor concentrations tend to be lower than those outdoors. Others such as NO tend to have similar concentrations indoors and outdoors if there are no significant combustion sources indoors (e.g., Weschler *et al.,* 1994). In the case of hydrocarbons, the concentrations of compounds that do not have significant indoor sources tend to be about the same as the outdoor concentrations. For example, Lewis and Zweidinger (1992) measured VOC in 10 homes in winter and showed that the concentrations of ethene, benzene, 2-methylpentane, methylcyclopentane, 2,2,4-trimethylpentane, and 2,3-dimethylbutane indoors were within experimental error of those outdoors. There are, however, some specific outdoor sources that can lead to higher concentrations of certain VOCs indoors than in the general outdoor air environment. For example, gases generated in landfills or from petroleum contamination can migrate through the soil and groundwater to adjacent buildings and homes to give larger indoor concentrations, particularly in basements and crawl spaces, than otherwise expected (Moseley & Meyer, 1992; Hodgson *et al.,* 1992; Fischer *et al.,* 1996).

In one such case, the total hydrocarbon concentration was measured to be 120 ppm in

a crawl space beneath the floor of a school where petroleum contamination was present from adjacent sources, compared to < 80 ppb outdoors (Moseley & Meyer, 1992). Although concentrations in various rooms were lower, they were still elevated compared to outdoors, ranging from 0.13 to 3.4 ppm. The use of pesticides *outside* buildings can also lead to enhanced concentrations of these compounds indoors. For example, Anderson and Hites (1988) measured the concentrations of chlorinated pesticides indoors and found elevated levels inside, e.g., a factor of 7 times higher for y-chlordane compared to outdoor levels. One home that had the highest indoor concentrations had been treated with chlordane about a decade earlier, presumably by subsurface injection from which the pesticide migrated into the house through cracks in the basement walls.

Enhanced levels of chlorpyrifos were observed indoors in homes where soil surrounding the home had been treated on a regular basis. Another source of VOC is motor vehicle emissions, which can be drawn into buildings from outdoors or parking garages (e.g., Perry & Gee, 1994; Daisey et al., 1994). For example, motor vehicles were major sources (responsible for > 75%) of 12 of 39 individual compounds measured in a dozen buildings by Daisey *et al.* (1994). Of the 12 compounds, 5 were alkanes and 7 were aromatics. Similarly, Baek *et al.* (1997) report that vehicle emissions are important VOC sources indoors in Korea during the summer in homes and offices, as has been reported in the United States (e.g., Hodgson *et al.,* 1991; Daisey *et al.,* 1994).

Building materials: Emissions associated with building materials are major contributors to indoor levels of VOC. New buildings often have higher concentrations of certain compounds compared to older buildings. For example, enhanced levels of n-dodecane, n-decane, and n-undecane, the xylenes, and 2-propanol have been measured in new buildings, and the total VOC concentration is generally larger (by factors of 4-23) compared to established buildings (Brown *et al.,* 1994). Kostiainen (1995) identified more than 200 individual VOCs indoors in 26 houses. In addition, they compared the VOC concentrations in normal houses to those where complaints of odours or illness had been registered. A number of different VOCs were present at increased concentrations in the houses with complaints compared to the normal houses; these included a variety of aromatic hydrocarbons, methylcyclohexane, n-propylcyclohexane, terpenes, and chlorinated compounds such as 1,1,1-trichloroethane and tetrachlorethene. Carpets are a major source of VOCs in homes. For example, Sollinger *et al.* (1993, 1994) have identified 99 different VOCs emitted from a group of 10 carpet samples, and Schaeffer *et al.* (1996) identified more than 100 different VOCs emitted from the carpet cushion alone. Emissions come not only from the carpet fibres but also from the backing materials and the adhesives used to bind the carpet to the backing. As a result, the individual compounds emitted by carpets can vary substantially, depending on the carpet construction. Many of the compounds emitted are known to be used in the manufacturing processes (e.g., e-caprolactam is used in Nylon-6 production) and /or are common solvents Emissions of VOC from carpets tend to decrease with time and increase with temperature.

The dependence of VOC emissions from building materials on relative humidity is more complex, with some emissions increasing with relative humidity, but others not. For example, Sollinger *et al.* (1994) report that the VOC emissions from carpets did not change with relative humidity over the range from 0 to 45% RH. On the other hand, the emissions of formic and acetic acids from latex paints have been reported to increase dramatically with

relative humidity; for example, for one paint sample the emission rate for acetic acid almost tripled when the relative humidity was changed from 4-5% to 5-23% (Reiss *et al.,* 1995b). A number of different aldehydes have been measured indoors (Crump & Gardiner, 1989; Lewis & Zweidinger, 1992; Zhang *et al.,* 1994; Daisey *et al.,* 1994; and Reiss *et al.,* 1995a), some of which are directly emitted and some of which are formed by chemical reactions indoors of VOCs such as styrene.

Of these, there is an enormous amount of evidence for direct emissions of HCHO from building materials. Interest in formaldehyde emissions and levels in homes and other buildings stems from its well-known health effects, which include possible human carcinogenicity and eye, skin, and respiratory tract irritation (Feinman, 1988). Formaldehyde is emitted from urea-formaldehyde foam insulation as well as from resins used in reconstituted wood products such as particleboard and plywood (Meyer and Reinhardt, 1986); urea-formaldehyde resins comprise about 6-8% of the weight of particleboard and 8-10% of mediumdensity fiberboard (Meyer and Hermanns, 1986). Other sources include permanent press fabrics (such as draperies and clothing), floor finishing materials, furniture, wallpaper, latex paint, varnishes, some cosmetics such as fingernail hardener and nail polish, and paper products (Kelly, 1996; Howard *et al.,* 1998a, 1998b).

Many measurements of HCHO have been made in indoor air environments. In conventional homes, average concentrations are typically about 10-50 ppb (Stock, 1987; Zhang *et al.,* 1994; Reiss *et al.,* 1995a). Sexton *et al.* (1989) measured concentrations of HCHO in 470 mobile homes in California and found geometric mean concentrations of 60-90 ppb, although maximum values of over 300 ppb were recorded in some cases. In a similar study in Wisconsin, levels up to 2.8 ppm were measured (Hanrahan *et al.,* 1985). Higher levels are typically found in mobile homes because of the reconstituted wood products (e.g., particleboard and plywood) used in their construction. Interestingly, HCHO does not appear to be a significant product of natural gas combustion, as levels in dwellings with and without gas stoves turned on are not significantly different (e.g., Pitts *et al.,* 1989; Zhang *et al.,* 1994). Temperature is again an important determinant of HCHO levels.

Human activities: There are many sources of VOCs associated with human activities in buildings. For example, mixtures of C_{10} and C_1~ isoparaffinic hydrocarbons, which are characteristic of liquid process copiers and plotters, have been identified in office buildings in which these instruments were in use (Hodgson *et al.,* 1991). Emissions of a number of hydrocarbons and aldehydes and ketones have been observed during operation of dry-process copiers; these include significant emissions of ethylbenzene, o-, m-, and p-xylenes, styrene, 2-ethyl-l-hexanol, acetone, nnonanal, and benzaldehyde (Leovic *et al.,* 1996). Enhanced levels of acetaldehyde in an office building in Brazil were attributed to the oxidation of ethanol used as a cleaning agent (Brickus *et al.,* 1998), although levels outdoors were also enhanced due to the use of ethanol as a fuel. Pyrocatechol has been measured in an occupational environment where meteorological charts are mapped on paper impregnated with this compound (Ekinja *et al.,* 1995), and p-dichlorobenzene is observed when mothballs containing this compound are in use (e.g., Tichenor *et al.,* 1990; Chang and Krebs, 1992). Elevated concentrations of the n-C_{13} to n-C_{18} alkanes and branched-chain and cyclic analogs were measured in a building having a history of air quality complaints; the source was found to be volatilisation from hydraulic fluids used in the building elevators

(Weschler *et al.,* 1990). Enhanced levels of chlorinated compounds have been observed indoors due to human activity as well. For example, increased levels of perchloroethylene have been observed from unvented dry-cleaning units (Moschandreas & O'Dea, 1995) and volatilisation of chlorinated organics such as chloroform from treated tap water can occur (McKone, 1987). Other sources include the use of household products. For example, chloroform emissions have been observed from washing machines when bleach containing hypochlorite was used (Shepherd *et al.,* 1996). It is interesting that emissions of organics associated with the use of washing machines are decreased when the machine is operated with clothes inside (Howard and Corsi, 1998).

Of course, activities such as smoking result in enhanced levels not only of nicotine (e.g., Thompson *et al.,* 1989) but also of a variety of other gases associated with cigarette smoke (e.g., California Environmental Protection Agency, 1997; Nelson *et al.,* 1998). For example, using 3-ethenylpyridine as a marker for cigarette smoke, Heavner *et al.* (1992) estimated that 0.2-39% of the benzene and 2-49% of the styrene measured in the homes of smokers was from cigarette smoke. Humans emit a variety of VOCs such as pentane and isoprene (e.g., Gelmont *et al.,* 1981; Mendis *et al.,* 1994; Phillips *et al.,* 1994; Jones *et al.,* 1995; Foster *et al.,* 1996). In addition, emissions from personal care products have been observed. Decamethylcyclopentasiloxane (D5), a cyclic dimethylsiloxane with five Si-O units in the ring, and the smaller D4 analog, octamethylcyclotetrasiloxane, are used in such products as underarm deodorant and antiperspirants at concentrations up to 40-60% by weight (Shields and Weschler, 1992; Shields *et al.,* 1996).

Increased concentrations of D5 have been measured in offices and are correlated to human activity, as expected if personal care products were the major source (Shields and Weschler, 1994). In some cases, increased concentrations attributable to emissions from silicone-based caulking materials were also observed (Shields *et al.,* 1996). The use of pesticides indoors can lead to very large concentrations not only of the pesticide but of the additional VOCs used as a matrix for the pesticide, which represent most (>95%) of the mass of the material as purchased. For example, Bukowski and Meyer (1995) predict that VOC concentrations immediately after the application of a fogger could reach levels of more than 300 mg m^{-3}!

2.5 Ozone

Because O_3 decomposes on surfaces, indoor levels are usually lower than those outdoors due to the decomposition that occurs as the air passes through air conditioning systems and impacts building surfaces (Reiss *et al.,* 1994; Finlayson-Pitts, 1999). The measured ratio of indoor-to-outdoor concentrations of ozone vary from 0.1 to 1, but are typically around 0.3-0.5 (e.g., Druzik *et al.,* 1990; Hisham & Grosjean, 1991; Liu *et al.,* 1993; Weschler *et al.,* 1989, 1994; Gold *et al.,* 1996; Jakobi & Fabian, 1997; Avol *et al.,* 1998; Drakou *et al.,* 1998; Romieu *et al.,* 1998). Buildings with low air exchange with outside air tend to have lower ratios, ~0.1-0.3 (Druzik *et al.,* 1990; Weschler *et al.,* 1994; Romieu *et al.,* 1998). For example, Gold *et al.* (1996) estimate that at outdoor ozone concentrations of 170 ppb in Mexico City, the indoor-to-outdoor ratio of O_3 at a school was 0.71 ± 0.03 with the windows and doors open, which maximised the exchange with outside air, 0.18 ± 0.02 with the windows and doors closed and the air cleaner off, and 0.15 ± 0.02 with the windows and doors closed and the air cleaner on. There are some additional sources of O_3 indoors.

These include dry-process photocopying machines, laser printers, and electrostatic precipitators (e.g., Leovic *et al.,* 1996; Wolkoff, 1999). Indeed, it is not unusual to detect O_3 by its odour during operation of some copy machines and laser printers. In the "indoor environment" in cars, ozone levels tend to be significantly less than in the surrounding area. For example, Chan *et al.* (1991) report that in-vehicle O_3 concentrations during commutes in Raleigh, North Carolina, were only about 20% of those measured in the local area at a fixed station. There are several contributing factors to these low concentrations. One is that NO concentrations are higher near roadways, so that O_3 is titrated to NO_2 by its rapid reaction with NO. A second is that O_3 can decompose on the surfaces of the automobile air conditioning system. A similar titration effect has been observed inside homes where there are combustion sources of NO.

2.6 Particles

With the epidemiological studies suggesting increased mortality associated with particles, there has been increasing interest in indoor particle concentrations compared to outdoor levels (Finlayson-Pitts, 1999). A number of studies have examined this over the years and are summarised in a review by Wallace (1996). In general, if there are no indoor sources of particles, the levels indoors tend to reflect those outdoors. For example, application of a mass balance model to measurements of indoor and outdoor particle concentrations in Riverside, California, indicated that 75% of $PM_{2.5}$ and 65% of PM_{10} in a typical home were from outdoors (Wallace, 1996). Similar conclusions were reached by Koutrakis *et al.* (1991, 1992) for homes in two counties in New York. For example, they report that 60% of the mass of particles in homes is due to outdoor sources. However, the contribution to various individual elements in the particles varies from 22% for copper to 100% for cadmium. There are some differences in indoor levels of particulate matter in areas with low outdoor compared to high outdoor levels. In the case of high outdoor levels, the indoor concentrations tend to be somewhat lower than those outdoors; for example, Colome *et al.* (1992) report that the ratio of indoor-to-outdoor median concentrations of PM_{10} is 0.7 in residences in southern California.

On the other hand, when outdoor levels are low, indoor levels tend to be higher Night time mass concentrations indoors tend to be smaller than those during the day, probably because of the decreased activity. Interestingly, when individuals wear personal exposure monitors to measure their actual exposure to particles, the measured mass concentrations tend to be higher than those measured with fixed monitors located indoors. A major source of increased particles indoors is cigarette smoking. (e.g. Spengler *et al.,* 1981; Quackenboss *et al.,* 1989; Neas *et al.,* 1994). In addition to the contribution to the *mass* concentrations of indoor particles, cigarette smoke is of concern because of the mutagens, carcinogens, and toxic air contaminants that are emitted (Löfroth *et al.,* 1991; Chuang *et al.,* 1991; California Environmental Protection Agency, 1997; Nelson *et al.,* 1998). Thus, a variety of both gaseous and particulate polycyclic aromatic hydrocarbons (PAH) and compounds (PAC) have been identified in buildings with cigarette smoke (Offermann *et al.,* 1991; Mitra & Ray, 1995). Indeed, in the homes of smokers, almost 90% of the total PAH was from tobacco smoke (Mitra and Ray, 1995).

Higher levels of mutagenic particles have also been shown to be associated with indoor air containing cigarette smoke (e.g., Lewtas *et al.,* 1987; Löfroth *et al.,* 1988, 1991; Georgiou

et al., 1991). Other significant sources identified in a number of studies are cooking, the use of paraffin heaters, wood burning, and humidifiers. For example, a study carried out under the auspices of the U.S. Environmental Protection Agency, the TEAM study (Total Exposure Assessment Methodology), indicated that an increase in PM m of ~10-20 / μg m^{-3} could be attributed to cooking (Wallace, 1996). This source will obviously depend on the amount of cooking, the types of cooking, and the ventilation. For example, Löfroth *et al.* (1991) measured emissions of particles ranging from 0.07 to 3.5 mg per gram of food cooked, depending on the particular food. Baek *et al.* (1997) measured indoor and outdoor concentrations of particles in homes, offices, and restaurants in Korea and report ratios of 1.3, 1.3, and 2.4, respectively.

The higher value in restaurants, even those using only gas and not charcoal, suggests a significant contribution from cooking. Paraffin heaters can be significant sources of particles under some circumstances. For example, paraffin heaters were reported to contribute to indoor $PM_{2.5}$ in homes in Suffolk County, New York, but not Onondaga County; wood stoves and fireplaces and gas stoves did not contribute in either case (Koutrakis *et al.,* 1992; Wallace, 1996). A similar conclusion was reached in a study of eight mobile homes in North Carolina (Mumford *et al.,* 1990). However, it should be noted that even where paraffin heaters do not contribute significantly to particle *mass* concentrations, they may still be important in terms of health effects. This is because of the composition of the particles emitted, which include polycyclic aromatic compounds and other mutagenic species, as well as sulfate (Traynor *et al.,* 1990). For example, Traynor *et al.* (1990) studied the emissions from unvented paraffin space heaters and identified a number of PAHs (naphthalene, phenanthrene, fluoranthene, anthracene, chrysene, and indeno[c,d]pyrene) and nitro-PAHs (1-nitronaphthalene, 9-nitroanthracene, 3-nitrofluoranthene, and 1-nitropyrene), in addition to a host of other gaseous species.

Baek *et al.* (1997) also reported increased levels of a number of gases indoors in homes and offices in Korea due to the use of paraffin heaters. In studies of indoor air in eight mobile homes, Mumford *et al.* (1991) identified the PAHs and nitro- PAHs measured in emissions from paraffin heaters by Traynor *et al.* (1990), as well as a number of compounds that may be animal carcinogens, such as cyclopenta[c,d]pyrene, benz[a]anthracene, benzofluoranthenes, benzo-[a]pyrene, and *benzo[ghi]perylene.* While the mass concentrations of PM10 did not increase with the paraffin heater on in six of the eight homes studied, the particles in five of the homes had increased mutagenicity using TA98 with or without $9 added. In short, not only the mass emissions but also the nature of the compounds emitted must be taken into account in assessing the health effects of indoor particles.

Where indoor heating and cooking involves the use of coal or biomass, indoor particle concentrations can be extremely large. For example, Florig (1997) and Ando *et al.* (1996) report that in China typical indoor total suspended particle (TSP)concentrations can be in the range from 250 to 900/μg m^{-3} in homes using coal and 950-3500 / μg m^{-3} in those using biomass fuels. These levels can be compared to annual average outdoor concentrations of 250-410 / μg m^{-3}. The high concentrations associated with coal burning combined with the mutagenic nature of the emissions have been suggested to be responsible for enhanced lung cancer in China (Mumford *et al.,* 1987). Similarly, Davidson *et al.* (1986) measured TSP concentrations of 2900-42,000/ μg m^{-3} in homes in Nepal that used biomass fuels, compared

to outdoor levels of 280 / μg m $^{-3}$. For particles with diameters less than 4 /μm, the levels ranged from 870 to 14,000/ μg m $^{-3}$. Similar conclusions regarding the relative indoor and outdoor concentrations have been reached in studies of office and commercial buildings. For example, Ligocki *et al.* (1993) measured indoor and outdoor concentrations of particles and their components at five museums in southern California. The indoor-to-outdoor ratios of particle mass varied over a wide range, depending to a large extent on the ventilation and filtration systems in use. Ratios varied from 0.16 to 0.96 for particles with diameters less than 2.1/μm and from 0.06 to 0.3 for coarse particles with diameters greater than this.

2.7 Asbestos and manmade mineral fibers

Asbestos is known to cause a number of diseases after occupational exposure (Brown & Hoskins, 1993). Before the hazards associated with the inhalation of these mineral fibers were understood these exposures were often very large with frequent reports of dust clouds so great that visibility in the workplaces was considerably reduced. This type of exposure is quantitatively quite different from those in the general environment that have provoked a response which in some quarters approaches hysteria. In the USA at least there is massive expenditure on asbestos removal, management and litigation.

Asbestos is a collective, trivial, name given to a group of highly fibrous minerals that are readily separated into long, thin, strong fibers occurring on sufficient large bulk deposits for their industrial exploitation. Asbestos minerals were usually used for their insulating properties, or in a composite, where they added strength, as in cement, or increased friction, as in brake shoes. Chrysotile, or white asbestos has counted for over 90% of the world trade in asbestos minerals. It is a serpentine mineral while the others (amosite (brown asbestos); crocidolite (blue asbestos); anthophyllite; tremolite; and actinolite) are all amphibole minerals. Amphibole asbestos has grater acid and water resistance than chryusotile and was used where these properties made it more suitable. Sometimes users would be unaware of the differences between the types of asbestos and so different minerals could have been used for a single application.

Recently the concern over the health effects of asbestos has been extended to another group of fibrous materials- the man-made mineral fibers (MMMF). While this term is self-explanatory a variety of types are produced with diverse chemical compositions, properties and uses. While sometimes referred to as ‘asbestos substitutes’ the majority of uses for the manmade fibres are relatively novel and ones for which the natural fibers are unsuitable. For example refractory ceramic fibers are resistant to considerably higher temperatures than are any of the natural fibres. The development of synthetic fibrous insulation materials has been given a great impetus in recent years by the need for more thermally efficient buildings and industrial processes.

MMMF can be made from most types of glass, from rock such as basalt, diabase and olivine and from various types of slag. Ceramic fibers can be made from kaolin or from pure silica and other oxide starting materials. The MMMF have been classified into four broad groups based on the manufacture and use: continuous filament glass fiber made by extrusion and winding processes, insulation wool (including ceramic fiber), and special purpose fibers. The non-continuous fibers are made by dropping molten material onto spinning disks or by air or steam jet impingement on a stream of the molten material. They contain a wide range

of fiber sizes and are contaminated by small glassy balls called shot which often account for 50% of the product by weight.

2.8 Microbial pollutants

Microbial pollution is a risk to health and is associated with allergic illnesses. Published results indicate that 20% of the population can be sensitised by airborne fungal spores in the UK, while 40% of the inspected houses in Germany suffer from mould-related problems (Waubke & Kusterle, 1990). The medical consequences of immune response, allergic reactions, endotoxins, mycotoxins, and epidemiology have been extensively studied by Miller (1990), Morey (1990), Gravensen et al. (1990) and Burge et al. (1990). Similarly, Legionnaires' disease and Pontiac fever are associated with wet cooling towers and domestic hot-water systems in complex buildings.

Accordingly to the official published figures, some 560,000 people need treatment because of indoor pollution due to mites and mould in damp houses (House of Commons Environment Committee, 1991). Indoor airborne allergic components come from two sources: outdoor air-borne spores moving inside and allergic components originating inside the dwelling.

The source of biological growth within buildings is associated with moisture and the formation of microclimates; it also depends upon the type of the buildings and their ventilation. Mould fungi thrive on surfaces on which there is nourishment and suitable humidity, for example on damp water pipes, windows and walls in kitchens and bathrooms, in central air-conditioning systems, circulation pumps, blowers, ventilation ductwork and air filters, central dehumidifiers, and inside damp structures. Allergenic substances can be airborne and inhaled, such as pollen, fungus and dust, digested, such as mouldy food or drink. Investigations suggest that airborne allergies cause more problems throughout the world than all other allergies combined. Additionally, cross-infection from patient to patient is of great concern in hospitals. The medical field that treats allergies recognizes the following allergenic diseases: asthma, allergic rhinitis, serous otitis media, bronchopulmonary aspergillosis, and hypersensitivity pneumonitis.

Allergic load and cocktail effect: For some people, an allergic reaction in the indoor environment may be triggered by non-biological factors, such as chemicals or other indoor air pollutants, emotional stress, fatigue or changes in the weather. These factors burden allergic people further if they are suffering from allergic reactions to biological contaminants. This combination is known as 'allergic load'. Microbial contaminants propagated within the health care establishment are particularly aggressive to patients due to reduced immune system resistance.

Recently, attention has been focused on the cocktail effect of chemicals present in indoor air. Volatile organic compounds may be produced from the use of wood preservatives and remedial timber treatment chemicals, moth-proof carpets, fungicides, mouldicide-treated paints, furnishing materials such as particle board and foamed insulation which may emit formaldehyde. Biological pollutants alone or in synergetic effect with any of the above-mentioned volatile organic compounds may produce symptoms such as stuffy nose, dry throat, chest tightness, lethargy, loss of concentration, blocked, runny or itchy nose, dry skin, watering or itchy eyes or headache in sensitive people. The 'sick building syndrome' (SBS)

or tight building syndromes may arise from a variety of causes. Because of the uncertainties about the causes of SBS and the rising levels of health related problems in buildings there is an increasing use of the term building-related illness (BRI) to cover a range of ailments which commonly affect building occupants.

3. EXPOSURE FACTORS OF INDOOR AIR POLLUTANTS

3.1 General

The types and quantities of pollutants found indoors vary temporally and spatially. Depending on the type of pollutant and its sources, sinks and mixing conditions, its concentration can vary by a factor of 10 or more, even within a small area.

Human mobility constitutes an important kind of complexity in the determination of exposure to air pollutants. Human activity patterns differ between midweek and weekend, between one season and another, and between one part of one's life and another. Activity patterns determine when and how long one is exposed to both indoor and outdoor pollutants. Therefore, in reviewing the factors that influence air-pollution exposures, we have specifically separated them into two major components: time (activity) and concentration (location).

Information on the time spent in various activities is summarised first, and then the variations in concentration often encountered in different locations. Unfortunately, most of the studies discussed were not longitudinal and thus do not offer information on seasonal differences in time spent indoors and outdoors or on regional differences in activity patterns.

Outdoor concentrations of pollutants and rates of infiltration affect the concentrations to which people are exposed indoors. Building construction techniques, as they vary geographically, and their effect on pollution infiltration are particularly important. But the measurement techniques available are limited; the need for additional studies is discussed. The rates of infiltration on a neighbourhood scale have been studied by only a few researchers. Although their work has focused on energy conservation, their findings can easily be applied to the study of impact on indoor pollution.

Patterns of human behaviour and activity determine the time spent in any specific location, and thus knowledge of them is essential in estimating exposures of populations to pollutants. As indicated by Ott (Ott, 1995), a large number of variety of studies in which data on human activities were collected from population samples have been completed over the past 50 ye.

When one examines the literature on human activities, the term "time budget" ("zeitbudget" , "budget de temps") is encountered often. A time budget produces a systematic record of how time is spent by a person in some specified period, usually 24 h. It contains considerable detail on a person's activities; including the locations in which the activities take place (Michelson, 1973).

One way of obtaining time budget information from the populations surveyed is to ask each respondent to maintain a diary of his or her activities over a 24-h period or longer. In another approach, the so-called "yesterday" survey approach, the interviewer asks each responder about his or her activities on the preceding day.

Several summaries of the historical development of time-budget research have been published (Chapin, 1974; Converse, 1968; Ottensman, 1972). Ott (Ott, 1995) discussed the literature on activity patterns in the context of estimation of exposure to air pollution. Owing to the small number of field monitoring studies, the geographic distribution of indoor air pollutants has not been determined. However, it is instructive to review the geographic distribution of the major factors that affect variations in the concentrations of pollutants and their impact on the quality of the indoor environment. Outdoor air quality, air-infiltration rates, and sources of emission of indoor air pollutants are the major factors. Outdoor air quality has been studied with respect to some pollutants, and the geographic distribution of these few pollutants is well understood. Descriptive statistics published annually by EPA and state and local air-quality agencies furnish much scientific information useful in discerning regional and local differences in concentrations of carbon monoxide, total suspended particles, ozone, NOx, sulfur dioxide, sulfates, and others. It should be noted that the geographic distribution of some criteria pollutants has been studied and is easily accessible from the literature; information on non-criteria pollutants is sparse and often collected and analyzed by questionable methods.

Concentrations of chemically non-reactive pollutants in residences generally correlate with those outdoors. Distribution of indoor air quality is extremely difficult to describe on a geographic scale, because indoor air quality is determined by complex dynamic relationships that depend heavily on occupant activity and highly variable structural characteristics. Weather, which has a regional character, influences indoor air concentrations of some chemicals, such as formaldehyde, and biologic contaminants, such as bacteria and molds. Therefore, the influence of relative humidity and other weather-related conditions affecting indoor environmental quality needs to be studied geographically. Research specifically addressed to geographic distribution of indoor air quality is needed.

3.2 Air infiltration

Typically, the air-infiltration rate for American residences is assumed to be 0.5- 1.5 ach. This assumption is supported by the results of several energy and air-quality studies that experimentally determined the range of ventilation rates for typical residences to be between 0.7 and 1.1 ach (Moschandreas & Morse, 1979). However, the sample that yielded the data is small, and statistical documentation for such statements is not strong.

The quality of indoor air is a function of outdoor air quality, emission from indoor sources, air-infiltration rates, and occupant activity is likely to vary within each metropolitan and suburban area, is indeed within each neighbourhood. Within a metropolitan area, it has been shown that an urban complex leads to the so-called urban heat reservoir (American Society of Heating, Refrigerating and Air-Conditioning engineers. ASHRAE, 1972). Urban characteristics-- such as city size, density of buildings, and population-- correlate with such meteorological factors as temperature, pressure and wind velocity (Gibson, & Cawley, 1977; Kostiainen, 1995). The urban heat island affects both urban pollution patterns and meteorological characteristics that affect the infiltration rates of buildings. Thus, although the exact nature of the impact on indoor air quality is not known, it is fair to expect that the heat island to have an impact on the indoor environment that is likely to be adverse. Also, the variations due to mechanical ventilation, structural differences, and air infiltration may vary within a neighbourhood as a function of such factors as house orientation, tree barriers,

and terrain roughness.

Occupant activity, air-infiltration rates, the indoor sources of pollutants and their chemical natures are some of the factors that cause variations within a city. A study (Moschandreas et al., 1980) in the Boston metropolitan area obtained indoor air samples from 14 residences under occupied "real-life" conditions for 2 week each. The indoor air character not only was driven by outdoor concentrations, but was greatly affected by other factors, such as indoor activities.

Wind speed, temperature difference, pressure differential, terrain characteristics (roughness and barriers, such as trees and fences), building orientation, and structure characteristics may be affected by the location of one residence relative to another within a neighbourhood.

The indoor air quality of an individual building is often characterised by the 24-h average for the concentration of one pollutant measured at one sampling location. Because the activity patterns of persons are such that more time is spent in some indoor areas than in others, the question arises (Moschandreas et al., 1978): "Do indoor zones (independent areas) with distinct pollutant patterns exist?" At issue here is whether sampling from one monitoring zone is sufficient to characterise the air quality of an entire building.

3.3 Indoor air quality

In an extensive analytic study of indoor air quality, Shair and Heitner (1974) assumed that there are no pollutant gradients in the indoor environment. The experimental database of Moschandreas and co-workers (1980) verified that the gradients in concentrations of several gaseous pollutants in the residential environment are negligible. J.D. Spengler, R.E. Letz, J.B. Ferris, Jr., T. Tibbets, and C. Duffy reported (at the annual meeting of the Air Pollution Control Association, 1981) on weekly nitrogen dioxide measurements in 135 homes in Portage, Wisconsin. On the average, kitchen concentrations were twice those in bedrooms in homes that had gas stoves. A study of the air quality in a scientific laboratory by West (1977) showed an almost uniform distribution of an intern tracer continuously released in the room. Similar experiments performed by Moshandreas et al. in residential environments showed that equilibrium is reached throughout a house within an hour. Episodic release of sulphur hexafluoride tracer gas also illustrates this point. The source location was the living room; adjacent locations were the kitchen and the hall. Episodic release of this inert gas in 24 residences was followed by uniform indoor distributions within 30 min (Moschandreas et al., 1978; Peterka & Cermak, 1977). The one-zone concept does not require instantaneous mixing, because it is based on the behaviour of hourly average pollutant concentrations.

Moschandreas and associates (1980) used a different database derived from the monitoring of 14 indoor environments in the Boston metropolitan area. Analysis of variance was used to reach the following conclusions:

Pollutants (ozone and sulphur dioxide) generated principally outdoors have little or no interzonal statistical difference indoors.

Pollutants with strong indoor generation have interzonal statistical differences in residences with gas facilities and offices, but not in electric-cooking residences. In general, the observed differences are not large, and the health differences are not expected to be

serious.

Depending on indoor activity and outdoor episodic pollutant activity, the indoor arithmetic 24-h average may or may not adequately represent the variation of hourly indoor concentrations. Although more than one zone would be preferable, hourly pollutant concentrations obtained from one indoor zone adequately characterise the indoor environment.

The most important factors that influence exposure to indoor air pollutants are the one described under. It should be noticed that these conclusions are not applicable to short-lived pollutants. Contaminants associated with tobacco smoke, bathroom odours, allergens, and other pollutants related to dust are expected to vary considerably in a given residence. Additional documentation is needed to determine the extent of this variation.

3.4 Site characteristics

The characteristics of a building site that influence indoor air quality are addressed as three related subjects: air flow around buildings, proximity to major sources of outdoor pollution, and type of utility service available.

The air flow around a building has been shown to be determined by the local characteristics of the geometry of surrounding buildings (Peterka & Cermak, 1977), the location and type of surrounding vegetation (White, 1995), the terrain (Geiger, 1965), and the size and shape of the building itself. Pollutants can be transferred by the air flow from the street level, over the façade of the building and onto the roof (Cermak, 1976). Field tests of isolated buildings have been used to develop scaling coefficients for both isothermal and and stratified cases of surface wind pressures, turbulence, and dispersion (Davenport, 1960.). Air flow around the building creates low pressure on the leeward side and/or the sides adjacent to the windward face, as well as the roof. Air pollutants released from stacks, flues, vents, and cooling towers in the region can re-enter the building through make-up air intakes for ventilation (Cermak, 1976).

Trees and forests have been generally studied as shelter belts in an agricultural context. Shelter belts affect air flow around buildings. When an air current reaches a shelter belt, part of it is deflected upward with only a slight change in velocity, part passes through the crowns of the trees with very low velocity, and part is deflected beneath the canopy with rapidly decreasing velocity (Federer, 1971). The changes in velocity of air flow outside may change the infiltration rate and thus affect indoor air quality.

The location of a building relative to a major outdoor pollution source can affect indoor air quality. For example, buildings near major streets or highways often have high carbon monoxide and lead concentrations, owing to the infiltration of these pollutants.

The type of utility service available is also related to the site of the building and may affect the character of its indoor environment. The availability of particular fuels (e.g., natural gas and oil) influences the types and concentrations of pollutants (e.g., combustion products) emitted by space-and water- heating. Service moratoria, development timing, and development scale are institutional elements that contribute to the variability of utility services and thus can affect indoor air quality.

3.5 Occupancy

Occupancy factor that affect indoor air quality include the type and intensity of human activity, spatial characteristics of a given activity, and the operation schedule of a building.

Several human activities-such as smoking, cleaning and cooking- generate gaseous and particulate contaminants indoors. The number of occupants of a space and the degree of their physical activity (i.e., metabolic rate at rest or under intense activity) are related to the production of various pollutants, such as carbon dioxide, water vapour, and biologic agents. If the only source of indoor carbon dioxide is that caused by occupants, ventilation rates may be proportional to the number of people and their metabolic rates (Mclntyre, 1980). Although studies have shown no constant relationship between carbon dioxide concentrations and the concentrations of other pollutants, carbon dioxide concentration is often used as a general indicator of the adequacy of ventilation in an occupied space.

Building occupancy is often expressed as occupant density and the ratio of building volume to floor area. The importance of occupancy in indoor air quality is illustrated by the fact that the choice of natural or mechanical ventilation is based on occupant density and the spatial characteristics of the building under consideration.

Occupancy schedule and associated building use may affect the type, concentration, and time and space distribution of indoor pollutants. Because most buildings are unoccupied for substantial portions of each day, the manipulation of "operating schedule" is a means of controlling energy use (American Institute of Architects Research Corporation. Phase Two Report for the Development of Energy Performance Standards for New Buildings, 1979). Efforts to conserve energy through the design of ventilation systems can result to the degradation of indoor air quality. However, detailed studies relating ventilation capacity, occupancy schedules, energy requirements, and indoor air quality have only recently been implemented.

3.6 Design

Elements of building design that affect the indoor environment include interior-space design (space planning), envelope design, and selection of materials.

The evolution of space planning in many building types has resulted in flexibility in assigning functions to specific locations. However, this flexibility is accompanied by a decrease in the ability to predict exposure to air pollutants. In particular, "open-plan" offices and schools have serious technical problems of redundant service distribution, limited acoustic control, incomplete air diffusion, and incomplete pollutant dispersion indoors, compared with "fixed-plan" floor layouts.

Evaluation of the success of a floor plan in achieving space efficiency, structural economy, and energy efficiency is usually in terms of net area per occupant and ratio of net usable area to total area. Explicit planning for environmental quality must be included to ensure that spatial arrangements are acceptable to the occupants.

A building's structural envelope consists of both primary elements -foundations, floors, walls, and roofs- and secondary "skin" elements -facings, claddings, and sheathing. To various degrees, the function of these is to maintain the integrity of the structure under the stresses caused by structural load, wind pressure, thermal expansion, precipitation, earth movement,

and fire. The integrity of the building envelope is a major consideration in uncontrolled air movement into and out of the building –usually referred to as "infiltration". This is a major factor in indoor air quality. There has been no systematic survey of infiltration rates of buildings in the United States. The dominant factor in determining a building's infiltration rate is the total area of effective leakage, as measured with fan pressurisation. Following the leakage area in importance are the terrain and shielding near the building, the mean climatic conditions during heating (or cooling) periods, and the building height (Sherman, 1981). There is much evidence (Dickerhoff et al., 1980), both in the United States and in Europe, that houses in mild climates are "very leaky", whereas houses in severe climates are "tight".

Greater height of a building increases the "stack effect", or updraught, and exposes the building to higher wind speeds. Thus, higher wind pressures drive air through existing openings, referred to as "leakage", increasing the infiltration rate.

The dominant building factors that determine infiltration have not been identified, but a catalogue of leakage openings found in typical structures is as follows:

Walls: Leakage around sill plates (the openings at the bottom of wallboard), electric outlets, plumbing penetrations, and headers in attics for both interior and exterior walls. Windows and doors: Window type is more important than manufacturer in determining window leakage. This source of leakage tends to be overrated; it contributes only about 20% of the total leakage of a house Fireplaces: This includes dampers, glass screens, and fireplace caps. Heating and cooling systems: The variables include combustion air for furnaces, dampers for stack air draft, air-conditioning units, and location of ductwork. Vapour barrier and insulation penetrations. Utility accesses: This includes recessed lighting and plumbing and electric penetrations leading to attic or outside. Terminal devices in conditioned space: This includes leakage of dampers, especially those for large air-handling systems. Structural types: Examples are drop ceilings above cupboards or bathtubs, prism-shaped enclosures over staircases in two-story houses, and elevator and utility shafts that lead from basement to attic. Wall and ceiling materials and floor finishes are the constituents of the building interior. Modular components, weight, strength, thermal insulation, thermal stability, sound insulation, fire resistance, ease and speed of installation and ease of maintenance are among the criteria considered in the selection of materials for walls, ceiling and floors. But emphasis on first cost, ease of installation, maintenance and long service life has also led to the use of materials that may be sources of indoor contaminants.

3.7 Operations

Depending on the type of ownership (owner-occupied or developer-owned), building operation may vary considerably, and this variation may have an impact on indoor air quality. "Building operation" pertains to the following elements of a building: the building envelope, service and plant, building facilities, equipment and landscaping. Cleaning, preventive maintenance, and replacement and repair of defects are also included in building operation. The staff responsible for building operation includes management, engineering, and custodial personnel. The care responsibilities are operation of the heating, ventilation, and air-conditioning systems and building services, such as hot water, lighting and power distribution. Building operation has an impact on indoor air quality in numerous ways, but the magnitude of this impact is not known.

4. HEALTH EFFECTS OF INDOOR AIR POLLUTION (IAP)

As far as it concerns the health effects of IAP, it is very interesting to present the methods of studying health effects, the criteria for the assessment of the impact of IAP on the community and the diverse effects of IAP on human health (ECA, 1991).

4.1 Methods of studying health effects

Methods of studying health effects of indoor pollutants can be grouped into three broad categories:

a. Human studies, subdivided into observational and experimental studies.

Epidemiological studies of pollutants are mostly observational, i.e. the investigator has no means of experimentally exposing humans to pollutants, or of allocating subjects to exposed and unexposed groups. Critical issues are therefore the validity and precision of exposure assessment, and the control for confounding factors in these studies. Recent developments have stressed the importance of reducing exposure misclassification, and of studying restricted, well defined, homogenous populations to address these issues. The main advantage is that humans are studied under realistic conditions of exposure. By themselves, observational epidemiological studies are not usually sufficient to support causality of an observed association, so that additional information is needed from other types of studies. Experimental studies are among these; however, these are only suitable for studying moderate, reversible, short term effects in persons who are healthy or only moderately ill. Their main advantage is that exposure conditions and subjects election are under the control of the investigator.

b. Animal studies, which can be subdivided into a number of categories depending on their length (acute, subchronic, chronic) or end-point (morbidity, mortality, carcinogenicity, irritation, etc.). Here, the investigator has full control over exposure conditions and health effects studied. However, the principle limitations lie in the fact that extrapolation from the studied animal species to man is always necessary. Also, while in human populations health effects with low incidences are often of interest (e.g., specific cancers), it is not feasible to study very large groups of animals to detect these low incidences. In practice, therefore, animal experiments are often carried out using very high experimental doses to compensate for the relatively small number of animals used and as a consequence, an additional extrapolation from high to low doses is also often necessary.

c. In vitro studies, in which effects of pollutants on cell or organ cultures are studied. These studies have the advantage that they are less costly than animal studies, and that results can generally be obtained in a shorter period of time. They are useful for studying mechanisms of action, but it is not usually possible to predict effects on whole organisms from their results in a quantitative way.

4.2 Criteria for the assessment of the impact of IAP on the community

The process of risk characterisation for indoor pollutants occurs through several phases: hazard identification, exposure assessment, dose-effect evaluation, and finally qualitative and quantitative risk assessment. The final product of this process may be an individual risk estimate per exposure unit or the evaluation of the incidence of the concerned effects in a given population. The risk characterisation through a multi-stage process as described

above is particularly informative because, by dividing the analysis of the scenario of each pollutant into steps, it allows the separate recognition of the importance of each variable in the scenario and the prediction of the changes of frequency or severity of effects obtainable by modifying (increasing or decreasing) exposure.

For some types of IAP, our understanding of human health risk is well defined. For most indoor air pollutants, however, the risk assessment process has its limitations.

First, it has been applied successfully only to individual pollutants for which information is available for exposure and dose-response relationships and for which the effect is clear, certain, and measurable, such as mortality and cancer. Little progress has been made in applying the risk assessment process to environmental issues involving pollutant mixtures or effects for which the causes are difficult to ascertain precisely, such as in heart disease, allergic reactions, headache, and malaise. A different approach is needed for the assessment and characterisation of the risks associated with most indoor air pollutants.

A basic and simple criterion for assessing the importance of the health risk related to indoor pollution makes reference to the severity of the effect concerned and to the size of the population affected. Important issues for the community may come from severe health impacts, particularly when affecting a large segment of the population. Minor impacts, such as those related to discomfort or annoyance may, however, become important when a large number of individuals in the community are concerned.

4.3 The impact of IAP on humans' health

4.3.1 Respiratory health effects associated with exposure to IAP

Several effects on the respiratory system have been associated with exposure to IAP. These include acute and chronic changes in pulmonary function, increased incidence and prevalence of respiratory symptoms, augmentation of pre-existing respiratory symptoms, and sensitisation of the airways to allergens present in the indoor environment. Also, respiratory infections may spread in indoor environments when specific sources of infectious agents are present, or simply because the smaller indoor mixing volumes allow infectious diseases to spread more easily from one person to the next. The latter mechanism is particularly operative in schools, nursery schools, etc.

Observed changes in pulmonary function due to exposure to, e.g., tobacco smoke in the home, have mostly been due to acute or chronic airway narrowing leading to obstruction of air flow. This is measured as a reduction in the quantity of air that can be exhaled in one second after deep inspiration (FEVI), and a limitation in the various measures of air flow such as Peak Expiratory Flow (PEF), Maximum Mid Expiratory

Flow (MMEF), and Maximum Expiratory Flow at x% of Forced Vital Capacity (MEFx). In growing children, it has also been suggested that lung development could be impaired by exposure to IAP.

Asthma, manifested by attacks of excessive airway narrowing leading to shortness of breath and wheezing, can be caused or aggravated by exposure to allergens at home, but it has also been associated with exposure to substances such as nitrogen dioxide and environmental tobacco smoke (ETS). Bronchitis, manifested in inflammatory changes in the airways and mucus hypersecretion has been linked to high levels of ambient air pollution in

the past, and to exposure to ETS in the home in recent studies. Respiratory symptoms which have been associated with exposure to indoor air pollutants are symptoms mostly related to the lower airways such as cough, wheeze, shortness of breath and phlegm.

In contrast to the occurrence of chemical pollutants in indoor air, attention to which has grown considerably over the past two decades, the role of infectious agents in indoor air has been known for a long time. Infectious agents can be involved in the inflammatory conditions rhinitis, sinusitis, conjunctivitis and sinusitis, in pneumonia, in asthma and in alveolitis.

4.3.2 Allergic diseases associated with exposure to IAP

Allergic asthma and extrinsic allergic alveolitis (hypersensitivity pneumonitis) are the two most serious allergic diseases caused by allergens in indoor air. Allergic rhinoconjunctivitis and humidifier fever are other important diseases; it is not clear if or how the immunological system is involved in humidifier fever.

Allergic asthma is characterised by reversible narrowing of the lower airways. Pulmonary function during an attack shows an obstructive pattern in serious cases together with reduced ventilation capacity. Allergic asthma may be caused by exposure to indoor air pollutants, either acting as allergens or as irritants. Immunological specific IgE sensitisation to an airborne allergen is a major component of this disease, but non-specific hypersensitivity is also important for the asthmatic attacks occurring on exposure to irritants in the indoor air.

The prevalence of asthma varies considerably from country to country. Although asthmatic attacks seldom lead to death, the costs of medical care are considerable in terms of hospital admissions, medication, and lost work days.

Allergic rhinoconjunctivitis is also an IgE-mediated disease, but while asthma occurs in all age groups, allergic rhinoconjunctivitis is especially prevalent among children and young adults. The main symptoms are itching of the eye andlor the nose, sneezing, watery nasal secretion and some stuffiness of the nose. The severity of the symptoms varies with the exposure to the allergen. Individuals often suffer from both allergic asthma and allergic rhinoconjunctivitis and are seldom sensitive to only one allergen. Aeroallergens from house dust mites, pets, insects, moulds, and fungi in the indoor air have been shown to be associated with allergic asthma and/or rhinoconjunctivitis. Extrinsic allergic alveolitis, also called hypersensitivity pneumonitis, is characterised by recurrent bouts of pneumonitis or milder attacks of breathlessness and flu-like symptoms. Studies of the pulmonary function during an acute episode will usually show a restrictive pattern with a decreased diffusion capacity. The disease is believed to be an inflammatory reaction in the alveoli and bronchioles involving circulating antibodies and a cell-mediated immunological response to an allergen. For example it occurs in farmers as a result of handling mouldy hay (“farmer’s lung”) and in pigeon breeders due to bird droppings. However, the disease has also in a few cases been associated with exposure to IAP, most frequently related to humidifiers in homes and offices contaminated with bacteria, fungi, or protozoans.

Allergic asthma and extrinsic allergic alveolitis resolve with cessation of exposure to the allergen, but continued exposure in sensitised patients may result in permanent lung damage and death from pulmonary insufficiency.

Humidifier fever is a flu-like illness involving the immune system, in which X-ray abnormalities are usually absent. The exact cause is not clear. The disease may occur among persons exposed to humidification systems contaminated with microbial growth. The symptoms typically occur 4-8 h after the exposure on the first day back at work after a weekend, but resolve within 24 h. Despite continuous exposure the disease does not recur until after the next weekend. Even though pulmonary changes are seen during attacks of humidifier fever, the disease does not lead to permanent lung damage.

4.3.3 Cancer and effects on reproduction associated with exposure to IAP

Lung cancer is the major cancer which has been associated with exposure to IAP (radon or ETS). Asbestos exposure has been linked to cancer in workers and also in workers' family members, presumably due to asbestos fibres brought into the home on workers' clothing. However, there are no studies associating asbestos exposure in homes or public buildings from asbestos used as a construction material to the development of cancer. Effects on human reproduction have been associated with exposure to chemicals in the environment, but it is as yet unclear to what extent (if any) exposure to IAP is involved

4.3.4 Sensory effects and other effects on the nervous system associated with IAP

Sensory effects are defined as the perceptual response to environmental exposures. Sensory perceptions are mediated through the sensory systems and result in a conscious experience of smell, touch, itching, etc. Sensory effects are typically observed in buildings with indoor climate, problems because many chemical compounds found in the indoor air have odorous or mucosal irritation properties. Most indoor air chemicals with a measurable vapour pressure will be odorous when the concentration is high enough.

Sensory effects are important parameters in indoor air quality control for several reasons. They may appear as: (1) adverse health effects on sensory systems (e.g., environmentally-induced sensory dysfunctions); (2) adverse environmental perceptions which may be adverse per se or constitute precursors of disease to come on a long term basis (e.g., annoyance reactions, triggering of hypersensitivity reactions); (3) sensory warnings of exposure to harmful environmental factors (e-g., odour of toxic sulfides, mucosal irritation due to formaldehyde); (4) important tools in sensory bioassays for environmental characterisation (e.g.. using the odour criterion for general ventilation requirements or for screening of building materials to find those with low emissions of volatile organic compounds).

The senses responding to environmental exposure are not only hearing, vision, olfaction and taste, but also the skin and mucous membranes. As pointed out by WHO (1989), many different sensory systems that respond to irritants are situated on or near the body surface. Some of these systems tend to respond to an accumulated dose and their reactions are delayed. On the other hand, in the case of odor perception the reaction is immediate but also very much influenced by olfactory fatigue on prolonged exposures.

Responders are often unable to identify a single sensory system as the primary route of sensory irritation by airborne chemical compounds. The sensation of irritation is influenced by a number of factors such as previous exposures, skin temperature, competing sensory stimulation, etc. Since interaction and adaptation processes are characteristic of the sensory systems involved in the perception of odour and mucosal irritation, the duration of exposure influences the perception. Humans integrate different environmental signals to evaluate the

total perceived air quality and assess comfort or discomfort. Comfort and discomfort by definition are psychological and for this reason the related symptoms, even when severe cannot be documented without using subjective reports. Sensory effects reported 10 be associated with IAP are in most cases multisensory and the same perceptions or sensations may originate from different sources. It is not known how different sensory perceptions are combined into perceived comfort and into the sensation of air quality. Perceived air quality is for example mainly related to stimulation of both the trigeminus and olfactorius nerves.

Several odorous compounds are also significant mucosal irritants, especially at high concentrations. The olfactory system signals the presence of odorous compounds in the air and has an important role as a warning system. In the absence of instrumentation for chemical detection of small amounts of some odorous vapours, the sense of smell remains the only sensitive indicator system. It is well known that environmental pollution can affect the nervous system. The effects of occupational exposure to organic solvents can be mentioned as an example. A wide spectrum of effects may be of importance, ranging from those at molecular level to behavioural abnormalities. Since the nerve cells of the CNS typically do not regenerate, toxic damage to them is usually irreversible. The nerve cells are highly vulnerable to any depletion in oxygen supply.

4.3.5 Cardiovascular effects associated with IAP

Increased mortality due to Cardiovascular Diseases (CVD) has been associated with exposure to ETS in some groups of non-smoking women married to smokers. Some investigators have also addressed the question whether total mortality is influenced by exposure to ETS, but results have been contradictory. As any effect on mortality would not be expected to occur until after many years of exposure, a problem in these types of study is the accuracy and reliability of the exposure classification. Attempts have also been made to relate ETS to electrocardiographic abnormalities and cardiovascular symptoms, but results have been inconclusive.

Carbon monoxide (CO) exerts its influence primarily through binding to the haemoglobin (Hb) in blood. The affinity of CO to Hb is about 200 times higher than the affinity of oxygen to Hb, so that at relatively low levels of CO in the air. Oxygen is replaced by CO. The percentage of Hb bound to CO (O/O carboxyhaemoglobin) is a measure of recent exposure to CO. Organs with a high oxygen demand, such as the heart and the brain, are particularly susceptible to a reduced oxygenation caused by CO exposure. Early effects include reduction of time to onset of chest pain in exposed, exercising heart disease patients. At higher levels of exposure, myocardial infarctions may be triggered by CO.

4.3.6 Socio-economic costs of IAQ

Indoor air pollution, apart from the health impact, has socio-economic costs. The potential economic impact of poor indoor air quality is quite high, and has been estimated to be in the order of tens of billions of ECU per year in Western Europe. This includes costs of medical care, loss of income during illness, days lost due to illness, poor working performance and lower productivity. Labour costs are significantly greater per square meter of office space than energy and other environmental control costs (ECA, 1989). In the US, the loss in productivity for each employee which is attributable to IAQ problems is currently estimated to be 3% (14 minutes/day) and 0.6 added sick days annually. Other estimates have been made

by calculating the impact of IAQ on productivity. For instance, in Norway, the authorities estimate that the costs to society related to poor IAQ are in the order of 1 to 1.5 billion ECU per year or about 250 - 350 ECU per inhabitant. This estimation only includes costs related to adverse health effects requiring medical attention and does not include reduced working efficiency or job-related productivity losses. Thus, from an economic consideration, remedial action to improve indoor air quality is likely to be cost effective even if an expensive retrofit is required.

Finally, a preliminary estimate of the total annual cost of IAQ problem prevention and mitigation activities in the United States is approximately $16 billion with a range of $12 billion to $20 billion (BLS, 2005). This estimate does not include activities that were considered typical or routine in the early 1970s before public awareness of indoor air quality became more common. While not precise, this estimate does indicate that the level of expenditure is substantial. It is also apparent that expenditures are growing and the market is shifting within the various elements of the market.

5. BASIC CONTROL STRATEGIES OF INDOOR AIR QUALITY

A healthy indoor environment is an essential part of a sustainable building (Levin, 2003). It must address the ability of the indoor environment to support healthy, comfortable, and productive occupants. To do this, the focus on indoor environmental quality includes the whole indoor climate -- thermal, illumination, acoustic, and indoor air quality. The essential questions that must be answered are as follows:

How to protect, facilitate, or enhance human comfort, health, and productivity?

How to minimize the use of energy and other scarce and polluting resources to produce ventilation, heating, cooling and illumination?

How to minimize pollution emissions and accumulation in sinks?

How to protect ecosystems to preserve biodiversity and the services provided by natural systems?

There are some basic control methods for lowering concentrations of indoor air pollutants (Ashford & Caldart, 2008), which are described bellow:

Source Management includes source removal, source substitution, and source encapsulation. Source management is the most effective control method when it can be practically applied. Source removal is very effective. However, policies and actions that keep potential pollutants from entering indoor are even better than preventing IAQ problems. Source substitution includes actions such as selecting a less toxic art material or interior paint than the products which are currently in use. Source encapsulation involves placing a barrier around the source so that it releases fewer pollutants into the indoor air (e.g., asbestos abatement, pressed wood cabinetry with sealed or laminated surfaces). Local Exhaust is very effecting on removing point sources of pollutants before they can disperse into the indoor air by exhausting the contaminated air outside. Well known examples include restrooms and kitchens where local exhaust is used.

Other examples of pollutants that originate at specific points and that can be easily exhausted include science lab and housekeeping storage rooms, printing and duplicating

rooms, and vocational/ industrial areas such as welding booths. Ventilation through use of cleaner (outdoor) air to dilute the polluted (indoor) air that people are breathing. Generally, local building codes specify the quantity (and sometimes quality) of outdoor air that must be continuously supplied to an occupied area. For situations such as painting, pesticide application, or chemical spills, temporarily increasing the ventilation can be useful in diluting the concentration of noxious fumes in the air. Exposure Control includes adjusting the time of use and location of use. An example of time of use for school students would be to strip and wax floors on Friday after school is dismissed, so that the floor products have a chance to off-gas over the location of use deals with moving the contaminating source as far as possible from occupants, or relocating susceptible occupants. Air Cleaning primarily involves the filtration of particles from the air as the air passes through the ventilation equipment. Gaseous contaminants can also be removed, but in most cases this type of system should be engineered on a case-by-case basis.

As it has been clearly proven above, indoor air pollution is a major public concern issue, which can be characterized as "global environmental phenomenon". Also, it is obvious, that the causes which created this domestic environmental problem, such as modern way of living, decreased ventilation rates for energy conservation or increased use of synthetic materials in buildings, are not expected to be reduced (it is more probable that they are going to be increased). Nevertheless, the task of reducing levels of exposure to air pollutants is rather complex. It begins with an analysis to determine which chemicals are present in the air, at what levels, and whether likely levels of exposure are hazardous to human health and the environment. It must then be decided whether an unacceptable risk is present.

When a problem is identified, mitigation strategies have to be developed and implemented so as to prevent excessive risk to public health in the most efficient and cost-effective way. In addition, analyses of air pollution problems are exceedingly complicated. Some are national in scope (such as the definition of actual levels of exposure of the population, the determination of acceptable risk, and the identification of the most efficient control strategies), while others are of a more basic character and are applicable in all countries (such as analysis of the relationships between chemical exposure levels, and doses and their effects). So, it is very essential for governments of all countries- especially the governments of the more developed ones- to adopt and implement these policies, in order to effectively face this worldwide issue in combination with the need of energy saving, the use of new building materials and the modern trend of living.

6. CONCLUSIONS

At last, living in a healthy indoor environment is a human right. The principles below derive from the fundamental principles in the fields of human rights, biomedical ethics and ecological sustainability, and focus on interactions among them (WHO, 2000):

• Principle 1 Under the principle of the human right to health, everyone has the right to breathe healthy indoor air.

• Principle 2 Under the principle of respect for autonomy ("self-determination"), everyone has the right to adequate information about potentially harmful exposures, and to be provided with effective means for controlling at least part of their indoor exposures.

• Principle 3 Under the principle of non-maleficence ("doing no harm"), no agent at a concentration that exposes any occupant to an unnecessary health risk should be introduced into indoor air.

• Principle 4 Under the principle of beneficence ("doing good"), all individuals, groups and organizations associated with a building, whether private, public, or governmental, bear responsibility to advocate or work for acceptable air quality for the occupants.

• Principle 5 Under the principle of social justice, the socioeconomic status of occupants should have no bearing on their access to healthy indoor air, but health status may determine special needs for some groups.

• Principle 6 Under the principle of accountability, all relevant organizations should establish explicit criteria for evaluating and assessing building air quality and its impact on the health of the population and on the environment.

• Principle 7 Under the precautionary principle, where there is a risk of harmful indoor air exposure, the presence of uncertainty shall not be used as a reason for postponing cost-effective measures to prevent such exposure.

• Principle 8 Under the "polluter pays" principle, the polluter is accountable for any harm to health and/or welfare resulting from unhealthy indoor air exposure(s). In addition, the polluter is responsible for mitigation and remediation.

• Principle 9 Under the principle of sustainability, health and environmental concerns cannot be separated, and the provision of healthy indoor air should not compromise global or local ecological integrity, or the rights of future generations.

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MICROPLASTIC POLLUTION ON THE COAST OF INDIA

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ABSTRACT

Plastics have become indispensible in many areas of modern life, used for clothing, storage, transportation, packaging, construction and a host of consumer goods. One of plastics' greatest properties, its durability, is also one of the main reasons that plastics present a threat to the marine environment. Plastics in the marine environment are of increasing concern because of their persistence and effects on the oceans, wildlife, and especially on humans. Several broad classes of plastics are used in packaging: Polyethyelene (PE), Polypropylene (PP), Polystyrene (PS), etc. They potentially can enhance the benefits that both medical and scientific technology will bestow to humankind. Because of frequent inappropriate waste management practices, or irresponsible human behaviour, large masses of plastic items have been released into the environment, and thereby have entered the world's oceans. Moreover, this process continues, and in some places is even increasing. It is widely cited that 80% of marine plastic debris originates from land.

Different plastics have different fates once they end up in the sea based on their physico-chemical properties (e.g. density, polymer type etc.) and other major factors like UV radiation, the oxidative properties of the atmosphere and hydrolytic properties of seawater and hydrodynamics (effects of wind, wave and current) etc. The minute fragments of plastic debris derived from larger plastic debris, named microplastics, have been accumulating in the oceans at least for the last four decades. According to various researchers, the microplastics are the particles ≥1mm to ≤5mm. microplastics are considered bioavailable to organisms throughout the food-web. Their composition and relatively large surface area make them prone to adhering waterborne organic pollutants (e.g. POPs) and to leaching of plasticisers that are considered toxic. Ingestion of microplastics may therefore be introducing toxins to the base of the food chain (e.g. planktons, bivalves), from where there are potential possibilities of bioaccumulation to the higher level of food chain (e.g. fish, birds, human). At global scale high concentrations of micro plastics have been found at five oceanic gyres but in the coastal environment of India the distribution and fate of microplastic pollution has not studied so far.

Therefore, resolving the plastic debris problem is important. Solutions to the plastic debris problem can only be achieved through a combination of actions. Such actions include the following: Legislation against marine pollution by plastics must be enforced, recycling must be accentuated, alternatives (biodegradable) to current plastic products must be found, and clean-up of debris must proceed, if the marine plastic pollution problem is to eventually be resolved. Governments cannot accomplish this task on their own, and will need help and initiative from the public.

In our present study the main objectives of this study are as follows: 1) To monitor and assess the spatial and temporal distribution, sources, composition and pathways of micro-plastics in marine environment by using hydrodynamic and particle tracking model along India coast, 2) Identification and quantification of hazardous chemicals (e.g. POPs and plastic additives) adsorbed and transported by micro-plastics. 3) To determine the impact and bioaccumulation of toxic pollutants associated with microplastics, in the biota. This study is going to be the first research work on microplastic to provide the baseline data on detailed source, distribution and impact of microplastics in the Indian coastal and marine environment.

1. INTRODUCTION

Human activities are responsible for a major decline of the world's biological diversity, and the problem is so critical that combined human impacts could have accelerated the present extinction rates to 1000-10,000 times the natural rate (Lovejoy, 1997). In the ocean, the threat to marine life comes in various forms, such as overexploitation and harvesting, dumping of waste, pollution, entry of alien species, land reclamation, dredging and global climate change (Derraik, 2002). One particular form of human impact, which constitutes a major threat to marine life, is the pollution by plastic debris. Reports of plastic pollution in the ocean first appeared in the scientific literature in the early 1970s, yet even after 40 years, no rigorous estimates exist of the amount and origin of plastic debris entering the marine environment. There are two main sources of plastic into the marine environment: (1) maritime activities, such as commercial fishing and illegal dumping; (2) land-based sources such as river, storm water runoff, wastewater, inland litter blown to the sea and the litter people leave behind on beaches (Ryan et al 2009; Coe and Rogers, 1997). It is widely cited that 80% of marine debris originates from land; however, this figure is not well substantiated and does not inform the total mass of debris entering the marine environment from land-based sources. Global plastic resin production reached 288 million metric tons (MT) in 2012 (PlasticsEurope, 2012), 620% increase since 1975 (Jambeck et al., 2015). 192 countries with coasts bordering the Atlanta, Pacific and Indian oceans, Mediterranean and Black seas produced a total of 2.5 billion metric tons of solid waste. Of that, 275 million metric tons was plastic, and an estimated 8 million metric tons of mismanaged plastic waste entered the ocean in 2010 (Fig 1). In terms of mismanagement of plastic waste, India ranks 12th position among the top 20 most mismanaged countries in the world (Table1) (Jambeck et al., 2015).

Figure 1: Plastic waste inputs from land to the ocean in 2010

Table 1: List of top 20 most plastic waste mismanaged countries in the world (Jambeck et al, 2015)

Rank	Country	Econ. classif.	Coastal pop. [millions]	Waste gen. rate [kg/ppd]	% plastic waste	% mismanaged waste	Mismanaged plastic waste [MMT/year]	% of total mismanaged plastic waste	Plastic marine debris [MMT/year]
1	China	UMI	262.9	1.10	11	76	8.82	27.7	1.32–3.53
2	Indonesia	LMI	187.2	0.52	11	83	3.22	10.1	0.48–1.29
3	Philippines	LMI	83.4	0.5	15	83	1.88	5.9	0.28–0.75
4	Vietnam	LMI	55.9	0.79	13	88	1.83	5.8	0.28–0.73
5	Sri Lanka	LMI	14.6	5.1	7	84	1.59	5.0	0.24–0.64
6	Thailand	UMI	26.0	1.2	12	75	1.03	3.2	0.15–0.41
7	Egypt	LMI	21.8	1.37	13	69	0.97	3.0	0.15–0.39
8	Malaysia	UMI	22.9	1.52	13	57	0.94	2.9	0.14–0.37
9	Nigeria	LMI	27.5	0.79	13	83	0.85	2.7	0.13–0.34
10	Bangladesh	LI	70.9	0.43	8	89	0.79	2.5	0.12–0.31
11	South Africa	UMI	12.9	2.0	12	56	0.63	2.0	0.09–0.25
12	India	LMI	187.5	0.34	3	87	0.60	1.9	0.09–0.24
13	Algeria	UMI	16.6	1.2	12	60	0.52	1.6	0.08–0.21
14	Turkey	UMI	34.0	1.77	12	18	0.49	1.5	0.07–0.19
15	Pakistan	LMI	14.6	0.79	13	88	0.48	1.5	0.07–0.19
16	Brazil	UMI	74.7	1.03	16	11	0.47	1.5	0.07–0.19
17	Burma	LI	19.0	0.44	17	89	0.46	1.4	0.07–0.18
18*	Morocco	LMI	17.3	1.46	5	68	0.31	1.0	0.05–0.12
19	North Korea	LI	17.3	0.6	9	90	0.30	1.0	0.05–0.12
20	United States	HIC	112.9	2.58	13	2	0.28	0.9	0.04–0.11

*If considered collectively, coastal European Union countries (23 total) would rank eighteenth on the list

Plastics in the marine environment are of increasing concern because of their persistence and effects on the oceans, wildlife, and especially on humans. Several broad classes of plastics are used in packaging: Polyethyelene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET); and Polyvinyl chloride (PVC). Jambeck et al (2015)

estimated that plastic waste entering into the ocean is one to three orders of magnitude greater than the reported mass of floating plastic debris in high-concentration ocean gyresglobally. About 18% of the marine plastic debris found in the ocean environment is attributed to the fishing industry. Aquaculture can also be a significant contributor of plastics debris in the oceans. Virgin resin pellets, a common component of debris, enter the oceans routinely via incidental losses during ocean transport or through run-off from processing facilities (Ogata et al., 2009; Andrady, 2011). Different plastics have different fates once they end up in the sea based on their physico-chemical properties (Fig. 2).

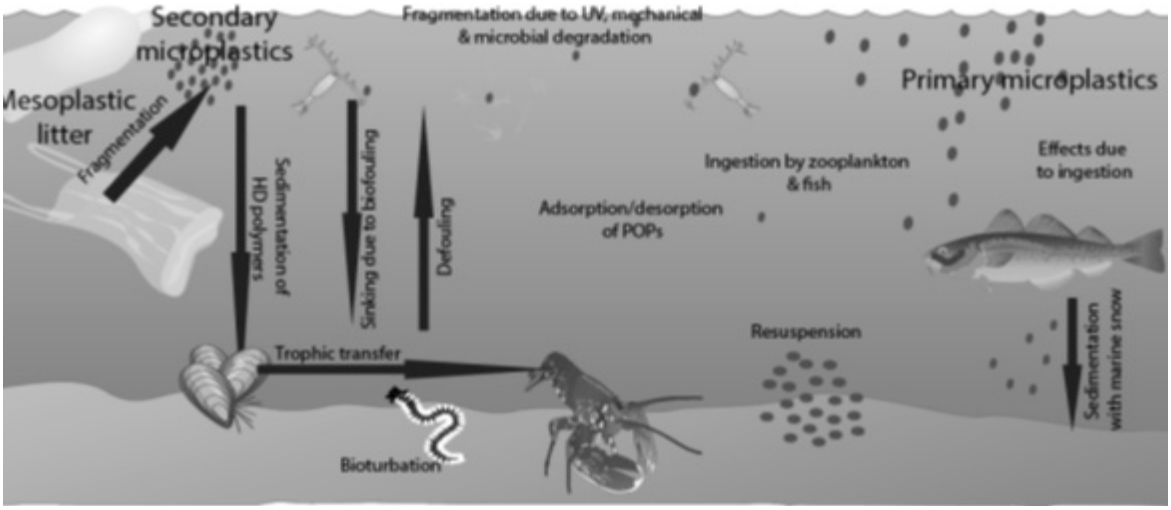

Figure 2: The fate and pathways and biological interactions of microplastics (Wright et al., 2013).

A recent significant finding is that minute fragments of plastic debris, termed microplastics, occur in oceans worldwide (Barnes et al., 2009). Microplastics, a form of man-made litter, have been accumulating in the oceans at least for the last four decades (Thompson et al., 2005). Microplastics include virgin resin pellets and smaller fragments of plastics derived from the larger plastic debris (Moore, 2008). The major factors for the formation of microplastics are UV radiation, the oxidative properties of the atmosphere and hydrolytic properties of seawater and hydrodynamics (effects of wind, wave and current) (Andrady, 2011; Webb et al., 2013). Microplastics are ubiqtous and abundant in marine environment, e.g., 72% of the plastics recovered from beaches in Portugal belonged to a size class ≤ 5 mm (Martins and Sobral, 2011). Similarly, plastic particles <1 mm accounted for 65% of total marine debris collected on beaches in the Tamar Estuary (UK) (Browne et al., 2010). Many authors have defined microplastics as particles smaller than 5 mm (e.g. Arthur et al. 2009) while others have set the upper size limit at 1 mm (e.g. Costa et al., 2010). The origin of microplastics could be attributed to two main sources: (a) direct introduction with runoff and (b) weathering breakdown of meso- and macroplastics debris. Microplastics include the micron-sized plastic particles, used as exfoliants in cosmetic formulations (Fendall and Sewell, 2009), those generated in ship-breaking industry (Reddy et al., 2006) and industrial abrasives in synthetic 'sandblasting' media (beads of acrylic plastics and polyester). A third category of microplastic, which is often kept separate from others, is "industrial plastic" which refers to resin pellets used as precursors in plastic manufacturing processes (Fig. 3).

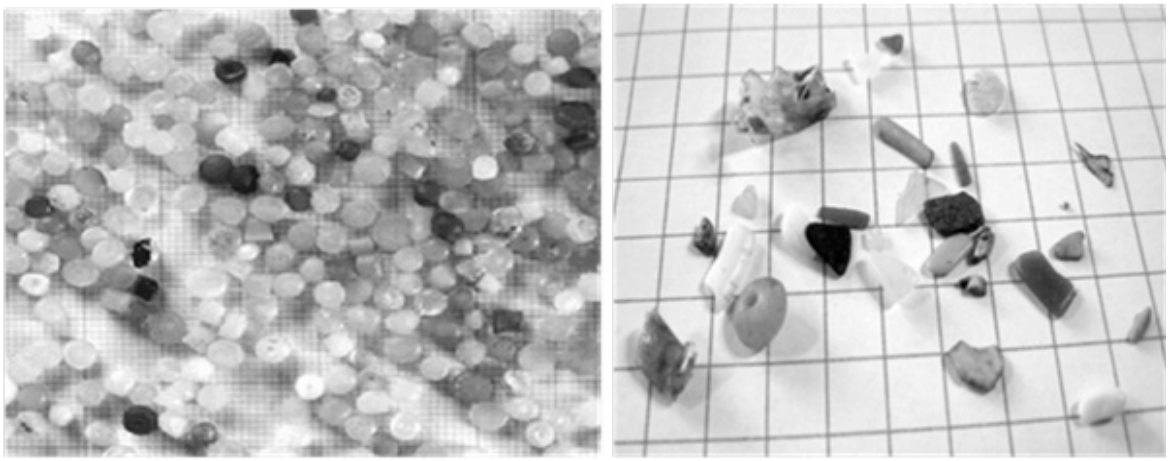

Figure 3: Plastic pellets and plastic fragments which are found on beaches.

Owing to their small size, microplastics are considered bioavailable to organisms throughout the food-web. Their composition and relatively large surface area make them prone to adhering waterborne organic pollutants (e.g. POPs) and to leaching of plasticisers that are considered toxic. Ingestion of microplastics may therefore be introducing toxins to the base of the food chain, from where there are potential possibilities of bioaccumulation (Teuten et al., 2009). Bivalves, for instance, will preferably ingest and process particles less than 40 μm, but larger particles (upto 600 μm) can be ingested and processed as well (Van Cauwenberghe and Janssen, 2014). Ingestion of plastics by birds (Mallory, 2008) and turtles (Mascarenhas et al., 2004) is extensively documented worldwide and at least 44% of marine bird species are known to ingest plastics (Rios and Moore, 2007). An unexpectedly high incidence of plastic debris was found in the North Pacific gyre (Moore, 2008). This interest has culminated in defining the topic as a high-priority research area in Marine Biology (Gregory, 2009 and Arthur et al., 2009). Over 660 marine species worldwide have been known to be affected by plastic waste in one way or other (GESAMP, 2015). Ingestion of microplastics by species in aquaculture or fisheries may potentially pose a risk for human food safety (Van Cauwenberghe and Janssen, 2014), excretion of microplastics in faecescan result in its sedimentation and thus affect biogeochemical cycles and the food supply to the benthos. Plastics contain additives, chemicals, which are added to improve the desirable properties of the plastic product. Many of these additives are known as hazardous substances and can leach from the plastic surface. Plastics once released into the environment can also accumulate toxic pollutants like persistent organic pollutants (POPs). POPs, the hazardous human-made chemical, that occur universally in sea water at very low concentrations, are picked up by meso-/microplastics via partitioning and the hydrophobicity of POPs facilitate their concentration in the meso-/microplastic litter at a level that is several orders of magnitude higher than that in sea water. Plastic particles have the potential to act as vectors for the transport and release of sorbed contaminants like POPs and PBT substances (persistent, bioaccumulative and toxic) and additives. POPs are organic compounds that are resistant to environmental degradation through chemical, biological and photolytic processes. They are potential cause for many adverse effects in marine life and humans (e.g. cancer, malformation, decrease in immune response, impaired reproductive ability). The extent of bioavailability of POPs dissolved in the microplastics to the biota (Moore, 2008) and their potential bio-magnification in the food web (Teuten et al., 2007) has not been studied in detail.

The current status of microplastic research describes that the oceanic habitats are polluted by plastic debris from pole to pole, found in the open ocean, on shorelines of even

the most remote areas (e.g. Arctic) (Zarfi and Matties, 2010) and in the deep sea. At global scale, several studies have identified large-scale convergence zones of plastic debris due to major ocean currents. High concentrations of micro plastics have been found at five oceanic gyres (North Atlantic, South Atlantic, South Indian, North Pacific and South Pacific). The occurrence and concentration of hydrophobic contaminants in marine microplastics and pellets has widely been reported (Ogata et al., 2009, Hirai et al., 2011, Heskett et al., 2012 and Mizukawa et al. 2013). However, extent of distribution, accumulation and fate of microplastics in the coastal environment of India has not been studied so far. Only some preliminary works have been done on abundance of plastic by weight and size on the Mumbai beaches (Jayasiri et al, 2013a,b) and along the Alang ship breaking yard (Reddy et al., 2006). So far no baseline data on micro-plastic pollution in the seas around India is available. The National Institute of Oceanography (NIO) has just initiated a study to understand the occurrence, source and characteristics of plastic debris (especially micro-plastics) and assessment of toxic pollutants (e.g. persistent organic pollutants, POPs) carried by micro-plasticsalong the coast of the Indian Ocean. Recently, leaders of G7 countries made a decision: plan to combat marine litter, especially plastics. G7 Ministers of Science met on 9 September 2015 in Berlin and made a decision to combat marine litter, especially plastics. The following major issues were discussed in this meeting:

- Scale of the quantity of plastic entering and accumulating in the oceans
- Forms in which plastic and its breakdown products are found
- Lifetime and fate of plastics and their breakdown products
- Impact of plastics and their breakdown products on marine species, including commercially fished and farmed species and finally human health?

The above background provides the importance of taking-up studies on microplastic pollution along the coastal and marine environment of India**.** This paper describes the microplastic sampling methods in beach, near-shore and offshore regions. The extraction, enumeration and identification methods and summarizes relative factors in the experiments, andtheir advantages and limitations.

2. METHODOLOGY

2.1. Collection of microplastics

2.1.1. Beach

Plastic resin pellets samples usually are collected from the high tide line from the sandy beaches. These are picked up by using solvent-rinsed stainless steel tweezers. Sediment with micro and meso plastic samples along the beach from the intertidal line to the high watermark (middle of intertidal to sub-tidal) are collected in a 1 x 1 m quadrate with 5 cm depth, using a stainless scoop, through a 1 mm sieve (Fig. 5). The collected plastic pellets and micro-plastics would be wrapped in aluminium foils and preserved at 4°C in the laboratory for further processing. Short sediment cores are collected at select sandy beaches to study the potential of plastic deposition rate and trends over the period of time.

Figure 4: Microplastic sampling on beach

2.1.2. Nearshore

In nearshore region, micro-plastics in the surface water are usually collected using neuston net (mouth size is 75 cm x 75 cm and length is 300 cm) with mesh size 100µm towed at the top 50 cm of the sea surface in an average speed of 2 knots for 30 min to 1 hour based on the availability (Fig. 5). The volume of filtered seawater (m^3) could be evaluated by a flow meter. Contents of the net would be washed into a sample jar and frozen at -30 degree C and transported by cooler box to the laboratory and would be stored at -30 ˚C until concentrations of microplastics are measured. The circular rosette of Niskin bottles attached around CTD or Bongo netare used for vertical water sampling based on the bathymetry. Both Niskin bottles and CTD have a stainless steel cable are connected to the Research Vessel's computer program system in real time. Each bottle is electronically tripped at selected depths. For the estimation of the micro-plastic density, three replicates (30 litres) would be collected for every site and for every temporal sampling. The pre-labelled bottle are used to collect water samples from the Niskin bottles. Sampling locations are selected according to hydrodynamic conditions (low hydrodynamic sites – lagoon and bay) and environmental features, both natural and anthropogenic.

Figure 5: Microplastic collection on sea surface by neuston net

2.1.3. Offshore

In the offshore region(100-300 km from shore), the microplastic samples are collected from surface and bottom waters using neuston net and circular rosette of Niskin bottles attached around CTD. Detailed procedure is given in 2.2.2.

2.1.4. Biological sampling

Fish, mussels and plankton (phyto and zoo) samples are collected from the select locations (near shore and offshore waters). Samples would be frozen within 2 h of capture and subsequently thawed out at room temperature prior to examination. Plankton samples are collected using neuston net and preserved on the spot with 2.5% formalin and transported to the lab for further analysis.

2.2. Analytical methods

2.2.1. Sorting, extraction and identification

Immediately after arrival, pellets are sorted using near-infrared spectrometer into PE, PP and other polymers. Among the PE pellets, yellowing pellets (yellowness of 40 or more) would be selected by the naked eye and subject to chemical analysis.

For sediment samples, 1L of 4.55 M sodium chloride solution is added to 250 g of sieved sediment samples and stirred for 2 min. The sediment is thenallowed to settle for 1 h before the supernatant is poured through a 30 μm mesh sieve. The extracted plastic particles are collected by hand under magnification (x45) using a dissecting microscope and watchmaker forceps. The debris are dried in an oven (40°C) to remove moisture. The length and weight of the plastic particles would be measured. The polymer types of microplastic particles are usually identified using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) in mid-infrared region (4000 - 400 cm^{-1}). FTIR can be conducted on individual fragments, pellets and fibres of unknown polymers and match them to a polymer database synthetic fibres ATR library. FTIR determines the structure of molecules through analysis of their absorption spectra. For samples that can be identified as plastic, the abundance, and mass and cross sectional area would be recorded. Fragments of polymer, polypropylene, polyethylene, polystyrene, polyamide and polyurethane are identified. Each sample is compressed to a minimum thickness using a diamond compression cell, which allows for maximum absorbance.

2.2.2. Filtration of water sample

A 50 ml of sub-sample collected by neuston net are placed in a separation funnel with 100 ml of filtered saturated 4.55 M sodium chloride solution. Buoyant smaller debris are extracted onto glass micro-fibre filter paper under vacuum. The collected water samples by Niskin water sampler would be filtered through glass microfiber filter paper (pore size 30μm) under vacuum filtration. The extracted materials are examined using a binocular microscope. The length and weight of the plastic particles would be measured. The polymer types of particles would be identified using ATR–FTIR.

2.2.3. Categorization

Plastic pellets are generally categorized based on the colour and polymer type. Microplastics in beaches, near and off-shore regions are categorized based on size, shape (e.g fibres, granules, plastic films and spherules), weight and polymer type.

2.2.4. Chemical analysis of microplastics (pellets)

Yellowing PE pellets (with a yellowness of 40) are selected for chemical analysis. Median POP values would be obtained by analyzing 5 pools of pellets (each pool consisting of five randomly selected pellets) from each location. POPs are extracted from pellets by soaking the pellets in hexane. The extracts are separated through fully activated silica gel columns into three fractions: Fraction I (*n*-alkanes and hopanes), Fraction II (PCBs and DDE) and Fraction III (DDT, DDD, 4 HCH isomers [α, β, γ, δ], and polycyclic aromatic hydrocarbons [PAHs]). All the fractions are quantified by GC–MS. PAH concentrations in the samples are recovery-corrected using recoveries of surrogates (i.e., deuterated PAHs) spiked before analysis.

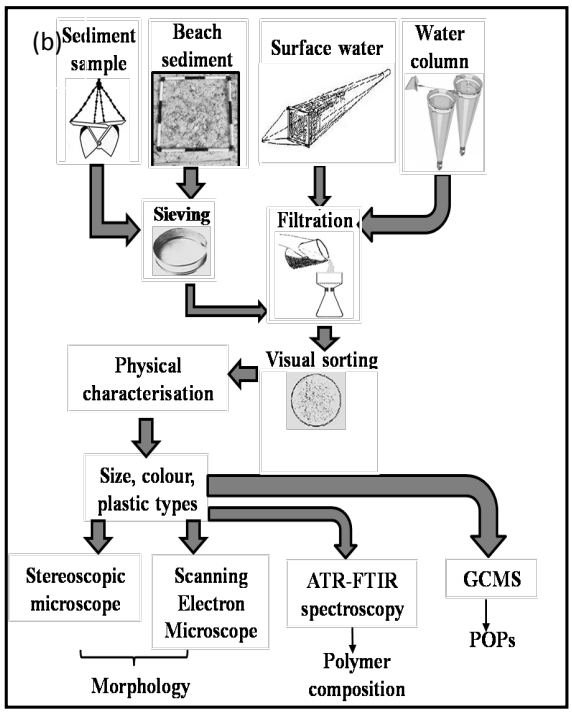

Figure 6: Overall methodology for microplastic analyses

2.2.5. Numerical modelling

Winds, tides and currents are the major driving forces which transport the floating plastic debris to the coastal and estuarine region. Therefore, a hydrodynamic model coupled with particle-tracking model would be set-up using suitable computational techniques to interpret the accumulation of plastic debris and the possible transport processes in the near and offshore waters.

2.2.6. Analysis of biota

The collected fish species are recorded their basic measurements including length, body weight and girth. Gastrointestinal tracts would be removed by dissection from each fish, from the top of the oesophagus and cut away at the vent. The digestive tracts are wrapped in aluminium foil and stored in plastic zip lock bags upto 2 h before transferring to clean petri dishes for inspection with a dissecting microscope. Every 10 mm of the digestive tracts are observed and ingested items, which do not resemble natural prey, are removed. The items are photographed and described according to maximum length, colour, and shape (fragment, fibre, bead and film). These items would be examined using FTIR spectroscopy to confirm and identify the polymer type (Lusher, 2013).

2.2.7 In-situ experiment

The abundant fish in Indian coastal waters are selected as a model fish species to achieve the baseline information regarding the accumulation of POPs associated with health effects in fish via a chronic dietary exposure to low-density polyethylene (LDPE). Polyethylene has greater affinity for POPs than other mass-produced polymers, and it is the largest component

of plastic produced globally (29%); also one of the most common polymers recovered as aquatic debris. Fish are exposed to three treatments: a negative control (no LDPE), a virgin-plastic (LDPE virgin pre-production plastic) and a marine-plastic treatment (LDPE deployed in an urban bay or harbor). Fish species are exposed to 10% plastic (by weight) mixed into treatment diets and sprinkled at the top of each treatment tank. Diet and plastic dissociated at the surface and thus fish are exposed to plastic similar to the way they are in the wild (i.e., floating in the water column). After a two months of dietary exposure, the concentrations of PAHs in the tissue of fish exposed to marine-plastic are measured using GC-MS and that hepatic stress are observed using histopathology (Rochman et al., 2013).

3. **CONCLUSION**

Having a huge coastline (7,517 km) and poor waste management, India contributes significantly to plastic pollution in global ocean. According to a report(Economic times report, 2013), plastics industry in India is expected to touch Rs 1.7 lakh crore levels on the back of multiple factors like increasing demand for the material in production of goods and its usage in packaging of various items. The demand for plastics is likely to increase, and the current level is almost 20 million metric tonnes (MMT). It is estimated that about 10,000 tonnes per day (TPD) of plastics waste is generated i.e. nine per cent of 1.2 lakhs TPD of municipal solid waste (MSW) in India. According to the Central Pollution Control Board (CPCB) of India, 40% of plastic waste is not recycled, and this waste is a source of continuing plastic pollution in the environment. Hence, considering the rising demand for plastics in India, it is essential to assess the source, distribution and fate of plastic and its further environmental consequences in terms of using as a media of toxic pollutant transfer. No baseline data for the occurrence and quantification of micro-plastics in coastal, estuarine and nearshore regions are available. Knowledge on mechanisms of pollutants sorption onto micro-plastics from environment and desorption (bioaccumulation) into the marine biota are still scare. At CSIR-NIO, we have initiated micro-plastic research work for monitoring the abundance, distribution, sources and potential adverse effects of micro-plastics in the coastal and marine environments of India.In this study, the methodology of microplastic sampling (from beaches, nearshore and offshore rgions), extraction, identification, chemical analyses. Thisreview suggests that monitoring microplastics needs standardized protocols for extraction, identificationand quantification and that further research on the effects of microplastics to human health is needed.

4. **ACKNOWLEDGMENTS**

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DYNAMIC AND BEHAVIOR OF ULTRAFINE PARTICLES IN URBAN ATMOSHERE: TOXICITY AND DEPOSITION IN HUMAN RESPIRATORY SYSTEM

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ABSTRACT

Aerosol pollution in urban environments has been recognized to be responsible of important pathologies of the cardiovascular and respiratory systems, it has also been associated to increased mortality and hospital admissions. In this perspective, great attention has been addressed to Ultra Fine Particles (< 100 nm), since they efficiently penetrate into the respiratory system and are capable of translocation from the airways into the blood circulation. High aerosol size resolution measurements are important for a correct assessment of the deposition efficiency in the human respiratory system as well as time resolution is another important requisite. Time resolved aerosol particle number size distributions have been measured in downtown Rome. FMPS and SMPS measurements have been carried out at the INAIL's Pilot Station, located in downtown Rome, in an area characterized by high density of autovehicular traffic. The two instruments have allowed investigating deeply the urban aerosol in the range 5.6-560 nm and 3.5-117 nm, respectively. Furthermore, moving from these data the total dose of particles and ultrafine particles deposited in the regions of the respiratory system (head, tracheobronchial and alveolar in the different lung lobes, have been estimated. Dosimeter estimates were performed with the Multiple-Path Particle Dosimetry model (MPPD v.2.1). The paper discusses the aerosol doses deposited in the respiratory system of individuals exposed in proximity of traffic. During traffic peak hours, about 6.6×1010 particles are deposited into the respiratory system. Such dose is almost entirely made of UFPs. According to the greater dose estimated, right lung lobes are expected to be more susceptible to respiratory pathologies than left lobes.

KEY WORDS: Particle; Aerosol distribution; FMPS; Urban area; Deposition dose; MPPD.

1. INTRODUCTION

Aerosol pollution in urban environments has been recognized to be responsible of important pathologies of the cardiovascular and respiratory systems, it has also been associated to increased mortality and hospital admissions. New regulations state that for fine particulate matter (PM2.5) there is yet no identifiable threshold below which PM2.5 would not pose a risk. Therefore, a general reduction of its concentrations in the urban background should be pursued to ensure that large sections of the population benefit from improved air quality. More recently, the International Agency for Research on Cancer (IARC) has considered outdoor pollution as a leading environmental cause of cancer deaths. Furthermore, particulate matter has been classified as carcinogenic to humans (Group 1). Within this context, the importance of the measurements of aerosol size distribution resides in that the doses deposited in the human respiratory system strictly depend on the particle sizes.

In this perspective, great attention has been addressed to Ultra Fine Particles (UFPs, < 100 nm) [1-3], since they efficiently penetrate into the respiratory system and are capable of translocation from the airways into the blood circulation. Many studies show that particle toxicity increases with decreasing their size, emphasizing the role of submicrometric particles, in particular of ultrafine particles (< 100 nm). In fact, particles greater than 2.5 μm are quickly removed through dry and wet deposition on the time scale of hours whereas submicrometer particles may reside in atmosphere for weeks, penetrate in indoor environment and be long-range transported. High aerosol size resolution measurements are important for a correct assessment of the deposition efficiency in the human respiratory system as well as time resolution is another important requisite.

Starting from such considerations, time resolved aerosol particle number size distributions have been measured in downtown Rome. FMPS and SMPS measurements have been carried out at the INAIL's Pilot Station, located in downtown Rome, in an area characterized by high density of autovehicular traffic.

2. EXPERIMENTAL PART

The aerosol measurements were carried out at the INAIL's Pilot Station, located in downtown Rome, in an area characterized by high density of autovehicular traffic, at 40 m above the ground level. The site is located in an area characterized by high traffic density, in a two-lane street whose aspect ratio H/W (H: building height, W: street width) is about 3.

Aerosol number size distributions were measured using a Scanning Mobility Particle Sizer (SMPS, model 3936, TSI, Shoreview, MN USA) equipped with an Electrostatic Classifier (model 3080, TSI) and a 3085 Differential Mobility Analyzer (DMA, model 3085, TSI), and a water-base Condensation Particle Counter (CPC, model 3786, TSI), for the measurement of particle number concentration. An hour aerosol data collected during the morning traffic peak period has been selected to carry out dosimetry estimates.

The Planetary Boundary Layer (PBL) mixing properties are important in determining the atmospheric pollutant levels. It is possible to obtain valuable information about PBL dilution by monitoring a ground-emitted and chemically stable compound, i.e. Radon. Its emission rate varies according to the soil composition, moisture content, porosity and permeability, but the variations can be considered negligible in a time scale of some days and a space scale of some kilometers [5-9]. Natural radioactivity has been assessed using a

PBL Mixing Monitor (FAI Instruments, Fonte Nuova, Italy).

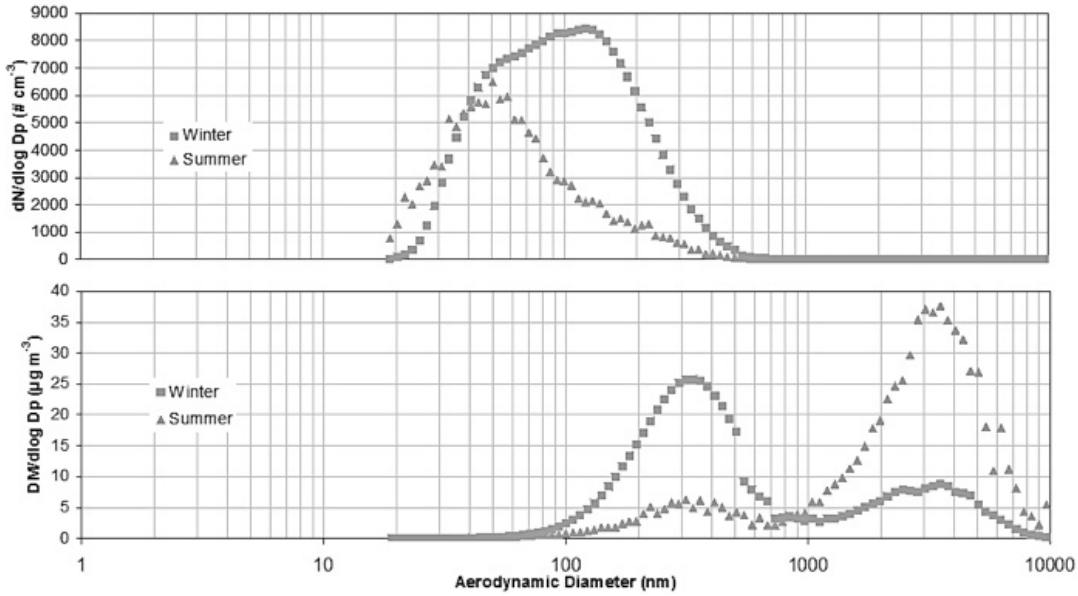

Figure 1: Seasonal average size dimensional distribution in downtown Rome.

In summer the number distribution of the fine mode displays a maximum value at lower diameters than on winter, reflecting the greater contribution due to the photochemical particle formation from gas-phase. Particles above 700 nm do not affect the number size distributions whereas they heavily influence the mass distributions not only in the accumulation mode but also, above all in the coarse mode [16]. In weekend the concentrations are lower, about 25,000 # cm^{-3}, than in the workdays, about # 35000 cm^{-3}, following the trend related to the traffic cycle. In big urban areas, the main sources to submicrometer aerosol are the combustion processes [1].

In downtown Rome, UFPs varies about from 70 to 95 % of total particle number concentration, with lower contributions observed during nighttime hours. In other locations as well, around 80 % of the total particles are under 100 nm [17-21]. In particular, maximum values of UFP number concentration are measured between 8 and 10 a.m. and at about 6 p.m., during periods of maximum autovehicular traffic intensity, on work days, whereas during the week-ends they were observed in the first afternoon (between 1 and 2 p.m.) suggesting a greater contribution from photo-oxidation of Volatile Organic Compounds (VOCs) than from autovehicular traffic. Minimum values are almost invariably observed during nocturnal hours, (between 2 and 3 a.m.) when the effect due to the reduction of the autovehicular traffic emission overcomes the decrease of the atmospheric mixing height.

The petrol and diesel engines contribute to the particle formation in the nucleation mode (below 50 nm). Scientific interest in these particles is related to their high number concentration and their composition.

Deposition velocities of small particles (< 0.1 μm) are mainly determined by Brownian diffusion, whereas for larger particles (> 2 μm) interception, impaction and gravitational settling become important [22]. Minimum values of deposition velocities are for particles

in the range 0.1-2 μm, where neither Brownian diffusion nor impaction or interception are effective mechanisms [22]. For such reasons the persistence in the atmosphere of nuclei mode particles is relatively low with respect to larger-sized particles.

The Figure 2 shows measurements performed by 1-s temporal resolution. The data are obtained by means of FMPS: it can be seen the total particles from 6 to 523 nm and the channels to 11 and 45 nm, for comparison it also shows the total particles (from 6 to 523 nm) averaged over a period of 5 minutes. It is evident that within a few seconds an abrupt increase of the numerical concentrations of about an order of magnitude occurs.

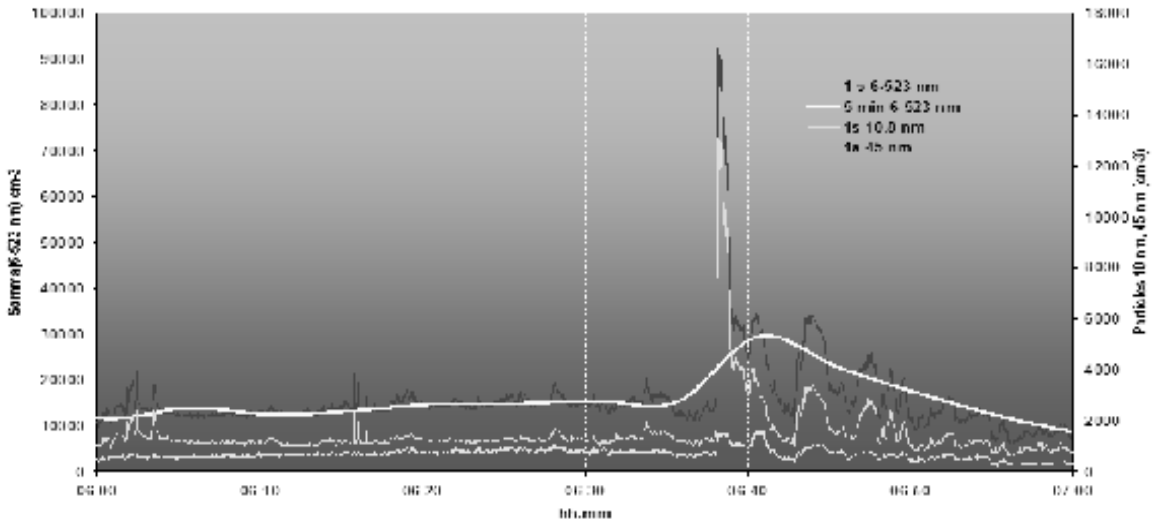

Figure 2: Measurements performed by FMPS (1-2 resolution) and SMPS (5-min resolution).

Figure 3 is an example of how the variations of the aerosol concentrations and size distributions can be fast. After 7 seconds (and even less) the mode distribution passes from a maximum peak at 30 nm to a maximum at 20 nm with a concentration increase of approximately 10 times.

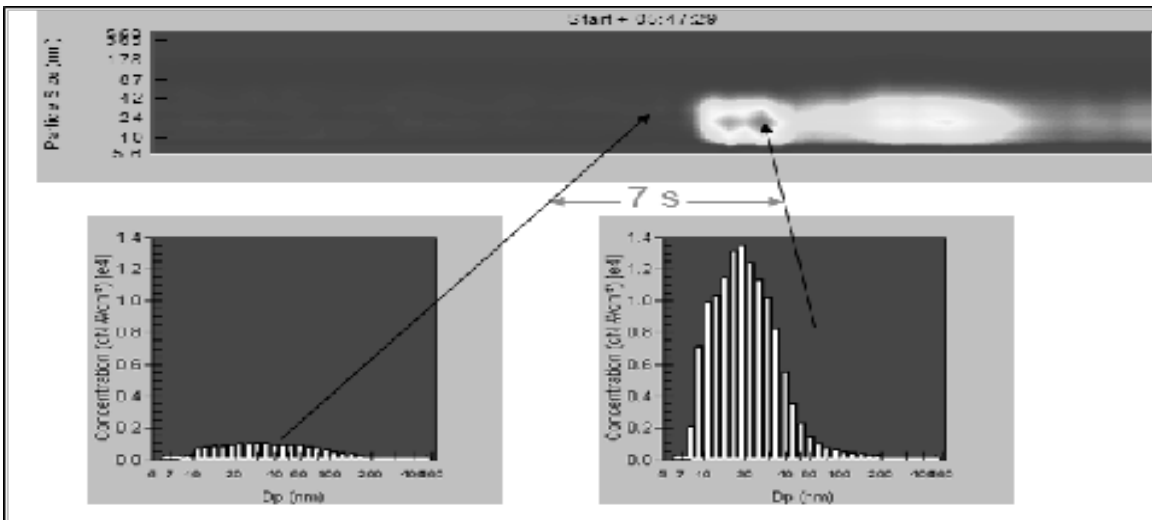

Figure 3: An example of how the variations of the aerosol concentrations and size distributions can be fast.

In relation to the rapidity with the concentration increase and the limited duration of the phenomenon, the variation observed could be due to the presence of new pollutant, obviously linked to the exhaust combustion; on the other hand, the distribution mode decreases suggesting the contribution of ultra fine particles in the nucleation mode.

The atmospheric processes are dependent on the dynamic evolution of the boundary layer; on the other hand, the atmospheric pollutant measurement is based on observations carried out at ground level. An interesting approach for describing the pollutant evolution may be given by Radon concentration measurements [9].

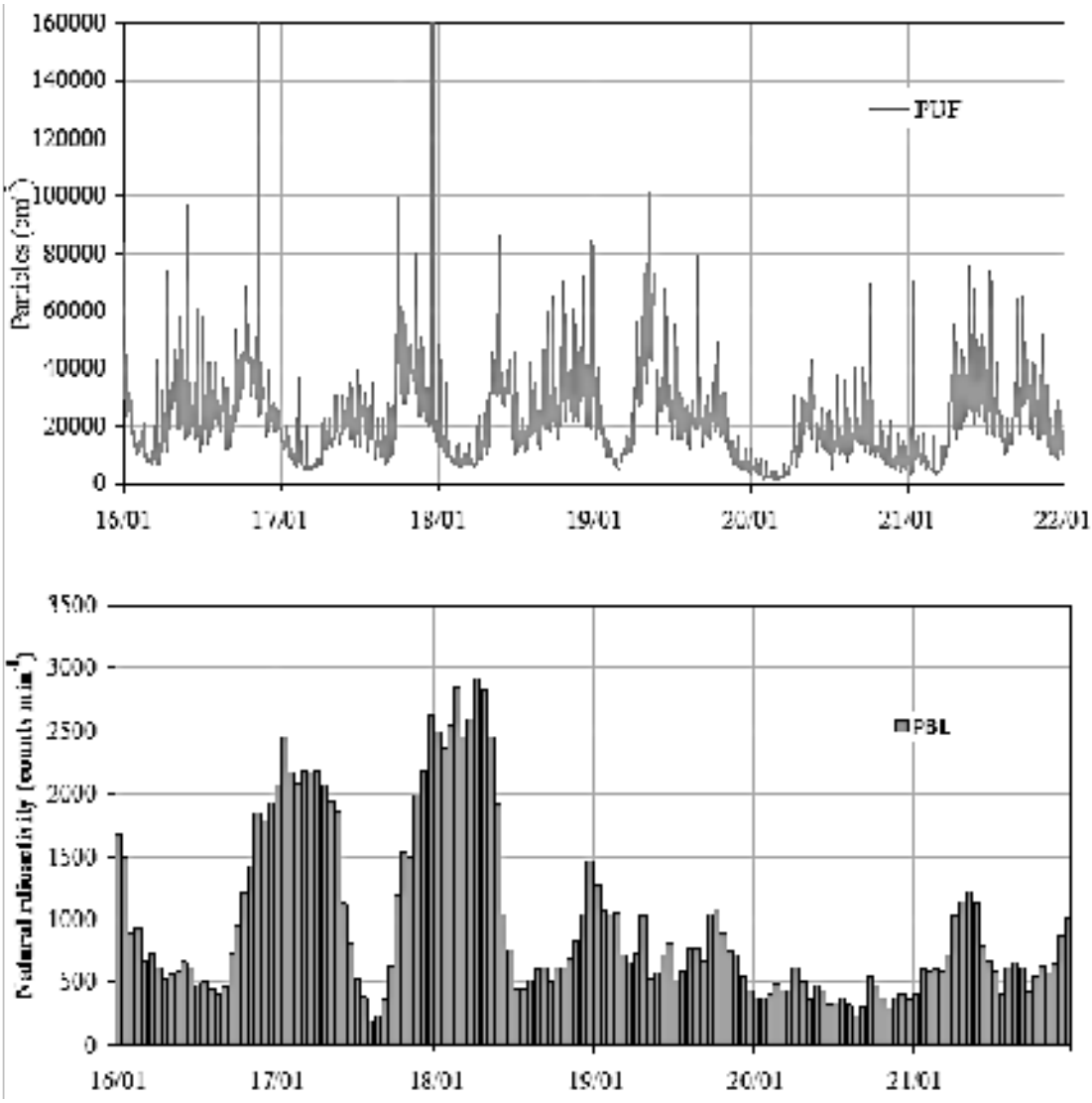

Figure 4: Ultrafine Particles (above) and natural radioactivity (below) trends in downtown Rome.

Radon emission may be considered almost steady source and it is spatially homogeneous over some kilometers. Therefore, the temporal evolution of Radon and its decay products (beta radiation) are not affected by any chemical transformation and are only dependent

on the dynamic of the boundary layer. In this term, the natural radioactivity measurements display the remixing properties of the low boundary layer. A typical occurrence applied to the UFP behavior is reported in Figure 4.

Figure 5 shows the temporal trend of total (5.6-560 nm) particle concentration throughout a 12 hours time span together with the percent contribution of UFPs. We selected one-hour size distribution data to carry out dosimetry estimates. During such time interval total particle concentration ranged from 2.6×10^4 particles$\times$cm^{-3} and 5.8×10^5 particles$\times$cm^{-3}, with an average value of 7.2×10^4 particles$\times$cm^{-3}.

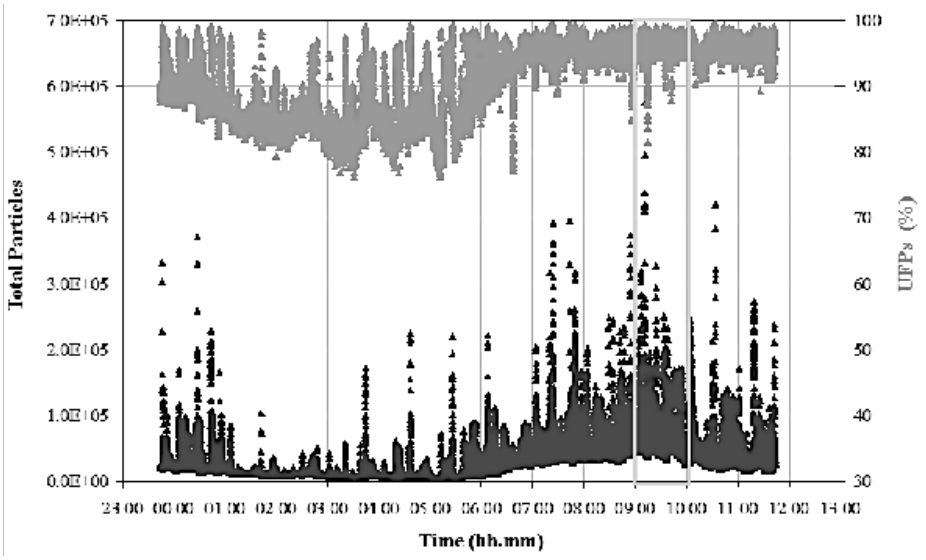

Figure 5: Temporal trends of 5.6-560 nm particle number concentration and of UFP (as %) contribution in downtown Rome.

Based on such data, one-hour regional cumulative doses D^R_h deposited in head, tracheobronchial and alveolar regions are reported in Figure 6.

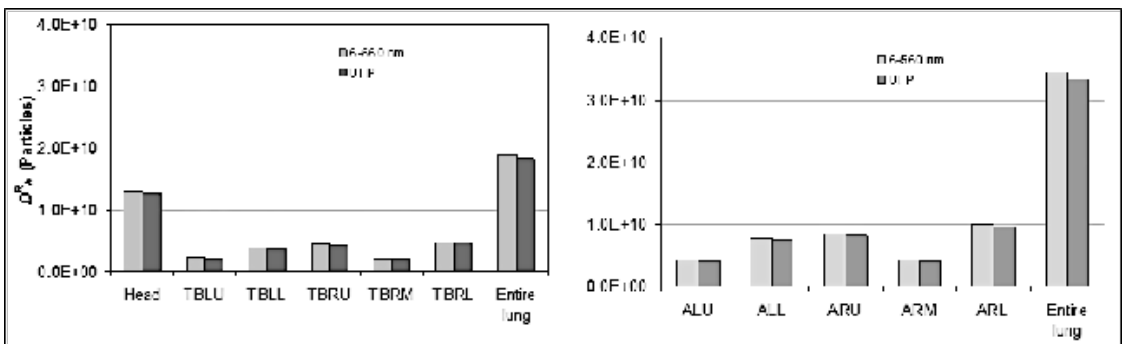

Figure 6: One hour regional cumulative doses (D^R_h) in Head, tracheobronchial left-upper lobe (TBLU), TB left-lower (TBLL), TB right-upper (TBRU), tracheobronchial right-middle (TBRM), TB right-lower (TBRL), alveolar left-upper (ALU), alveolar left-lower (ALL), alveolar right-upper (ARU), alveolar right-middle (ARM), alveolar right-lower (ARL) regions.

In the contour plots of Figure 7, the cumulative doses of particle deposited (D^R_c) are reported as function of time and particle diameter for t the five lung lobes both in tracheobronchial and alveolar regions, respectively.

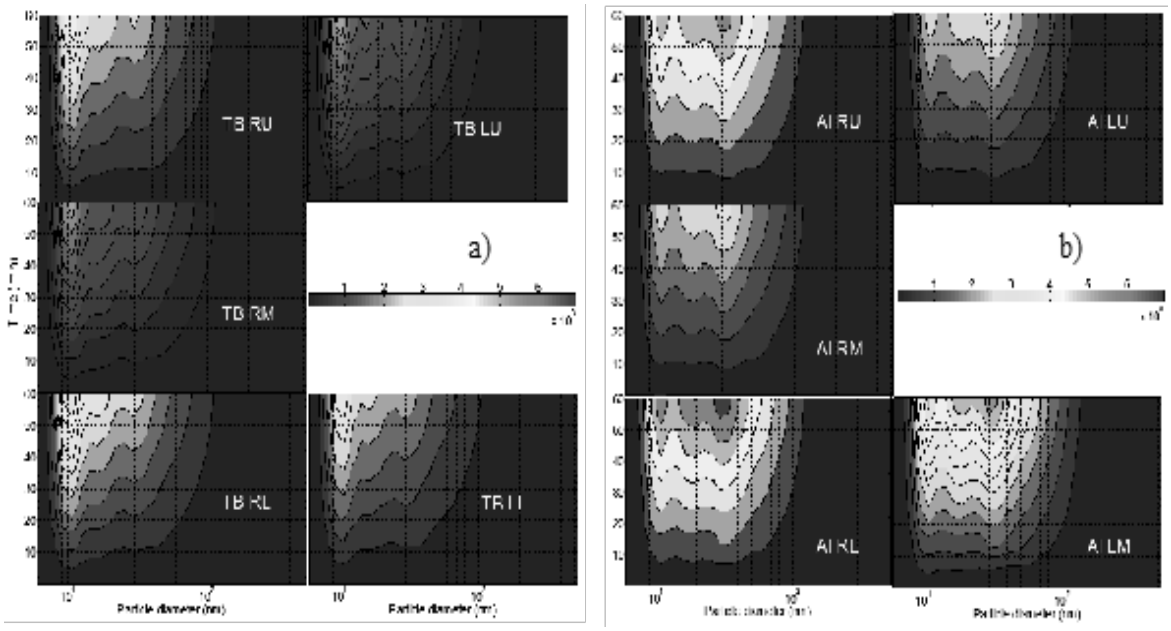

Figure 7: Cumulative dose DRc for lung lobes in tracheobronchial (TB) (a) and alveolar (AI) regions, as function of time and particle diameter.

At the end of one hour exposure period the size distributions of particle deposited doses follow a trimodal distribution with modes at 10, 16 and 29 nm for the H and TB region and 10, 19 and 29 nm for the alveolar region. 1.5×10^9, 8.9×10^8 and 7.0×10^8 particles are deposited in the H region respectively for 10, 19 and 29 nm particle diameters.

It is worth observing that the mode at 10 nm represents the main contribution to the cumulative dose deposited in H and TB regions whereas for the Al region the greater contribution is due to the mode at 29 nm (Figure 7b). In particular, for the daytime and traffic site considered, about 28 % of the particles below 10 nm are deposited in the head region. These particles are formed by the nucleation of semivolatile organic compounds in vehicle exhaust. Their persistence in the atmosphere is relatively low with respect to larger-sized particles [2]. They are responsible for the high frequency contribution observable in the temporal trend of total particle concentration (Figure 5) [1, 23-26].

From the health point of view, UFPs may translocate from the blood circulation to other organs such as liver, spleen, kidneys, heart and brain [27]. The most likely mechanism is from deposits on the olfactory mucosa of the nasopharyngeal region of the respiratory tract and subsequent translocation via the olfactory nerve [28]. Particles on entering the brain may possibly entail neurodegenerative consequences. Histological evidence of neurodegeneration has been reported in both canine and human brains exposed to high ambient PM levels, suggesting the potential for neurotoxic consequences [29].

4. CONCLUSION

This paper would like to deeply review the Ultrafine Particles in downtown of a big urban area. PM atmospheric aerosol in fine mode could be described by granulometric size distribution below 1 μm: in this way, it should be underlined the higher importance of the number concentration for the human health than the mass concentration for aerosol < 1 μm. This paper also evidences how the autovehicular traffic is the main source of Ultrafine Particles in downtown urban area as well as the total UFP concentration following a daily trend governed by both the evolution of the atmospheric mixing height and the variation of autovehicular traffic intensity. Based on aerosol number-size distributions measured with 1s time resolution, the aerosol doses deposited in the respiratory system have been estimated for individuals exposed in proximity of traffic. During traffic peak hours, after one-hour exposure about 6.6×10^{10} particles are deposited into the respiratory system. Such dose is almost entirely made of UFPs and is asymmetrically deposited into the lung lobes, more in right than in left lung lobes.

5. ACKNOWLEDGMENTS

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CHAPTER - VI

ECOTOXICOLOGICAL IMPACTS OF LANDFILL LEACHATE AND ITS TREATMENT USING GREEN COAGULANTS

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ABSTRACT

Disposal of solid waste in the landfill is a practice that still brings serious impacts to the environment through generation of leachate, a dark-colored, strong odour and highly toxic liquid. Landfill leachate was found to be the cause of high toxicity in samples obtained downstream from landfills or in groundwater contaminated with landfill leachate. Landfill leachate may contain many persistent compounds such as heavy metals and organic micro-pollutants. Landfill leachate that contains Persistent Organic Pollutants (POPs) is a great threat to human and environment due to POPs persistency in the environment, long range transportability and bio magnification properties. This study was conducted to evaluate the efficiency of Guar gum and Xanthan gum in treating POPs in landfill leachate. Bis(2-ethylhexyl) phthalate was the main POPs detected in leachate. The results of POPs treatment using Guar gum and Xanthan gum was compared with common coagulant, alum. Gas chromatography–mass spectrometry (GC-MS) was used to identify and determine the concentration of organic pollutants before and after leachate treatment. This study found that 86% and 100% of bis(2-ethylhexyl) phthalate were effectively removed from leachate using Guar gum and Xanthan gum, respectively at pH12. Optimisation of the experiment involving different pH, Guar gum dosage and mixing speed was done by using Box Behnken design. Scanning Electron Microscopy (SEM) and fourier transform infrared spectroscopy (FTIR) studies were carried out on the flocs to study the changes involved in the treatment process. Xanthan gum was found to be more effective than Guar gum and alum in treating POPs in landfill leachate. Therefore Xanthan gum is recommended as an option in treating POPs because it is a green natural coagulant with biodegradability characteristic and involved lower treatment cost.

KEY WORDS: Persistent Organic Pollutants (POPs); bis(2-ethylhexyl) phthalate; Xanthan gum; Guar gum.

1. INTRODUCTION

Waste management has become a serious issue all over the world. About 95% of the municipal solid wastes in Malaysia are disposed of into the landfills. Solid waste disposal by landfill poses a threat to environment through the disposal of leachate (MohdRaihanTaha*et al*, 2011). Landfill leachate that contains persistent organic pollutants (POPs) is a big threat to groundwater systems and are projected to have hazardous effects in the long term if proper management strategies of the landfills are not put in place by those responsible (Nomngongo*et al*., 2012).

Persistent Organic Pollutants (POPs) are carbon-based organic chemical substances, which are intentionally or non-intentionally released to the environment (World Health Organization, 2008), which can also be found in leachate from landfills. POPs are a subset of the persistent, bio-accumulative, and toxic (PBT) category, which are natural or anthropogenic in nature and resist biological, chemical, and photolytic degradation (O'Sollivan and Megson, 2014). POPs are also lipophilic, have long-range of transport leads to global pollution; accumulate in food chain, have acute, high level toxicity is well characterized.These properties of unusual high persistence and semi-volatility, coupled with other characteristics, have resulted in the presence of POPs all over the world, even in regions where they have never been used (Mehmetli and Koumanova, 2007).

Increasingly affluent lifestyles, continuing industrial and commercial growth in many countries around the world in the past decade has been accompanied by rapid increases in both the municipal and industrial solid waste production (Renou, *et al*., 2008).Municipal wastes in Malaysia are mostly disposed of to the landfill. Solid waste disposal by landfill poses a threat to environment through the formation of leachate. Although landfill leachate have been proved to be toxic and recalcitrant, landfilling still remains one of the main methods for municipal and industrial solid waste disposal (Lopez, *et al*., 2003). Sanitary landfill leachate is a highly and complex polluted wastewater. Its quality is the result of biological, chemical and physical processes in landfills combined with the specific waste composition and the landfill water regime. Landfill leachate that contain persistent organic pollutants (POPs) are a big threat to groundwater systems and are projected to have hazardous effects in the long term if proper management strategies of the landfills are not put in place by those responsible.

POPs are long-lived organic compounds that become concentrated as they move through the food chain. They have toxic effects on animal reproduction, development, and immunological function (Wania and Mackay, 1996). POPs are typically `water-hating' (hydrophobic) and `fat-loving' (lipophilic) chemicals. In aquatic systems and soils they partition strongly to solids, notably organic matter, avoiding the aqueous phase. They also partition into lipids in organisms rather than entering the aqueous milieu of cells and become stored in fatty tissue. This confers persistence on the chemical in biota since metabolism is slow and POPs may therefore accumulate in food chains. Importantly, POPs have the propensity to enter the gas phase under environmental temperatures. Hence, they may volatilize from soils, vegetation and water bodies into the atmosphere and because of their resistance to breakdown reactions in air travel long distances before being re-deposited.

Thus, some effective treatment strategies should be implemented on leachate and other substances which contain POPs. The treatment could either be physical, chemical or biological as individual treatment or as combinations.

2. MATERIALS AND METHODS

2.1 Coagulants

Landfill leachate from Jeram Sanitary landfill was treated with three different types of coagulants which are Guar gum, Xanthan gum and Alum. Food grade Guar gum, Xanthan gum and analytical grade Alum ($KAl(SO_4)_2 \cdot ١٢H_2O$) were used in the study. Guar gum is a plant based biopolymer which is usually used in food processing industry (Sen Gupta and Ako, 2005). Guar gum is a straight chain galactomannan obtained from the seed of the guar plant (*Cyamopsistetragonoloba*). It is a high molecular weight polysaccharide with sugars galactose and mannose. Xanthan gum is an anionic polysaccharide produced by the bacteriumXanthomonascampestris(Sun et al., 2007 and Verma et al., 2012). It is produced by the fermentation of glucose by the X. campestris bacterium. After the fermentation, polysaccharide is separated from the growth medium with the help of solvent separation technique, dried and ground into a fine powder (Verma et al., 2012). Solution of Guar gum and Xanthan gum were prepared using 0.1 g of Guar gum and Xanthan gum powder dissolved in 100ml of distilled water, respectively. Guar gum and Xanthan gum powder were slowly added into a beaker containing distilled water and shaken continuously to ensure an evenly wetted solution. Solutions were prepared every day before the experiment to avoid the growth of moulds.

2.2 Sample collection

Leachate samples were collected from Jeram Sanitary Landfill. Raw samples were collected at the outlet of the leachate pipe before it enters into the leachate treatment plant. The samples were immediately transported to the laboratory after collection, then stored at 4 °C before being analysed.

2.3 Methods for coagulation and flocculation experiment

Coagulation and flocculation experiments were performed usingJar test apparatus (Phipps and Bird PB-900 Programmable Jar Tester). Guar gum and Xanthan gum solutions were prepared on the same day of experiment to prevent the growth of moulds. Approximately 250mL of leachate was used for each test. The pH was adjusted by 1N H_2SO_4 and NaOH solutions. The mixing process was carried out in three phases. In the initial phase or the flash mixing phase, the propeller speed was set at 200rpm for 5 minutes. Flocculent was added into the sample after 1 minute of flash mixing. It was followed by two more stages of slow mixing of 10 minutes each first at 60 rpm followed by 40 rpm. The flocs were allowed to settle for 30 minutes after mixing. The supernatant obtained after 30 minutes of settling was used for extraction and analysis of POPs.

2.4 Sample extraction and instrumental analysis

Landfill leachatewas collected and analysed by GC-MS. 100 mL of sample was extracted using liquid-liquid extraction (LLE) method. LLE was repeated three times using 50mL

of analytical reagent grade methylene chloride, CH_2Cl_2 for each extraction. 15g of granular sodium chloride, NaCl was added into separating funnel.The bottom organic layer was filtered through approximately 20 g of granular anhydrous sodium sulfate. It was filtered into a 250 mL round bottom flask. The organic extract was condensed until near dryness at 40° C with Eyela N-1100 rotary evaporator. 1mL of CH_2Cl_2 was added and the extract was kept in a chromatographic vial.

The extracted compounds were analysed with Shimadzu GCMS-QP2010 Plus. It was operated using GCMSolution software. Samples were analysed on DB-5MS capillary column (30m X 0.25mm i.d and 0.25µm film thickness). GC operating conditions were as follows: oven parameters, 70 °C, hold for 2min, increased at 25 °C/min to 150 °C/min, hold for 0 min, ramped at 3 °C/min to 200 °C, hold for 0 min, rose at 8 °C/min to 280 °C, hold for 15 min. The temperature of the inlet was 230 °C and the injection volume was 2µL (splitless).

2.5 Experimental design and data analysis

Preliminary testscarried out with different pH values and dosages of Guar gum enabled the establishment of the optimisation design by using the Box-Behnken experimental design. The advantages of the use of this design include fewer experiments and good predictability. The design summary of the Box-Behnkendesign used is presented in Table 1. Mixing speed, pH and dosage of Guar gum were selected as independent variables. The experimental results were analysed with Design Expert 7 software. This software can improve the existing design as well as create a new design. The experimental design of this study is shown in Table 2.

Table 1 : Experimental design summary.

Factors	Name	Type	Low actual	High actual	Central values (zero level)
A	pH	Numeric	4	12	8
B	Guar gum dose	Numeric	3 mg L^{-1}	5mg L^{-1}	4 mg L^{-1}
C	Mixing speed	Numeric	150 rpm	250 rpm	200 rpm

Table 2 : Design of experiment.

Run	pH	Guar gum dose (mg L^{-1})	Mixing speed (rpm)
1	4	4	150
2	8	4	200
3	4	5	200
4	8	4	200
5	8	5	150
6	12	4	250

7	12	4	150
8	4	4	250
9	8	4	200
10	12	3	200
11	8	3	150
12	8	4	200
13	8	3	250
14	4	3	200
15	8	4	200
16	12	5	200
17	8	5	250

2.5 Scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis

SEM operating with the SEI detector of a JEOL JSM-7001F SEM was performed to compare the images of flocs after treatment involving the use of guar gum and alum. The FTIR spectra of the flocs and leachate were obtained using a Perkin Elmer spectrophotometer (FTIR-Spectrum 400).

3. RESULTS AND DISCUSSION

3.1 POPs removal using Guar gum and alum

The mean concentrations of bis(2-ethylhexyl) phthalate in leachate was 7.98 mg L^{-1}. The results for POPs removal when Guar gum and Xanthan gum were used are presented inFig. 1. POPs were treated using Guar gum and Xanthan gum at dosages of 2.5, 3.0, 4.0, and 5.0 mg L^{-1}. About 85.91% and 100.00 % of bis(2-ethylhexyl) phthalate were efficiently removed using Guar gum and Xanthan gum at a dosage of 4.0 mg L^{-1},respectively. The percentage of POPs removal initially increased from 2.5 mg L^{-1} to 4.0 mg L^{-1} of Guar gum dose and then decreased at 5.0 mg L^{-1} after reaching the optimum concentration. This result indicates that re-suspension occurred because of the high concentration of Guar gum. Eventually, re-suspension led to the generation of repulsive forces between the flocculent and POPs and decreased the percentage of POP removal (Mishra and Bajpai, 2005). The results were compared with those obtained when a common inorganic coagulant, alum, was used. A similar trend was observed in POPs removal when alum was used as shown in Fig. 2. The highest removal percentages of bis(2-ethylhexyl) phthalate were observed at 0.5g L^{-1}. Based on the results, 4.0 mg L^{-1} of Guar gum or 0.5g L^{-1} of alum is required to remove more than 80% of POPs in leachate. This finding indicates that the concentration of alum must be 125 times higher than that of Guar gum to remove POPs in leachate effectively. Thus, Guar gum is a better option in treating POPs in leachate because a lower dosage is needed and the cost for treatment is decreased.

From an economic point of view, the treatment of POPs in landfill leachate by using Guar gum is more cost-effective compared with alum. The cost of guar gum powder is USD

58.20 per gram, whereas the cost of alum powder is USD 9.70 per gram. Although the cost of Guar gum powder per gram is higher than that of alum, the dosage of Guar gum used for leachate treatment was 125 times lower than that of alum. Thus, the cost of POP treatment is significantly reduced when Guar gum was used. The cost of POP treatment to treat leachate by using Guar gum is USD 0.23 per L. However, if alum was used, the cost to treat the same amount of leachate is much higher at USD 4.85 per L.

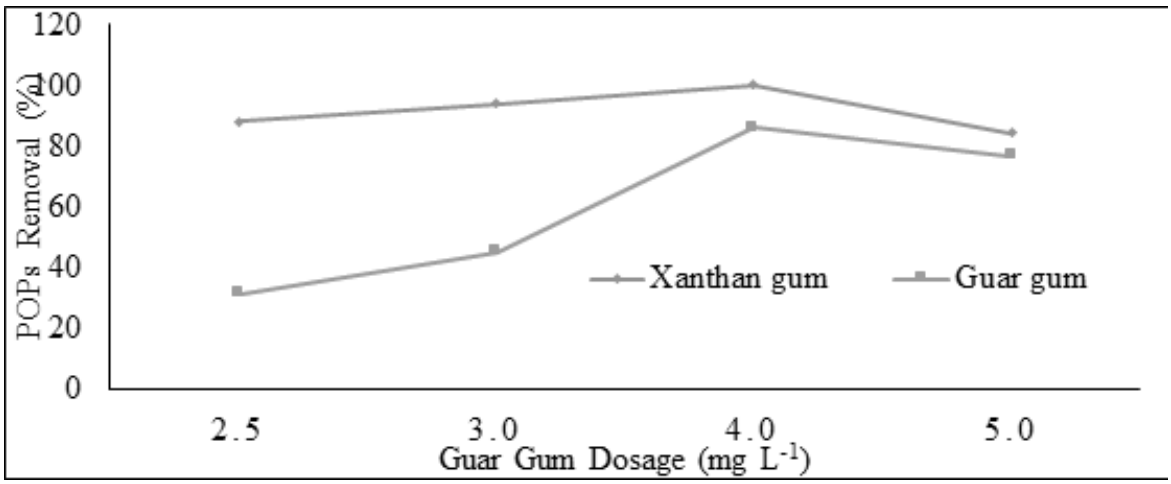

Figure 1: Percentage of bis(2-ethylhexyl) phthalate removal in leachate using Xanthan gum and Guar gum, at pH12 at various dosages.

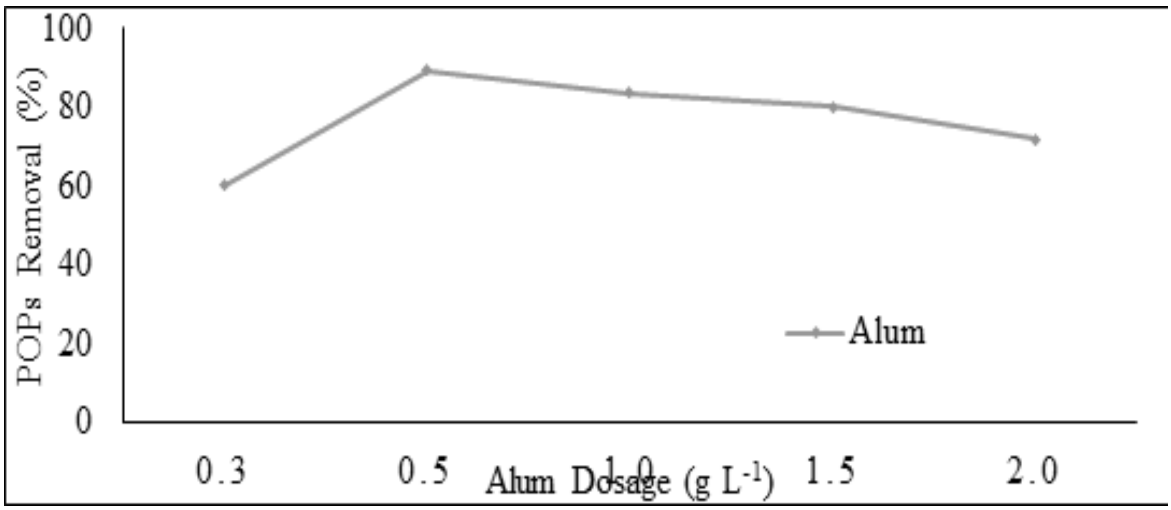

Figure.2: Percentage of POPs removal in leachate using alum, at pH12 at various dosages.

Various operating parameters, such as pH, Guar gum dosage and mixing speed were optimised for POP removal by using Guar gum. The results of ANOVA for bis(2-ethylhexyl) phthalate removal is shown in Table 3.Second-order polynomial equations was established for bis(2-ethylhexyl) phthalate removal in Equations 1

Bis(2-ethylhexyl) phthalate removal= +121.50000 - 1.06250 × pH + 152.12500 × Guar Gum Dose - 3.27250 × Mixing speed + 2.81250 × pH × Guar Gum Dose + 0.011250 × pH × Mixing speed - 0.020000 × Guar Gum Dose × Mixing speed - 0.68750 × pH2 -21.25000 × Guar Gum Dose2 + 7.70000E-003 × Mixing speed2 Eqn (1)

The ANOVA regression model shows that a quadratic model is suitable in predicting the removal rate of bis(2-ethylhexyl) phthalate via Fisher's F-test (F_{model} = 36.23), with a very low probability value (P model> F=0.0001), as recommended in a previous study (Liu et al., 2004). Values of "Prob> F" less than 0.0500 indicate that the model terms are significant. The chance that a "Model F-Value" this large could occur is only 0.01% because of the presence of noise.

Predicted R^2 and adjusted R^2 are used to determine the accuracy of the model. If the difference between the predicted R^2 and adjusted R^2 is more than 0.20, then an error in the data or model exists. From Table 3, the predicted R^2 of bis(2-ethylhexyl) phthalate (0.7638) are in reasonable agreement with the adjusted R^2 values of 0.9519.

Table 3

Statistical models obtained from the ANOVA for optimization of bis(2-ethylhexyl) phthalate removal from leachate.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	5276.51	9	586.27	36.23	< 0.0001	Significant
A-pH	264.50	1	264.50	16.34	0.0049	
B-Guar Gum Dose	3.12	1	3.12	0.19	0.6736	
C-Mixing speed	666.12	1	666.12	41.17	0.0004	
AB	506.25	1	506.25	31.29	0.0008	
AC	20.25	1	20.25	1.25	0.3002	
BC	4.00	1	4.00	0.24	0.6343	
A^2	509.47	1	509.47	31.49	0.0008	
B^2	1901.31	1	1901.31	117.52	< 0.0001	
C^2	1560.26	1	1560.26	96.44	< 0.0001	
Residual	113.25	7	16.17			
Lack of Fit	113.25	3	37.75			
Cor Total	5389.7647	16				
Std. Dev.	4.0222		R-Squared	0.9789		
Mean	76.8823		Adj R-Squared	0.9519		
C.V. %	5.2317		Pred R-Squared	0.7638		
PRESS	1812.0000		Adeq Precision	22.5288		

3.3 Effect of pH

The effect of pH on bis(2-ethylhexyl) phthalate removal is shown in Fig. 3. pH has a strong effect on POP removal efficiency. Increasing the pH from acidic to neutral results in a positive trend in the percentage of POP removal. Subsequently, the percentage of POP removal slightly decreases when the leachate sample is too alkaline. The optimal adjusted pH for POP removal is around pH 9. The influence of pH on the flocculation process can be considered as a result of two competitive forces between H^+ with the metal hydrolysis product and between OH^- with organic anions (Stephenson and Duff, 1996). At low pH, the removal rate is low because of the competition between hydrogen ions and metal hydrolysis products for organic ligands. Some of the organic acids do not precipitate; thus the POP concentration remains high in leachate. When the pH was increased, OH^-competes with the organic compound for metal adsorption sites and results in large amount of precipitate (Stephenson and Duff, 1996).

3.4 Effect of dosage

The results for bis(2-ethylhexyl) phthalate removal when Guar gum was used are presented in Fig. 3.shows that the percentage of bis(2-ethylhexyl) phthalate removal initially increases, then decreases after reaching the optimum concentration at 4 mg L^{-1} of Guar gum. This result is due to the low concentration of added Guar gum, which attaches to the colloidal particles by forming a bridge. Thus, POPs are removed in the leachate. However, the phenomenon changed beyond the optimum dosage. When Guar gum was added in excess, the particle surface cannot sustain biopolymer attachment (Tripathy and De, 2006). Thus, the particles become destabilised because of repulsive forces and result in a less effective POP removal.

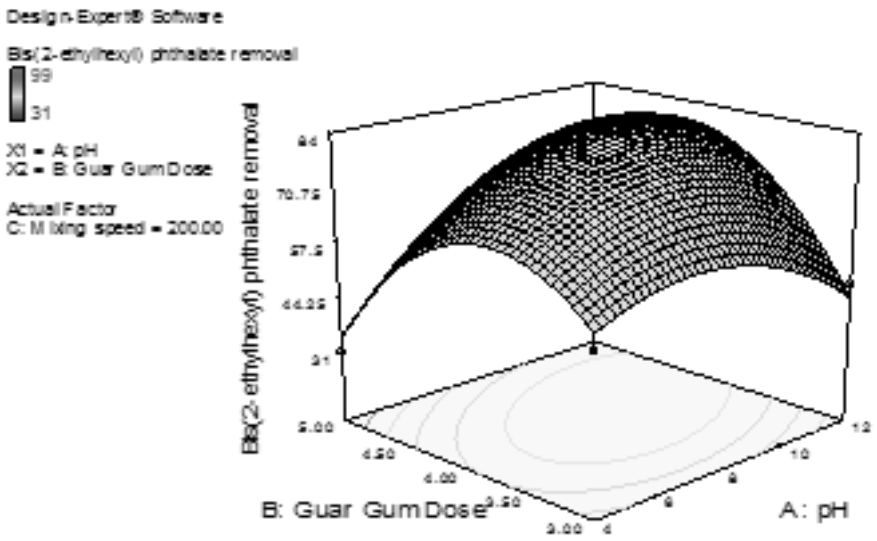

Figure. 3:. Response surface plot for bis(2-ethylhexyl) phthalate removal by Guar gum addition.

3.5 SEM micrographs

The surface structures of Guar gum and alum flocs from the leachate are presented in Fig. 4. The SEM images show that the Guar gum flocs have porous cross linkages and an amorphous surface structure. These characteristics enable Guar gum to effectively remove POPs from the leachate. The SEM micrographs also show that the flocs produced by Guar gum are more structured and denser than alum. The flocs produced by alum are somehow not well-distributed. This result shows that Guar gum is more effective in removing POPs compared with alum. Similarly, a previous research found that Guar gum is more effective than alum in treating waste water (Mukherjee et al., 2014).

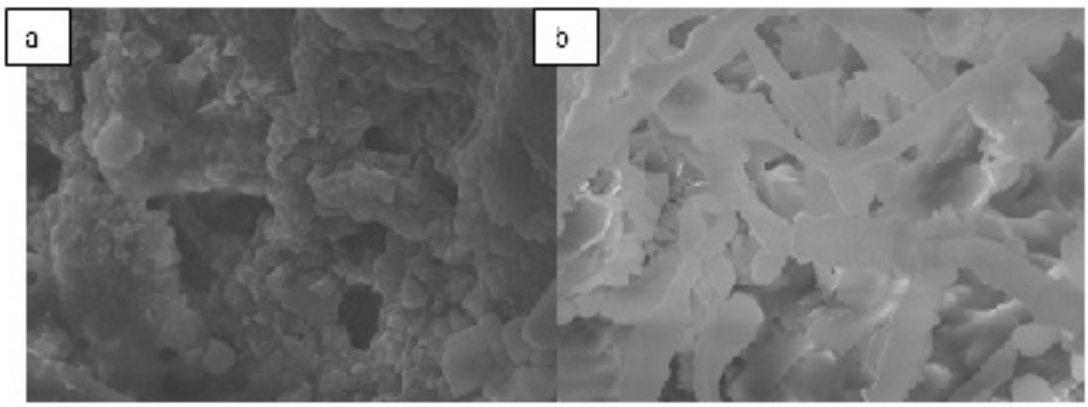

Figure. 4: SEM images of (a)Guar gum flocs from leachate and (b)Alum flocs from leachate.

3.6 FTIR analysis

FTIR analysis was conducted on leachate, guar gum powder, guar gum flocs with leachate and alum flocs with leachate, and the results are shown in Fig. 5. FTIR analysis was used in this study to identify functional groups. The infrared radiation (IR) spectrum of guar gum flocs with leachate is very similar with that of guar gum powder at wavenumber of 3300, 1640 and 1050 ms^{-1}, which correspond to the vibrations of -OH, C-C and C-O, respectively. The IR spectra of leachate and guar gum flocs with leachate are similar at the wavelengths of 1640 and 605 ms^{-1}, which correspond to the vibrations of C-C and C-Br. Several peaks were observed near wavelengths of 3200, 1100 and 615 ms^{-1} in alum flocs with leachate. These peaks indicate the presence of O-H, C-O and C-Br. Thus, a complex interaction occurred, which caused the different components of the leachate to attach to the coagulants and results in POP removal. Although the IR spectrum of guar gum flocs with leachate is similar to those of leachate and guar gum powder, several shifts in the wavenumber of the peak are observed, which are probably due to the formations of hydrogen bonds. Guar gum consists of long straight chains of α-D-mannopyranosylunits linked by β-D-(1-4)glycosidiclinkages at the ratio of 2:1(Iqbal et al., 2013). The free reactive hydroxyl groups in the Guar gum backbone can be substituted by different functional groups which enable the removal of POPs in leachate.

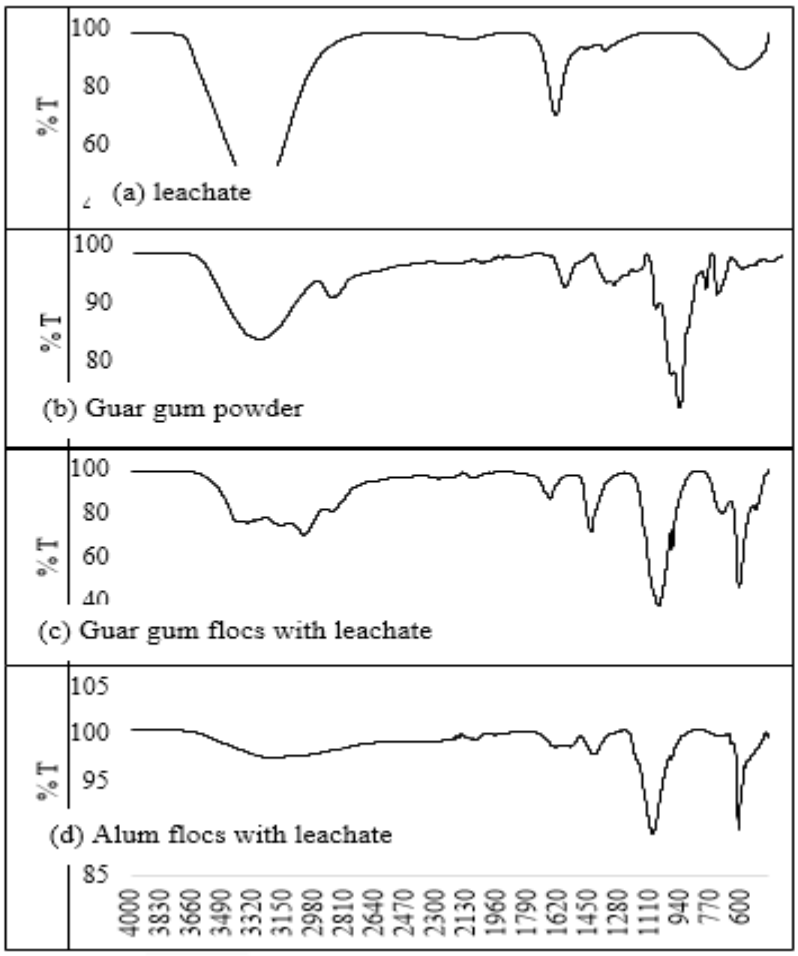

Figure. 5: FTIR spectra of (a) leachate, (b) Guar gum powder, (c) Guar gum flocs with leachate, (d) alum flocs with leachate

4. CONCLUSION

In this study, bis(2-ethylhexyl) phthalate in landfill leachate was effectively removed using Guar gum. Guar gum can remove more than 80% of POPs in landfill leachate at an optimum dosage of 4 mg L^{-1}. The optimisation of POP removal by using Guar gum, experimental design, mathematical modelling and data analysis was carried out using Design Expert 7 Software. The SEM images also proved that Guar gum, which has more void spaces and is well-structuredcan remove POPs more effectively compared with alum. About 86% and 100% of bis(2-ethylhexyl) phthalate were effectively removed from leachate using Guar gum and Xanthan gum, respectively at pH12. Xanthan gum is highly recommended as an option for treating wastewater because it is a biodegradable biopolymer, non-toxic, low-cost, easily available and produced in abundance.

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