Environmental Toxicology

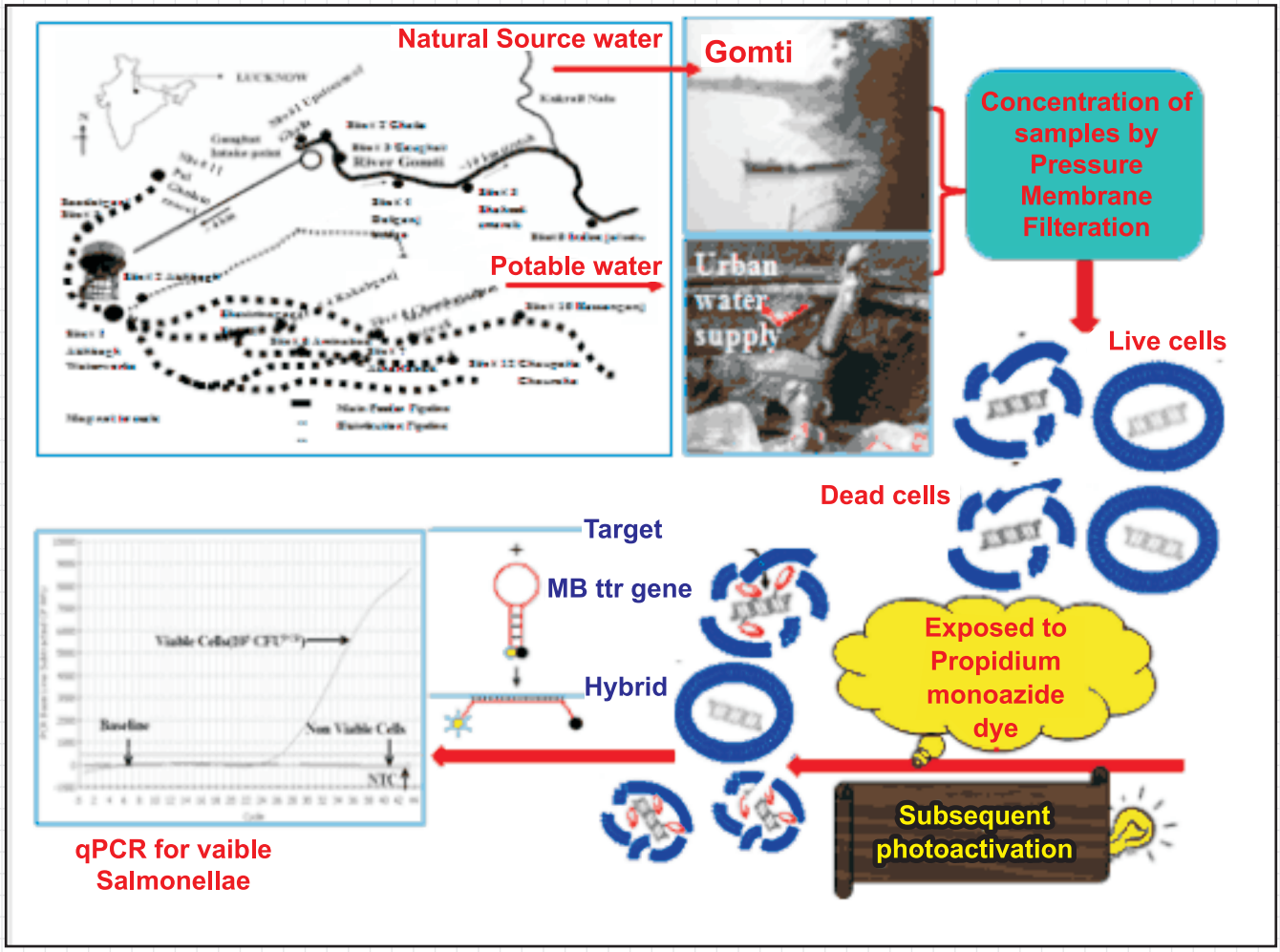
Determination of viable Salmonellae from potable and source water through PMA assisted qPCR

Resource constrained countries identified as endemic zones for pathogenicity of Salmonella bear an economic burden due to recurring expenditure on medical treatment. qPCR used for Salmonella detection could not discriminate between viable and non viable cells. Propidium mono azide (PMA) that selectively penetrates nonviable cells to cross-link their DNA was coupled with ttr gene specific q PCR for quantifying viable Salmonellae in source/potable waters collected from a north Indian city. Source water (raw water for urban potable water supply) and urban potable water exhibited viable Salmonellae in the range of $2.1X10^4$–$2.6X10^6$ and 2–7160 C.F.U/100mL, respectively. Potable water at water works exhibited DNA from dead cells but no viable cells were detected. PMA assisted q PCR could specifically detect low numbers of live Salmonellae in source and potable waters. This strategy can be used in surveillance of urban potable water distribution networks to map contamination points for better microbial risk management.

Singh et al.; Ecotoxicology and Environmental Safety; 2013; 93; 121-127

Use of transgenic GFP reporter strains of the nematode *Caenorhabditis elegans* to investigate the patterns of stress responses induced by pesticides and by organic extracts from agricultural soils

As a free-living nematode, *C. elegans* is exposed to various pesticides used in agriculture, as well as to persistent organic residues which may contaminate the soil for long periods. In a previous study of metal effects on 24 GFP-reporter strains representing four different stress-response pathways in *C. elegans*, parallel data on the responses of these same strains to several

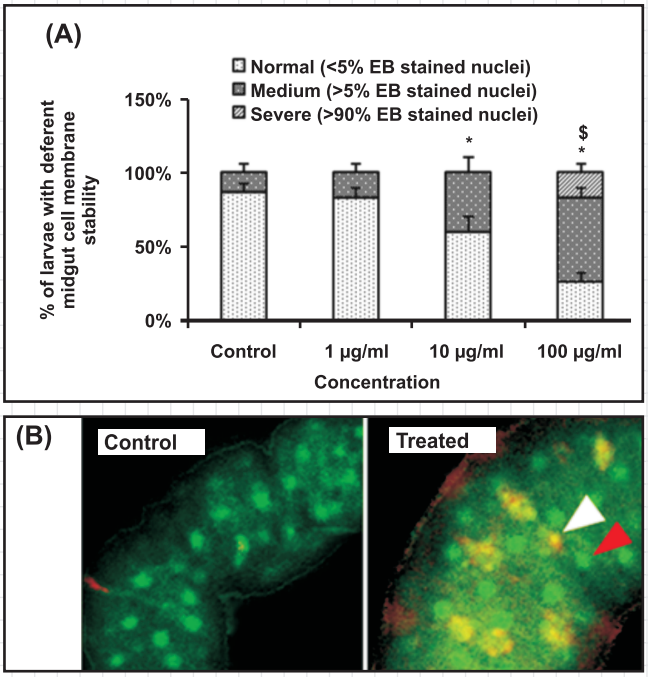


commonly used pesticides has been presented. Some of these, like dichlorvos, induced multiple stress genes in a concentration-dependent manner. Unusually, endosulfan induced only one gene (cyp-34A9) to very high levels (8-10-fold) even at the lowest test concentration, with a clear plateau at higher doses. Other pesticides, like diuron, did not alter reporter gene expression detectably even at the highest test concentration attainable, while others (such as glyphosate) did so only at very high concentrations. Five responsive GFP reporters were used to investigate the toxicity of soil pore water from two agricultural sites in south-east Spain, designated P74 (used for cauliflower production, but significantly metal contaminated) and P73 (used for growing lettuce, but with only background levels of metals). Both soil pore water samples induced all five test genes to varying extents, yet artificial mixtures containing all major metals present had essentially no effect on these same transgenes. Soluble organic contaminants present in the pore water were extracted with acetone and dichloromethane, then after evaporation of the solvents, the organic residues were redissolved in ultrapure water to reconstitute the soluble organic components of the original soil pore water. These organic extracts induced transgene expression at similar or higher levels than the original pore water. Addition of the corresponding metal mixtures had either no effect, or reduced transgene expression towards the levels seen with soil pore water only. The study concluded that the main toxicants present in these soil pore water samples are organic rather than metallic in nature. Organic extracts from a control standard soil (Lufa 2.2) had negligible effects on expression of these genes, and similarly several pesticides had little effect on the expression of a constitutive myo-3GFP transgene. Both the P73 and P74 sites have been treated regularly with (undisclosed) pesticides, as permitted under EU regulations, though other (e.g. industrial) organic residues may also be present.

Anbalagan et al.; Ecotoxicology; 2013; 22; 72-85

Cellular internalization and stress response of ingested amorphous silica nanoparticles in the midgut of *Drosophila melanogaster*

Amorphous silica nanoparticles (aSNPs) are used for various applications including food industry. However, limited in vivo studies are available on absorption/internalization of ingested aSNPs in the midgut cells of an organism. The study aimed to examine cellular uptake of aSNPs (<30nm) in the midgut of *Drosophila melanogaster* (Oregon-R$^+$) owing to similarities between the midgut tissue of this organism



Membrane destabilization by amorphous silica nanoparticles (aSNPs) in midgut cells of *Drosophila melanogaster*. (A) graphical representation and (B) confocal images showing AO/EB staining in the midgut cells of control and aSNPs exposed third instar larvae of *Drosophila melanogaster* after 36 h. Red and white arrows denote AO and EB stained nuclei respectively.

and human and subsequent cellular stress response generated by these nanoparticles. Third instar larvae of *D. melanogaster* were exposed orally to 1-100µg/mL of aSNPs for 12-36h and oxidative stress (OS), heat shock genes (hsgs), membrane destabilization (Acridine orange/Ethidium Bromide staining), cellular internalization (TEM) and apoptosis endpoints were evaluated. A significant increase was observed in OS endpoints in the midgut cells of exposed Drosophila in a concentration- and time-dependent manner. Significantly increased expression of hsp70 and hsp22 along with caspases activation, membrane destabilization and mitochondrial membrane potential loss was also observed. TEM analysis showed aSNPs-uptake in the midgut cells of exposed Drosophila via endocytic vesicles and by direct membrane penetration. aSNPs after their internalization in the midgut cells of exposed Drosophila larvae show membrane destabilization along with increased cellular stress and cell death. Ingested aSNPs show adverse effects on the cells of GI tract of the exposed organism thus their industrial use as a food-additive may raise concern to human health.

Pandey et al.; Biochimica et Biophysica Acta; 2013; 1830; 2256-2266

Biosorption of arsenic from aqueous solution using dye waste

The purpose of this study is to examine the removal of arsenic from water by biosorption through potential application of herbal dye wastes. Four different flower dye residues (after extraction of natural dye) viz. Hibiscus rosasinensis, Rosa rosa, Tagetes erecta, and Canna indica were utilized successfully for the removal of arsenic from aqueous solution. Batch studies were carried out for various parameters viz. pH, sorbent dose, contact time, initial metal ion concentration, and temperature. Data were utilized for isothermal, kinetic, and thermodynamic studies. Scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDAX), and Fourier transform infrared (FTIR) analyses of biomass were performed. The results showed that 1 g/100 ml for 5.0-5.5 h contact time at pH 6.0-7.5 with agitation rate 150 rpm provided 98, 96, 92, and 85 % maximum absorption of arsenic by R. rosa, H. rosasinensis, T. erecta, and C. indica, respectively, at initial concentration of 500 ppb. Data followed Langmuir isotherm showing sorption to be monolayer on heterogeneous surface of biosorbent. FTIR results showed apparent changes in functional group regions after metal chelation. SEM and EDAX analyses showed the changes in surface morphology of all test biosorbents. Herbal dye wastes, used as biosorbent, exhibited significant (85-98 %) removal of arsenic from aqueous solution. Hence, these biosorbents are cost-effective, easily available, eco-friendly, and comparatively more effective than other biosorbents already in use. These may be used to remove arsenic and other toxic metals from water.

Nigam et al.; Environmental Science and Pollution Research International; 2013; 20; 1161-1172

Potential environmental pollution hazards by coal based power plant at Jhansi (UP) India

Coal, a fossil fuel, is the largest source of energy for the generation of electricity in India. In order to study the potential environmental hazards of camed by coal based power plants, particulate matters were collected using Stack Monitoring Kit and gaseous pollutants by Automatic Flue Gas Analyzer. The morphological and chemical properties, mineralogical composition and particle size distribution have been determined by SEM–EDX, XRD and CILAS. The data revealed the presence of particulate matters, SO_2, NOx in the range of 236–315, 162–238, 173–222 mg/Nm^3 respectively. The emission of CO_2 was in the range of 43,004–60,115 Nm^3/h with an average of 52,830 Nm^3/h. Among the elements, Fe > Mn > Al > Zn > B > Ni > Cr > Cu were present in substantially higher proportion than Pb > Mo > Cd > Se > As > Hg. It was found that most of the elements were concentrated on fly ash surface rather than coal, bottom ash and pond ash. This variation may be attributed to the fineness of fly ash particles with large surface ratio to mass. Mineralogical studies of coal and fly ash by X-ray diffraction revealed the presence of mullite, quartz, *cristobalite and maghemite*. Presence of mullite and quartz found in fly ash indicate the conversion of complex minerals to mullite and quartz at high temperature. Transfer Coefficient was calculated to determine the ratio of the enrichment of trace elements in fly or bottom ash with respect to coal and pond ash.

Kisku et al.; Environmental Earth Sciences; 2012; 67; 2109-2120

Temporal distribution of fine particulates ($PM_{2.5}$:PM_{10}), potentially toxic metals, PAHs and metal-bound carcinogenic risk in the population of Lucknow City, India.

Ubiquitous fine particulates or particles can readily be bound to toxic metals and polycyclic aromatic hydrocarbons and are considered to be a great threat to human health. The purpose of this study was to assess the magnitude of air pollution risk to public health by determining four crucial parameters- inhalable particulates, metals in particulates and PAHs which are associated with PM_{10} in the air environment of Lucknow, India during 2007-09. The values of PM_{10} and $PM_{2.5}$ ranged between 102.3-240.5 and 28.0-196.9 µg/m^3 whilst the average PM_{10} was 1.7 times and PM was 1.5 times higher than their respective NAAQS of 100 and 60 µg/m^3 respectively. The estimated relative death rate and hospital admissions for each increase in the PM_{10} levels of 10 µg/m^3 ranged from 1.5-8% and from 3.9-8.0% (as per APHEA2 1990) respectively in persons > 65 yrs. Among the locations; AQ, AQ and AQ (with diversified activities and heavy traffic) recorded higher concentrations of both the particulate fractions than the AQ (residential area with low traffic). The average concentrations of Fe, Pb, Ni, Cu, Cr, Cd in PM_{10} were 219.4, 40.6, 35.1, 27.3, 22.2 and 16.2 ng/m^3 and that in $PM_{2.5}$ were 54.3, 33.9, 38.5, 29.4, 8.4, and 1.17 ng/m^3 respectively Regression analysis revealed that correlation of metals with $PM_{2.5}$ was stronger than PM. The ratio of metals adsorbed on surface of particles ($PM_{2.5}$:PM_{10}) reveals that $PM_{2.5}$ has more affinity for Ni, Cu and Pb and PM_{10} for Cd, Fe and Cr. Health risk due to carcinogenic metals bound to respirable particulates was predicted by estimating excess cancer risk (ECR).

The highest ECR value was estimated for Cr, 266.70 × 10^{-6}, which was associated with PM_{10} and 100.92 × 10^{-6} which was associated with $PM_{2.5}$, whereas lead has the lowest ECR value. Amongst PAHs, benzo(a)pyrene (51.96 ± 19.71 ng/m) was maximum in PM_{10} samples. Maximum concentrations of PM_{10}, $PM_{2.5}$, metals and PAHs were detected during winter, and the lowest was during monsoon. The higher prevalence of diseases among the population may be due to high concentration of particulates coated with toxic metals and PAHs present in air environment.
Pnadey et al.; Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering; 2013; 48; 730-745

Pollution in Lucknow city and its health implication on exposed vendors, drivers and traffic policemen

In this pilot study, an exposure–response assessment (aged 16 to 60 years, $n = 1,012$) was carried out related to eye problems due to vehicular fumes during the winter of 2003. Inhalable particles <PM_{10} and Pb level were monitored at 12 locations of Lucknow using a Respirable Dust Sampler and EPM 2000 filter. The range of PM_{10} particles varied from 144 to 305, 211–366 and 141–242 µg/m^3 while Pb levels were 0.02–0.93, 0.06–0.90 and 0.07–0.77 µg/m^3 during 2002, 2003 and 2004, respectively. The concentrations of PM_{10} exceeded alarmingly the prescribed standards by a factor of 1.3–3.2. A health survey demonstrated that symptoms were developed such as burning of eyes (in 20 % of drivers, 74.07 % of vendors and 77.47 % of traffic policemen), eye watering (in 51.45 % of drivers, 44 % of vendors and 72.77 % of traffic policemen), constant irritation and redness (in 33.49 % of drivers, 21.29 % of vendors and 26.12 % of traffic policemen) and impaired vision (in 25.01 % of drivers, 20.37 % of vendors and 23.5 % of traffic policemen). The main finding was the prevalence of symptoms of the eyes among the population exposed to automobile fumes.
Kisku et al.; Air Quality, Atmosphere & Health; 2013; 6; 509-515

Transcriptomic analysis provides insights on hexavalent chromium induced DNA double strand breaks and their possible repair in midgut cells of *Drosophila melanogaster* larvae

Hexavalent chromium [Cr(VI)] is a well known mutagen and carcinogen. Since genomic instability due to generation of double strand breaks (DSBs) is causally linked to carcinogenesis, we tested a hypothesis that Cr(VI) causes in vivo generation of DSBs and elicits DNA damage response. We fed repair proficient *Drosophila melanogaster* (Oregon R(+)) larvae Cr(VI) (20.0µg/ml) mixed food for 24 and 48h and observed a significant ($p<0.05$) induction of DSBs in their midgut cells after 48h using neutral Comet assay. Global gene expression profiling in Cr(VI)-exposed Oregon R(+) larvae unveiled mis-regulation of DSBs responsive repair genes both after 24 and 48h. In vivo generation of DSBs in exposed Drosophila was confirmed by an increased pH2Av immunostaining along with the activation of cell cycle regulation genes. Analysis of mis-regulated genes grouped under DSB response by GOEAST indicated the participation of non-homologous end joining (NHEJ) DSB repair pathway. We selected two strains, one mutant (ligIV) and another ku80-RNAi (knockdown of ku80), whose functions are essentially linked to NHEJ-DSB repair pathway. As a proof of principle, we compared the DSBs generation in larvae of these two strains with that of repair proficient Oregon R(+). Along with this, DSBs generation in spn-A and okr [essential genes in homologous recombination repair (HR) pathway] mutants was also tested for the possible involvement of HR-DSB repair. A significantly increased DSBs generation in the exposed ku80-RNAi and ligIV (mutant) larvae because of impaired repair, concomitant with an insignificant DSBs generation in okr and spn-A mutant larvae indicates an active participation of NHEJ repair pathway. The study, first of its kind to our knowledge, while providing evidences for in vivo generation of DSBs in Cr(VI) exposed Drosophila larvae, assumes significance for its relevance to higher organisms due to causal link between DSB generation and Cr(VI)-induced carcinogenesis.
Mishra et al.; Mutation Research; 2013; 747-748; 28-39

Biosorption of Arsenic in drinking water by submerged plant: *Hydrilla verticilata*

Evaluation of the biosorption efficacy of submerged aquatic plant *Hydrilla verticilata*, a submerged aquatic plant for arsenic uptake from drinking water was utilized successfully. To estimate the removal efficacy of arsenic and to determine the nature of reaction studies with various parameters viz. pH, sorbent dose, contact time, initial metal ion concentration, and temperature were carried out and data obtained were utilized to plot Lagergren graph along with pseudo-second-order graphs for kinetic studies. The study showed 96.35 % maximum absorption of arsenic by *H. verticilata* at initial concentration of 100 ppb with 0.5 g of biomass/100 ml for 5 h contact time at pH 6.0 with 150-rpm agitation rate. Data followed Langmuir isotherm showing sorption to be monolayer on homogeneous surface of biosorbent. The

negative values of $\Delta G°$ indicated spontaneous nature; whereas $\Delta H°$ indicates exothermic nature of system and negative value of $\Delta S°$ entropy change correspond to a decrease in the degree of freedom to the adsorbed species followed by pseudo-second-order adsorption kinetics. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) have been performed for characterization of metals on biomass. FTIR and SEM results showed apparent changes in functional group regions after metal chelation and the changes in surface morphology of biosorbent. This is a comparatively more effective, economic, easily available, and environmentally safe source for arsenic uptake from solution due to its high biosorption efficacy than other biosorbents already used.

Nigam et al.; Environmental Science and Pollution Research International; 2013; 20; 4000-4008

Extractability and phytotoxicity of heavy metals present in petrochemical industry sludge

Bioavailability of heavy metals present in industrial sludges and their subsequent phytotoxicity are crucial parameters to assess the associated health hazards and suitability for land application. The present study is an effort to determine the extractability of heavy metals present in different phases of the sludges, coming out of two different operations involved in petrochemical industry viz. spent caustic treatment (SCT) and waste water treatment (WWT) following the BCR sequential extraction procedure. The maximum amount of Cd and Cu was found associated with oxidizable phase, whereas Cr and Ni were best recovered in residual fractions of both the sludges. Maximum Pb was recovered in oxidizable and residual phase in the WWT and SCT sludges, respectively. The stabilization treatment undergone by sludges strongly influenced the heavy metal distribution and the phases to which they were associated. The total metal concentration in both the sludges did not exceed the limit set out by the European Legislation and was found as Cd = 0.449, Pb = 3.340, Ni = 6.530, Cr = 21.087, & Cu = 27.129 $\mu g\ g^{-1}$ and Cd = 0.549, Pb = 5.664, Ni = 7.161, Cr = 27.096, & Cu = 35.479 $\mu g\ g^{-1}$ in the SCT and WWT sludges, respectively. Phytotoxicity of the sludges was assessed against the germination index and the relative root and shoot growth. Sludge leachates did not adversely affect the seed germination and the early seedling growth of Mung (*Phaseolus mungo*) and Gram (*Cicer arietinum*), indicating that these metals were concentrated in the non-bioavailable fractions of sludges.

Kumar et al.; Clean Technologies Environmental Policy; 2012; doi 10.1007/s10098-012-0559-1