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Persistent organic pollutants: potential threat to ecological integrities in term of genotoxicity and oxidative stress

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Abstract

As a consequence of both increasing population and industrialization in agro-economic sector, Pakistan has inevitably confronted by multi-complex environmental challenges. Owing in part to poor regulatory framework, pollution due to persistent organic pollutants (POPs) has caused serious problems throughout the country. Resultantly, extensive use of POPs is causing vigorous deterioration of environment and human health. The current study addresses: (1)
sources and distribution patterns of various POPs classes in environmental compartments of Pakistan; (2) their respective occurrence in living organisms; and (3) the general information on associated ecological effects and toxicity assessment by meta-analysis for local fauna and flora. Based on the study, it can be concluded that the environment of Pakistan is highly contaminated by organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dechlorane plus (DP), and polychlorinated naphthalenes (PCNs) which is further supported with the meta-analysis. Nevertheless, unavailability of environmental quality standards and food safety for POPs render it a forthcoming challenge of multi-compartment toxicity exposure. Therefore, strategies must be planned for risk assessment of biologically active POPs while the POP waste inventory should be elevated, along with the necessary measures to promote appropriate handling and treatment of POP as a matter of prime importance.

**Keywords**

*Ecological risk assessment, Geno-toxicity, POPs*
1. Introduction

Twentieth century has witnessed unprecedented advances in industry based economy. Modern industry involves a variety of synthetic chemicals to be used in manufacture of everyday products including pesticides, food, dyes, cosmetics, medicines, etc. (Arslan et al., 2015). Although, many of these chemicals have been proved to be beneficial, however, studies have reported their harmful effects on living organisms and the environment. Among those, contamination of environment with persistent organic pollutants (POPs) is of great concern. POPs are known for their ability to persist in the environment due to long half-lives (Keithmaleesatti et al., 2007), transport for an extensive range in the environment (Park et al., 2011) and bio-accumulation in the food web (Nakata et al., 2002). Their significance in the environment was first reported in 1960’s by Racheal Carson’s landmark book “Silent Spring”; highlighting premature death of birds due to dichlorodiphenyltrichloroethane (DDT) contaminated food (Carson et al., 1962). Soon after, several studies recognized them as major threat to ecology at each trophic level of the food web (Radomski et al., 1968; Wurster et al., 1965; Innes et al., 1969).

During 1960s to 1990s, developments in analytical procedures made it easier to analyze the variety of POPs in different compartments of the environment (de Boer and Fiedler, 2013). As a result, in 1995, United Nations Environmental Program’s (UNEP) Governing Council suggested remedial action to address the presence of POPs as a risk to human health and the environment (Jennings and Li, 2015). The regulation was step forwarded by the Stockholm Convention on Persistent Organic Pollutants in 2001, in which toxicological impacts of the POPs
were recognized. This international treaty aimed to eliminate or at least restrict the production and usage of POPs, along with phasing out of POPs containing commercial products/chemicals. The Convention came into force in 2004, ninety days after it has been ratified by fifty signatory nations. As of 2014, the Convention has 179 Parties, including Pakistan (Ahad et al., 2010; Fiedler et al., 2013; Alamdar et al., 2014; Ahlgren, 2014).

Initially, the convention banned twelve POPs, referred as “dirty dozen”, due to their persistence and toxicity to the biotic elements of the ecosystem. These compounds can be categorized into three groups on the basis of their usage, i.e., a) organochlorine pesticides (OCPs) (aldrin, dieldrin, chlordane, endrin, heptachlor, DDT, hexachlorobenzene (HCB), mirex, toxaphene); b) industrial chemicals (HCB, polychlorinated biphenyls (PCBs)); and c) unintended by-products (HCB; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs) (Johansen, 2003, Xu et al. 2013; Haffner and Schecter, 2014). In later years, the list did not remain limited to 12 compounds as in May 2009, the Conference of the Parties (COP) to the Stockholm Convention added nine new compounds at its fourth meeting (i.e., i) α-hexachlorocyclohexane, ii) β-hexachlorocyclohexane, iii) chlordecone, iv) hexabromobiphenyl, v) hexa- and hepta- bromodiphenyl ether, vi) lindane (gamma-hexachlorocyclohexane), vii) perfluorooctane sulfonic acid and perfluoroctane sulfonyl fluoride, viii) pentachlorobenzene, ix) tetrabromodiphenyl ether and pentabromodiphenyl ether) to the list. In 2011 and 2013, the COP at its fifth and sixth meeting respectively, added two new compounds (i.e. Endosulfan and Hexabromocyclododecane) to the POPs list; summarizing twenty three compounds as of today (UNEP, 2009a; UNEP, 2015a). The usage, toxicity and persistence of POPs are listed in Table 1.
The POPs which have been mainly produced synthetically and are released into environment through anthropogenic activities are accounted to be associated with adverse impacts to the biota (Koč et al., 2007; Burger et al. 2007; Dickhut et al., 2009). The exposure of POPs to living organisms, primarily to humans have been reported to cause multiple acute and/or chronic reproductive, carcinogenic, neurotoxic, pathological and immunological problems (Yamashita et al. 1993; Shaw et al., 2006; Kalyoncu et al., 2009; Sharma et al., 2009). They may also cause endocrinal disruption in wildlife and humans (Tanabe et al., 2002; Letcher et al., 2010).

Despite of the fact that Pakistan became signatory of this convention in 2001; however, still there is a lack of precise management and legislation plan to monitor and control the POPs level in various environmental sectors of the country (Malik et al. 2011a; Eqani et al., 2012a). Moreover, to date, these compounds have not been even incorporated to the NEQS (National Environmental Quality Standards) hence reflecting unavailability of the standards at local context (Eqani et al., 2012a). Currently, only few studies associated with the ecotoxicological aspect of POPs in Pakistan are available (Malik et al., 2011a,b; Ali et al., 2013b; 2014; Mahmood et al., 2014b, 2014d, 2014e, 2015b; Mumtaz et al., 2015; 2016; Eqani et al., 2015; Robinson et al., 2016). Therefore, this review compiles the current state of knowledge on POPs level in Pakistan along with their knock on effects on ecosystem. Moreover, we conclude the paper with some recommendations and research needed in this particular field in Pakistan.
2. Toxic impact of POPs on ecological integrities

While, writing about potential risks of POPs to local ecology, we would like to clarify that there are only limited studies available on this topic in the region (SI Table 2-9). However, the chemical nature of POPs makes it inevitable to consider all possible risk pathways that can cause harm to environmental elements and thereby can be speculated in all ways considering examples of other countries. As many of the POPs are biologically active in nature, their presence may cause harmful effect to living organisms (Ankley et al., 2007; Corcoran et al., 2010). The harmful effects of POPs are not limited to target organisms but they also affect non-target populations. Most of them raise extensive toxicological concerns, particularly when bind with other pollutants and form complex mixtures. This can exert unwanted effects on exposed populations leading to the physiological, biochemical, behavioral and developmental stress responses in different trophic levels of food web (Beketov et al. 2013). Recent studies have measured the extent of harmful effects in an ecosystem based on three main factors namely pollutant toxicity, concentration, and exposure period, i.e., acute (short term) or chronic (long term). Matrix of these factors result into different levels of harmful effects, for example, chronic exposure at low concentration and acute exposures at high concentration may cause significant damages to exposed fauna and flora. However, acute toxicity at low concentration is less likely to cause any significant impact (Wu et al., 2008a). To date, different studies have been conducted to elucidate both acute and chronic effects of variety of POPs (Gregoraszczuk and Ptak, 2013; Byer et al., 2015). The chronic effects of POPs toxicity are widely acknowledged and being investigated at large still in modern world (Ibrahim et al., 2011; Bussolario et al., 2012; McLeod et al., 2014; Pinzone et al., 2015; Elliott et al., 2015). It is reported that insulin
resistance, obesity and endocrine disruption is greatly associated with chronic exposure of POPs in humans (Ibrahim et al., 2011; Gregoraszczuk and Ptak, 2013). On the other hand, acute toxicity is mostly reported from in vivo studies performed on captive animals, nevertheless renders difficult to generalize the possible adverse effects at lower concentrations (Letcher et al., 2010).

2.1. Toxicity and resulting oxidative stress mechanism in plants

Plants are the single most important biotic factor of earth ecosystem, for they serve as host to many other biotic factors (microbes and arthropods), and also are the most important source of food, fiber and shelter (Blomqvist et al., 2000; Grime, 2006). Plants are the source of raw material for the most important economic sectors on the planet such as agriculture, pharmaceutical, textile and food industry. Hence, a slight toxic effect to plants resulting in deteriorated quality or reduction in yield can have drastic consequences. It can literally wreak havoc to the food supply and industrial processes world over (Liener, 2012). Therefore, phytotoxicity of any level from anything must be taken very serious, and strategies must be devised in advance to avoid or minimize potential risks (Kabata-Pendias, 2010). Moreover, some of the POPs have log $K_{ow}$ values lower than 3.5 and, therefore, are readily taken up by the aquatic and terrestrial plants rendering their entry in the food web (Table 1) (Yebra-Pimentel et al., 2015).

The POPs can impart negative effects from a variety of reasons. Firstly, the toxicity to plants begins at germination stage as many of the pollutants directly affect the plant embryo by altering the growth conditions (Pereira and Macías, 2010). Secondly, if the embryo is able to
survive, presence of pollutants inhibits plant growth by affecting several biochemical pathways such as chlorophyll synthesis, enzymatic reactions, plant-microbe interactions, etc. One reason behind these inhibitions is the disturbance of cell redox status due to production of reactive oxygen species (ROS) (Brame et al., 2014). The presence of ROS results into major changes in electron transport chain particularly in the chloroplast and mitochondrial membranes leading to dismantling of plasma membrane, ion leakage, chlorophyll synthesis inhibition, biological molecules degradation, lipid peroxidation and DNA-strand cleavage (Carrasco-Gil et al., 2011).

Thirdly, they may accumulate within the plant’s tissues and organs, for example, in consistence with literature, Kacálková and Tlustoš (2011) reported uptake and accumulation of PCB congeners from soil to maize (*Zea mays* L.), sunflower (*Helianthus annuus*), poplar (*Populus nigra x P. maximowiczii*) and willow (*Salix x smithiana*) in POPs contaminated site. As a result, the chronic exposure of POPs can deteriorate the plant health. Nevertheless, extent of phytotoxicology also depends on plant species in addition to the nature of compound.

As far as plants are influenced by any radical change in their host environment, the host environment is also influenced by any radical change in the physical, physiological or biological properties of plants they are hosting (Orcutt and Nilsen, 2000; Wardle et al., 2004). One reason behind this toxicity is due to direct effects of carcinogenic nature of ROS. Wettasinghe and Shahidi (2000) have reported that ROS play a very important role in the health of higher animals including human beings as the occurrence of ROS in foods is inevitable due to the biological nature of foods (Choe and Min, 2005; Wettasinghe and Shahidi, 2000). In humans, ROS may interact with proteins, lipids, sugars, and vitamins resulting into the production of undesirable volatile compounds, destruction of essential fatty acids, amino acids and vitamins, and formation
of carcinogens. Moreover, ROS can change the functionalities of proteins, lipids, and carbohydrates by forming oxidized dimers and trimers rendering harmful effects to consumers (Chi et al., 2013). In Pakistan, in context to plants, only a few studies have been conducted to assess the levels of POPs in two major cereal crops, i.e. wheat and rice in the region (SI Table 2-5).

2.2. Toxicity to animals

Besides, direct effects of POPs on fauna has also been widely reported extensively especially on microorganisms, nematodes, insects, rodents, birds and higher animals. The POPs may have direct or indirect effects on fauna species as well. Environmental disturbances due to their persistence constitute a threat to the fauna populations. The avifauna population declines have been reported in past studies (Elliott and Harris, 2002; Bonier et al., 2007), characterized with reproductive failure due to thinning of eggshell, poor hatchability and chick survival due to POPs acute exposure (Senthilkumar et al., 2002). Although in past few decades, the POPs production and usage have been started slowly disappearing from the global environment (Elliott and Elliott, 2013), however their levels remained amply high to limit the birds of prey colonization in some ecosystems (Elliott et al., 2005). In Pakistan, three studies have been conducted to assess the POPs levels in relation with the colonial water birds eggs, prey and feathers (SI Table 6-9).

Contamination of the aquatic ecosystem with POPs may occur from direct discharge of waste and wastewater into rivers, lakes and coastal areas (Eqani et al., 2012a,b). POPs from various sources may also enter the aquatic ecosystem via atmospheric deposition (Eqani et al., 2014).
Due to POPs atmospheric transportation without being degraded, many of the POPs have even found in the polar fauna of Arctic (Kumar et al., 2002; Andersen et al., 2015). The low solubility of POPs in water tends to accumulate them in fatty tissues of aquatic species, particularly in fish (Eqani et al., 2015). The contaminated fish consumption by human is an important route for the POPs transfer to human in developing countries (Moon et al., 2009; Adu-Kumi et al., 2010; Mezzetta et al., 2011). Though it accounts only 10% of the human diet yet it is a vital source responsible for 90% of human’s POPs exposure (Liem et al., 2000). POPs are toxic to human health due to their high lipophilic properties (Wong et al., 2005). At the same time, some POPs may biomagnify up the food chains, by a factor typically between 2 and 10, even sometimes by a factor as high as 100 (Leonards et al., 2008) and occur in greatest concentrations in top level of the food chain (Elliott and Elliott, 2013). Hence, the POPs in the environment may lead to bioaccumulation and potential negative effects in multiple levels of the foodweb.

Secondly, the POPs may be broken down (and/ or reassembled) into more toxic metabolites. For example, two relative recent metabolites of PBDEs, i.e. Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) methoxylated polybrominated diphenyl ethers (MeO-PBDEs) are more toxic than parent PBDEs (Legradi et al., 2014). Similarly, the hydroxylated metabolites of PCBs (OH-PCBs) are more toxic than PCBs (Soechitram et al., 2004; Berghuis et al., 2014). Moreover, the toxicity index was found higher in the chronic experiments. This is not the only case; as sometimes the respective metabolites are more concentrated than the parent compound. In Pakistan, limited number of studies has been conducted to report the level of POPs in the riverine fish species (SI Table 8-9), human serum (Table 2) and pet samples (Table 3).
2.3. Geno-toxic behaviors of POPs

Interestingly, harmful effects of POPs are not limited to physiological changes in community structure but it may also lead to genetic modifications. It has been established that chronic exposure of highly toxic pollutant at higher concentration could be highly genotoxic and hence can cause extinction of species at much shorter time. This could be due to the loss of genotypes determining a necessary trait(s) (Ribeiro and Lopes, 2013). Recently, the phenomenon has been recognized as genetic erosion hypothesis (Hoffmann and Willi, 2008). Many studies have reported chemical induced genetic erosion in both laboratory and field exposed organisms (Dixon et al., 2007). For example, Nowak et al. (2008) reported that the tributyltin exposure resulted into rapid decrease in genetic diversity of chironomid populations in the laboratory experiments. Furthermore, this genetic erosion could happen to both somatic and germinal cells hence, harmful effects may appear in the same generation or in trans-generations, respectively. In case of somatic cells genotoxicity, physio-pathological changes such as impairment of vital metabolic function may result into reduction of average fitness of the affected group whereas, germinal toxicity can pass detrimental damage to the next generation (Kurelec, 1993). In a laboratory experiment, Hebert and Murdoch (1998) illustrated that the damages in germinal cells are passed on to the next generation with detrimental effects on fitness due to non-viable offspring. The germinal toxicity may also cause infertility or reduced fertility resulting into disappearance of the species at large (Craig et al., 2011; Lewis and Ford 2012). For instance, the chronic exposure of organochlorine pesticide methoxychlor may lead to ovarian toxicity and hence adverse reproductive outcomes (Wang et al., 2010). Similarly, Joshi (2007) reported that the higher concentration of Chlorpyrifos causes significant reduction in epididymal and testicular
sperm counts with decrease in serum testosterone concentration and reduced fertility, i.e. 85%. Nevertheless, no study available on Pakistan’s context highlighting genetic toxicity to any of the living organisms but the nature of POPs makes it inevitable to speculate genetic erosion leading to micro-evolution in smaller invertebrates to higher organisms including plants.

3. **Meta-analysis; toxicity assessment**

Besides qualitative assessments, POPs toxicity to local fauna and flora is also evaluated using a meta-analysis approach; after comparing with the available standards (e.g., Punjab food standards). The meta-analysis revealed that mean concentration of DDT in the cereal crops varied significantly with respect to the standards; while concentration of heptachlor and chlordanes did not vary significantly which suggest their lesser toxicity respectively. Similarly, animal flesh, HCH concentration appeared to have higher significance for toxicity whereas DDT concentration were not significant here. This initial assessment emphasizes the importance of cross-validating management recommendations in toxicity assessment along with the development of local standards for other group of pollutants.

4. **POPs detected from the environmental matrixes of Pakistan and their probable impacts**

4.1. **Organochlorine (OCs) contaminants**

The organochlorine (OCs) contaminants are of major concern among all of the POPs classes. The records from past two decades indicate that their usage have been declined significantly from the developed countries; however, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are still used in the developing countries, including Pakistan,
mainly due to their low cost and versatility in industrial sector (Zhang et al., 2013; Eqani et al., 2012a; Syed and Malik 2011; Eqani et al., 2011; Tariq et al., 2007). The fate of OCs in the environment is governed by their physicochemical properties and regional climatic conditions, including events of high precipitation, wind storms, and temperatures (Farooq et al., 2011). The two most common organochlorine (OCs) contaminants that have been even widely reported in environmental matrixes of Pakistan are organochlorine pesticides and polychlorinated biphenyl (SI Table 1-8).

4.1.1. Organochlorine pesticides (OCPs)

The organochlorine pesticides (OCPs) are non-polar and semi-volatile chemical compounds mainly comprising of carbon, hydrogen, and chlorine (Idowu et al., 2013). Alike other POPs, these compounds are of global concern due to their persistence, bioaccumulation, and adverse effects on the ecosystem (Parween et al., 2014). The OCPs can be categorized into four distinct classes: a) diphenyl alephatic (e.g. DDT, dicofol), b) cyclodienes (e.g. heptachlor, dieldrin), c) cyclohexanes (e.g. hexachlorocyclohexane [HCH]), and d) chlorinated benzenes (e.g. hexachlorobenzene [HCB]) (Rashid, 2011).

Varieties of OCPs were extensively used worldwide during 1950s to the mid-1980s especially in countries with agricultural based economy (Maurya and Kumar, 2013). Approximately 70% of the population in Pakistan is directly or indirectly associated with agriculture, hence, consumption rate of agrochemicals along with spraying practices was accordingly increased (Tariq et al., 2004; Azmi et al., 2006; Sultana et al., 2014). Temporal survey elucidates that, in Pakistan, the usage of agro-pesticides in 1954 was 254 metric tons per
year which was later raised to 16,226 metric tons per year in 1976-77, and ultimately reached to 78,132 metric tons per year in 2003 (Syed and Malik, 2011). This boom was attributed to the transfer of pesticide import and distribution industry from the public sector to private sector, leading to local production of OCPs such as DDT and HCH (Malik et al., 2011a). The pesticides consumption in Pakistan is increasing with an annual rate of 6% which is further expected to increase manifolds in the upcoming years (WWF, 2007; Ministry of Finance, 2015).

Over the last five decades, OCPs usage in Pakistan was not limited to the agricultural activities only (Eqani et al., 2012a;2012b);, but their application can also be seen extensively in control of insect-borne epidemics, domestic pests and termites (Malik et al., 2011a). Principally, only 1% of the applied pesticides remained effective to reach its target pest whereas ~99% of the compound escaped, being persistent, to non-target environment through surface runoff, spray carry-over, or residue retention on food (Miller, 2004; Zhang et al., 2011). Resultantly, a significant content of the OCPs was released into the environmental compartments whose presence is reported by different studies (SI Table 10-12).

Another important source of the OCPs in the environmental matrices of Pakistan is direct dumping of thousands of kilograms of OCPs, particularly DDT, within or around the vicinity of demolished factories of OCPs in different areas of the country (Eqani et al., 2012a). Although OCPs were initially imported from Europe; however, to meet the high demand of DDTs and other OCPs, significant amount of OCPs were produced locally in different regions of the country (e.g., Nowshera (Khyber Pakhtunkhwa), Lahore and Kala Shah Kaku (Punjab), and Karachi (Sindh)) (Tariq et al. 2007). However, the banning of DDT production and usage after
Stockholm Convention resulted into decommissioning of many of the units in Pakistan, finally lead to dumping in massive quantities in the surrounding of the units in the absence of any appropriate legislation (Eqani et al., 2012a). Since then, the pollutants inhabit in Pakistan’s soil render one of the major source of bioaccumulation in the food web (discussed later).

Currently, Pakistan holds one of the largest stock of obsolete pesticides, chemical storages, demolished factories and dumping sites in the populated cities (Jan et al., 2009; Syed and Malik, 2011). The obsolete pesticides are estimated to be 128 tonnes in Baluchistan, 179 tonnes in KPK, 2016 tonnes in Sindh, 3805 tonnes in Punjab, and 178 tonnes in the possession of Federal Department of Plant Protection (Ahad et al., 2010). These outdated pesticides contain thousands of kilograms of DDT in stockpiles located in different provinces of the country (Jan et al., 2009).

The improper storage due to damaged and/or corroded containers, rotten sacks and rough handling of obsolete pesticides is another source of OCPs in the environment. It may lead to leaking out of the compounds into various environmental media such as water, sediments, and biotic life (Eqani et al., 2011). However, to date no policy has been developed to prevent the illegal practice and unsafe storage of the banned POPs (Ahad et al., 2010). The severe contamination in the neighborhood of such storage facilities and dumping sites has reported in different areas of Pakistan (Ahad et al. 2010; Syed and Malik 2011).

For an instance, Ahad et al. (2010) reported level of OCPs in soil and water around obsolete pesticides stores from various areas across Pakistan, including Nowshera, Bunair, D.I. Khan, Faisalabad, Multan, Lodhran, Ahmadpur Sharqia, Sahiwal, Kalashakaku, Wazirabad,
Simriyal, Karachi, Hyderabad, Nawabshah, Dadu, Larkana, Khairpur, and Nara. Similarly, OCPs concentrations in surface soils from obsolete pesticide burial ground and its surrounding were reported from Hyderabad (Alamdar et al., 2014) and Kalashah Kaku (Syed and Malik 2011; Syed et al., 2014a) areas. However, in Pakistan, the mass application of the OCPs in recent decades has resulted in the contamination of all the environmental compartments including water, air, soil and biota (Eqani et al., 2012a). Alike other parts of world, several studies in Pakistan have reported the contamination of different compartments of the environment with OCPs (SI Table 10-12).

4.2. Polychlorinated biphenyls (PCBs)

The polychlorinated biphenyls (PCBs) are a class of semi-volatile, chlorinated POPs that enters into environment as a consequence of anthropogenic activities (Wimmerová et al., 2011). They are classified into 209 congeners which can be divided into two distinct groups according to their toxicological behavior; (i) dioxin-like PCBs (DL-PCBs) and (ii) non dioxin-like PCBs (NDL-PCBs) (Herceg-Romanic’ et al., 2014). The DL-PCBs are considered to be most toxic to ecosystem among the 209 PCB congeners (Arslan-Alaton and Olmez-Hanci, 2013).

Due to their dielectric, thermal stable, and oxidation resistance properties, PCBs have been extensively used in manufacturing of transformers, circuit breakers, voltage regulators, capacitors, plastics, lubricants, pigments/paints, adhesives, and other metallic coating compounds (EPA, 2004; Park, 2000). World Health Organization (1993) estimated about 1.2 million metric tons (MT) production of PCBs worldwide during 1929--1977. In Pakistan, Eqani et al. (2012a) reported a total demand of 4000 MT/year of PCBs as a coolant and insulator in transformer oil
only, whereas the quantity of used transformer oil collected is at least 1500 MT/year. Therefore, release of PCBs from manufacturing and repairing units of electrical devices, particularly the transformer oil leakage while its repair, transportation, and storage of old transformers and electrical wastes are the major sources for its contamination in Pakistan (Eqani et al., 2013; 2014). Besides, open burning of e-wastes and untreated and uncontrolled disposal of industrial wastewater to the streams and rivers are another major sources of PCBs (Mahmood et al., 2014c). The problem is of serious concern to riverine ecosystem as rivers are considered to be easy disposal points for effluents in developing countries (Olayinka, 2004). The low water solubility and strong octanol--water partition coefficient of PCBs leads to the strong affinity to suspended particulate matter in the aqueous environment, ultimately accumulating in the bottom sediments and thus bioconcentration in benthic communities (Wei et al., 2008). In Pakistan, like OCPs and other POPs, no specific regulatory mechanism exists to monitor and control PCBs in various environmental matrices. However, few reports are available on the levels of PCB in different environment compartments in Pakistan (SI Table 13-16)

4.3. Polybrominated diphenyl ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are class of recalcitrant, semi-volatile and bioaccumulative halogenated (brominated) compounds. They share some properties with DDT, PCBs and their metabolites, including structural, environmental persistence, lipophilic and bioaccumulative nature, and toxicity (Wilford et al., 2004; Stapleton et al., 2006). Since 1960s, PBDEs have been used extensively as additive flame retardants in variety of polymer based industrial products such as electronic goods, foams, textiles, building materials, plastic, and
vehicles to prevent the fire spread (Wilford et al., 2004; Syed et al., 2013b). During past few decades, to raise the safety standards, demand of PBDEs have been increased significantly in industrial and domestic activities due to intensification in use of plastic and electronic components (Malik et al., 2011 b).

Though PBDEs have saved many lives and millions in property damage however they have been used without evaluating their health and environmental impacts leading to high levels of human exposure (Besis and Samara, 2012). It is estimated that up to 15% PBDEs is integrated in plastic and up to 30% PBDEs in polyurethane foam (WHO, 1994). Although the PBDEs are mixed into foams and plastics nevertheless they do not form chemical bonding, making them much more vulnerable to escape out of goods and products (Sjödin et al., 2003).

In past, three mixtures forms of PBDEs were commercially available worldwide viz; decabromodiphenyl ether (deca-BDE), pentabromodiphenyl ether (penta-BDE) and octabromodiphenyl ether (octa-BDE) (La Guardia et al., 2006). The environmental and human health concerns associated with them are of different nature namely carcinogenic, neurotoxicological, and endocrine disruption effects (Malik et al. 2011b). In 2009, the penta-BDE and octa- BDE have been added to the Annex A of Stockholm Convention on Persistent Organic Pollutants, with restriction to eliminate the production and usage of these chemicals (UNEP, 2009b). However, the formulations of deca-BDEs are still in use in most parts of the world (Malik et al. 2011b; Mahmood et al., 2015a). According to the Bromine Science Environmental Forum (BSEF), approximately 5000 metric tonnes of deca-BDE worldwide was sold in 2012 (BSEF, 2014; Mackintosh et al., 2015).
Moreover, despite of the fact that the use and production of the penta-BDE and octa-BDE have been restricted worldwide, their long historical usage in consumer products, of which numerous are still in use, is indicative of continued leaching into the environment (Kwan et al., 2013). In developing countries including China, India, Pakistan, Vietnam and the Philippines, the conditions are worst as they received about 50-80% of the e-waste generated in industrialized countries for recycling (UNEP, 2005). Consequently, recycling in the world inventory is a major ongoing source of PBDEs in developing countries (Bi et al., 2007; Wong et al., 2007). In Pakistan, the levels of PBDEs in various environmental matrixes, excluding the biosphere (discussed later) have been reported in last few years (SI Table 17). To date, only seven publications have reported the levels of PBDEs from surface water (Mahmood et al., 2015a), air (Mahmood et al., 2015b; Syed et al., 2013b; Ali et al., 2015b), surface sediments (Mahmood et al., 2015a; Ali et al., 2016a; Malik et al., 2014; Syed et al., 2013b) and soil (Mahmood et al., 2015b; Syed et al., 2013b; Ali et al., 2015b; Zehra et al., 2015).

4.4. Dechlorane plus (DP)

Dechlorane plus (DP), a highly chlorinated flame retardant was manufactured since mid-1960s as a substitute for Dechlorane (also called Mirex) (Kang et al., 2010). With the ban on the production and usage of PBDEs, the non-regulated flame retardants including DP substitutes them in most of the commercial products.

As an additive flame retardant, DP has been extensively used in household and industrial products. Its major application is in polymer materials that in terms of percentage composition ranges from 10% to 35% in commercial polymer products (Xian et al., 2011). The DP is mostly
used in electric cables and cable/wire coatings, electric plastic connectors in television and monitors, polymer roofing materials and furniture, and other polymeric systems (Betts, 2006; Qiu et al., 2007). The estimated global production of DP approximates about 5000 tonnes per annum (Ren et al., 2008). Therefore, DP may leach from these products and contaminate the surroundings.

The commercial formulation of DP contains two stereoisomers, i.e. syn-DP and anti-DP that constitute in the ratio of 1:3 (Luo et al., 2013). Currently, the DP and its analogs have bioaccumulation potential so may threaten the aquatic food webs (discussed later). The predicted bioaccumulation factor values for DP and its analog in fish exceeds bioaccumulation factor criteria set in the Annex D of Stockholm Convention, i.e. > 5000 (UNEP, 2001). Therefore, these chemicals are the contenders for Annex D evaluation under the United Nations Stockholm Convention on Persistent Organic Pollutants (Sverko et al., 2011).

In 2006, the pioneer detection of DP in Great Lakes Basin was milestone in environmental monitoring (Hoh et al., 2006). Though DP has been used for last 40 years, but still a little attention has been paid for its detection in different environmental compartments (Sverko et al., 2011). However, the DP has become a ubiquitous contaminant in all compartments of environment, including the biota (Sverko et al., 2011). Only few studies in Asia have reported the DP contamination in the environment, most of these studies are from China, where DP in air (Ren et al., 2008; Ma et al., 2009, 2011; Yu et al., 2011), soils (Wang et al., 2010; Yu et al., 2010; Ma et al., 2011) and human serum (Ren et al., 2009) has been detected. In Pakistan, to date, only five published studies detected the levels of DP in surface water (Mahmood et al.,
2015a), surface sediments (Ali et al., 2016a; Mahmood et al., 2015a; Syed et al., 2013b), soil and air (Ali et al., 2015b; Mahmood et al., 2015b; Syed et al., 2013b) (SI Table 18). The studies have reported leaching of DPs from burning, dumping and recycling of e-waste and uncontrolled industrial expansion as major cause of high level of this class of POPs (Ali et al., 2015b; Mahmood et al., 2015a). Thus, it is vital to pay significant attention to determine the levels, risk assessments and environmental fate of DP and its analogs.

4.5. Polychlorinated naphthalenes (PCNs)

The polychlorinated naphthalenes (PCNs) are other extensively concerned environmental contaminants due to their high persistency, bioaccumulative properties, and potent toxic in nature (Lee et al., 2007; Mahmood et al. 2014e). Since their detection from Arctic, the PCNs has gained aggressive anxiety in the environmental concerns (Lerche et al., 2002). In 1998, PCNs were considered as the contenders of POPs by United Nations Economic commission for Europe (UNECE) (Lerche et al., 2002) while in 2013, the Persistent Organic Pollutants Review Committee proposed di-,tri-,tetra-,penta-,hexa-, hepta- and octa-chlorinated naphthalenes to be included in Annex A and Annex C of Stockholm Convention on POPs. In May 2015, at the seventh meeting of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants, a decision is made to add polychlorinated napthalenes (PCNs) to the list of banned substances, i.e. Annex A (UNEP, 2015b).

The PCNs commercial production has been started about 100 years ago (Hayward, 1998), predominantly during the period of 1910--1970 and have been used in various industrial applications due to their best dielectric properties as well as low reactivity (Hanari et al., 2013).
The historical application of PCNs was as an insulator and coolant for thermal stability, combustion processes like metal refining and incineration, wood and coal burning, and various PCB associated applications may discharge PCN into the environment (Lee et al., 2007; Hogarh et al., 2012).

Industrial discharges from chloralkali industry, waste incineration units, copper smelters and magnesium production units are mainly responsible for PCNs emission (Kannan et al., 1998; Hanari et al., 2013). Coal combustion is considered to be another major source of PCNs emission (Orlikowska et al., 2009). In South Asia, in particular India and Pakistan, the coal consumption rate is very high with India globally at third rank in the terms of coal consumption (Xu et al., 2014). During the monsoon outbreaks, PCNs emitted by coal combustion are even transferred to the far regions (Mahmood et al., 2014e). Very little is known about the PCNs levels in Pakistan. Only three studies from two different area of Pakistan, i.e. River Chenab and its tributaries (Mahmood et al., 2014e; 2014f), Punjab Province (Xu et al., 2014) and Indus River Basin (Ali et al., 2016b) has reported the levels of PCNs in various environmental matrixes (SI Table 19). The studies from Pakistan have reported PCN levels in air significantly higher than in other sites worldwide and comparable to those reported from Asia (Xu et al., 2014). However, the PCNs levels in soil were comparable to those previously published from elsewhere (Mahmood et al., 2014f). The emission from industrial sources and biomass burning and re-emissions from various halowaxes are possible sources of PCN pollution in region (Xu et al., 2014; Mahmood et al., 2014e; 2014f; Ali et al., 2016b).

5. Conclusions
At present, the relevance of POPs and their impact on local ecosystems of Pakistan can be addressed by developing inventory of POPs in a given host environment, studying the interactions and fate discrimination in the ecosystems, and assessment of their functioning in plant/water/soil system. Moreover, the development and implementation of action plans for POPs, encompassing the monitoring programme, and national standards are of prime importance. The inventory of information primarily illustrating persistence, ecotoxicity, and endocrine disruption potential both individually and in complex compounds may help to resolve the threats beforehand. Their missing legacy and lack of standards for instruments should be devised at local context especially when the pollutants are overwhelmingly present. The measuring and monitoring of pollutant concentrations at the emission source within the environmental compartments and in the bodies of living organisms should also be given a prime importance. Online monitoring should be developed by authorities at sensitive locations where necessary otherwise the introduction of passive sampling procedures may also help to consider better monitoring especially when they are newly introduced to the environment. Besides, understanding of the ecosystem damages due to chronic exposure of POPs should be done at two or three tier levels in order to understand the effects on local ecology.
6. References


of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. PLoS ONE, 6, e25170.


Park, J.S., 2000. Atmospheric deposition of PAHs, PCBs, and pesticides to the South Texas Bays, Galveston Bay and Corpus Christy Bay. Ph.D. dissertation, Texas A & M University, College Station, TX, USA.


meeting (UNEP/POPS/COP.7/36). Published by the Secretariat of the Stockholm Convention on Persistent Organic Pollutants; Geneva.


Table 1. List of POPs under Stockholm Convention including their usage, toxicity, and persistence

<table>
<thead>
<tr>
<th>Group name</th>
<th>Generic name</th>
<th>Usage</th>
<th>Toxicity (LD$_{50}$)</th>
<th>Log $K_{ow}$</th>
<th>Persistence (half-life)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organo-Chlorine</td>
<td>Aldrin*</td>
<td>Insecticide</td>
<td>39 mg/kg</td>
<td>5.52</td>
<td>5 years</td>
<td>(Garten et al., 1983)</td>
</tr>
<tr>
<td>Pesticides</td>
<td>Dielhrn*</td>
<td>Insecticide</td>
<td>49 mg/kg</td>
<td>5.48</td>
<td>5</td>
<td>(Mackay, 1982)</td>
</tr>
<tr>
<td></td>
<td>Chlordane*</td>
<td>Pesticide</td>
<td>83--590 mg/kg</td>
<td>5.66, 5.62, 5.44</td>
<td>1--3 years</td>
<td>(Simpson et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>DDT*</td>
<td>Pesticide</td>
<td>113--800 mg/kg</td>
<td>6.2</td>
<td>2--15 years</td>
<td>(Weyens et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Endrin*</td>
<td>Pesticide</td>
<td>43.4 mg/kg</td>
<td>4.71</td>
<td>12--15 years</td>
<td>(Finizio et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>Heptachlor*</td>
<td>Pesticide</td>
<td>40--162 mg/kg</td>
<td>6.10</td>
<td>Up to 2 years</td>
<td>(Simpson et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Mirex*</td>
<td>Pesticide</td>
<td>740 mg/kg</td>
<td>6.89</td>
<td>Up to 10 years</td>
<td>(Mackay, 1982)</td>
</tr>
<tr>
<td></td>
<td>Classification</td>
<td>Concentration</td>
<td>Degradation half-life</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Toxaphene*</td>
<td>Pesticide</td>
<td>80--293 mg/kg</td>
<td>4.77 to 6.64</td>
<td>(Fisk et al., 1999)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCB**</td>
<td>Fungicide</td>
<td>19--245 mg/kg</td>
<td>5.23</td>
<td>(Mackay et al., 1982)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlordecone</td>
<td>Insecticide</td>
<td>126--132 mg/kg</td>
<td>4.5</td>
<td>(Cabidoche et al., 2009)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lindane (γ-HCH)</td>
<td>Insecticide</td>
<td>88--190 mg/kg</td>
<td>3.85</td>
<td>(Mackay, 1982)</td>
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<td></td>
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<tr>
<td>α, β- Hexachlorocyclohexane</td>
<td>Insecticide</td>
<td>88 mg/kg</td>
<td>3.89 and 3.95</td>
<td>(Isnard et al., 1988)</td>
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</tr>
<tr>
<td>Endosulfan</td>
<td>Pesticide</td>
<td>18--160 mg/kg</td>
<td>3.5</td>
<td>(DeLorenzo et al., 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial Chemicals</td>
<td>PCBs*</td>
<td>Insulating fluid</td>
<td>1010--4250 mg/kg</td>
<td>3.76 to 0.9--7.2 years</td>
<td>(Wu et al., 2008b)</td>
<td></td>
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<tr>
<td>Ingredient</td>
<td>Type</td>
<td>Concentration</td>
<td>Persistence</td>
<td>Breakdown</td>
<td></td>
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<tr>
<td>Commercial pentaBDE Flame retardant</td>
<td>2640--6200 mg/kg</td>
<td>6.64 to 6.97</td>
<td>10--20 days</td>
<td>(Rahman et al., 2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial octaBDE Flame retardant</td>
<td>--</td>
<td>5.5 to 8.9</td>
<td>14--70 days</td>
<td>(Rahman et al., 2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexabromocyclododecane Flame retardant</td>
<td>10 gm/kg</td>
<td>5.62</td>
<td>--</td>
<td>(Hayward et al., 2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unintended By-products</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctane sulfonic acid Coating</td>
<td>--</td>
<td>--</td>
<td>4 Years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctane sulfonyl fluoride Clothing</td>
<td>--</td>
<td>--</td>
<td>4 Years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioxins--dibenzodioxins* Byproduct of chlorine</td>
<td>22 μg/kg</td>
<td>4.20</td>
<td>20 years</td>
<td>(Wang and Wong, 2002)</td>
<td></td>
<td></td>
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<tr>
<td>Furans--dibenzofurans* Herbicide</td>
<td>22 μg/kg</td>
<td>--</td>
<td>20 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentachlorobenzene</td>
<td>Pesticide</td>
<td>125--1080 mg/kg</td>
<td>5.19</td>
<td>--</td>
<td>(Mackay et al. 1982)</td>
<td></td>
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</tbody>
</table>

* = indicates dirty dozen chemicals

** = falls in more than one group
Table 2. Concentration of various POPs in human serum samples of (a) mothers and children with urban and rural residential settings and (b) various occupational settings from Pakistan

<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Rural mothers</td>
<td>Rural children</td>
</tr>
<tr>
<td></td>
<td>(ng/g)</td>
<td>(ng/g)</td>
</tr>
<tr>
<td>PCB 118</td>
<td>&lt;0.5--2.5</td>
<td>1--7</td>
</tr>
<tr>
<td>PCB 153</td>
<td>1--5.5</td>
<td>&lt;1--7</td>
</tr>
<tr>
<td>PCB 138</td>
<td>0.5--2.0</td>
<td>1--8</td>
</tr>
<tr>
<td>PCB 180</td>
<td>0.2--2.7</td>
<td>0.4--3.9</td>
</tr>
<tr>
<td>PCB 170</td>
<td>0.2--1.1</td>
<td>0.3--1.6</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>1.5--14.5</td>
<td>2--30</td>
</tr>
<tr>
<td>ΣHO-PCBs</td>
<td>&lt;0.2--1.4</td>
<td>&lt;0.2--4</td>
</tr>
<tr>
<td>HCB</td>
<td>1.3--5.3</td>
<td>3--13</td>
</tr>
<tr>
<td>Compound</td>
<td>Units</td>
<td>PCP</td>
</tr>
<tr>
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<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>ΣHCHs</td>
<td>&lt;1--52</td>
<td>&lt;1--415</td>
</tr>
<tr>
<td>PCP</td>
<td>9--115</td>
<td>15--185</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>&lt;1--1290</td>
<td>75--2700</td>
</tr>
<tr>
<td>p,p'-DDT</td>
<td>1--40</td>
<td>&lt;1--75</td>
</tr>
<tr>
<td>ΣDDTs</td>
<td>b1--1300</td>
<td>80--2780</td>
</tr>
<tr>
<td>ΣOCPs</td>
<td>3--1330</td>
<td>95--2885</td>
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<tr>
<td>BDE 47</td>
<td>&lt;0.2--2.2</td>
<td>&lt;0.2--2.5</td>
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<tr>
<td>BDE 99</td>
<td>&lt;0.2--1.5</td>
<td>&lt;0.2--1.5</td>
</tr>
<tr>
<td>BDE 153</td>
<td>&lt;0.2--0.7</td>
<td>&lt;0.2--5</td>
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<tr>
<td>ΣPBDEs</td>
<td>0.2--4.2</td>
<td>&lt;0.2--5</td>
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<tr>
<td>2,4,6-TBP</td>
<td>&lt;5--15</td>
<td>&lt;5--25</td>
</tr>
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<td>ΣBPs</td>
<td>&lt;5--15</td>
<td>&lt;5--45</td>
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<tr>
<td>BTBPE</td>
<td>&lt;0.2--8</td>
<td>&lt;1--6.5</td>
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Table 3. Concentration of Organohalogenated contaminants in serum and hair samples of pets from Pakistan

<table>
<thead>
<tr>
<th>Analyte/s</th>
<th>Cat Serum (ng/g)</th>
<th>Dog Serum (ng/g)</th>
<th>Cat Hair (ng/g)</th>
<th>Dog Hair (ng/g)</th>
<th>References</th>
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<tr>
<td>PCB 101</td>
<td>1.5--25</td>
<td>1.7--3.6</td>
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<td>Ali et al. (2013b)</td>
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<td>PCB 118</td>
<td>1.7--26</td>
<td>1--8.5</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PCB 153</td>
<td>&lt;5--27</td>
<td>&lt;5--6</td>
<td>-</td>
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<tr>
<td>PCB 138</td>
<td>3.5--33</td>
<td>&lt;3--7.2</td>
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<tr>
<td>ΣPCBs</td>
<td>16--132</td>
<td>9--22</td>
<td>0.05--1.1</td>
<td>&lt;0.05--0.6</td>
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<td>4HO-CB 107</td>
<td>&lt;0.5--5.95</td>
<td>&lt;0.5--2</td>
<td>-</td>
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<tr>
<td>4HO-CB 146</td>
<td>&lt;0.5--7.2</td>
<td>&lt;0.5--4.6</td>
<td>-</td>
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<tr>
<td>4OH-CB 187</td>
<td>&lt;0.5--5.5</td>
<td>&lt;0.5--4</td>
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<tr>
<td>ΣHO-PCBs</td>
<td>&lt;0.5--21.5</td>
<td>2.2--16.8</td>
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<tr>
<td>PCP</td>
<td>&lt;10--10.90</td>
<td>2--13</td>
<td>1--21</td>
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<td></td>
<td>HCB</td>
<td>p,p′-DDE</td>
<td>∑DDTs</td>
<td>∑HCHs</td>
<td>TN</td>
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<td>&lt;2--6.9</td>
<td>&lt;1--2150</td>
<td>&lt;1--2175</td>
<td>&lt;1--11.5</td>
<td>&lt;0.5--5.7</td>
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<td>&lt;2--8.8</td>
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<td>&lt;1</td>
<td>&lt;1--11.5</td>
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<td></td>
<td>0.4--2.7</td>
<td>-</td>
<td>&lt;0.1--7.85</td>
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<td>&lt;0.35--5</td>
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<td>&lt;0.1--0.1</td>
<td>2--8.2</td>
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<td>∑PBDEs</td>
<td>HO-PBDEs</td>
<td>BTPBE</td>
<td>2,4-DBP</td>
<td>2,4,6-TBP</td>
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<tr>
<td></td>
<td>1--1280</td>
<td>0.6--4</td>
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<td>∑HO-PBDEs</td>
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<tr>
<td>BTPBE</td>
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<td>&lt;0.7--1</td>
<td>&lt;0.7--1.25</td>
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<tr>
<td>2,4-DBP</td>
<td>&lt;5--150</td>
<td>&lt;5--35</td>
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<tr>
<td>2,4,6-TBP</td>
<td>&lt;5--85</td>
<td>&lt;5--30</td>
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<tr>
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<td>&lt;1--22</td>
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<tr>
<td>∑BPs</td>
<td>20--335</td>
<td>&lt;5--150</td>
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</table>
Fig 1: Forrest plot illustrating relative toxicity due to A). cereal crops B). animal flesh; when compared to local food standards (Punjab).