Hexachlorocyclohexane toxicity in water bodies of Pakistan: challenges and possible reclamation technologies
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ABSTRACT
Pakistan is an agro-economy country where the hexachlorocyclohexane (HCH) pesticides is being used to improve the crop productivity, as a result, the risk of contamination of soil and sediment has been increased. HCH exhibits all the characteristics of persistent organic pollutants (POP), therefore; added to the list of ‘new POPs’ in 2009. This review report revealed that the major rivers of Pakistan such as Indus Basin, River Ravi, River Chenab and their tributaries all are contaminated with HCH and the highest residual concentration (4,090 ng/g) was detected in pesticide burial ground in Hyderabad city. Major sources of HCH contamination were identified as agricultural runoff, discharge of untreated industrial effluents and surface runoff. In order to manage HCH pollution, various ex-situ and in-situ remediation techniques along with their merits and demerits are thoroughly reviewed. Among these, microbial bioremediation is low cost, environment friendly, effective in-situ remediation technique for remediation of HCH. Overall, the information provided in this manuscript will provide a future reference to the scientific community and bridge the knowledge gap between HCH release in environment and their mitigation through proper treatment method.

Key words | Hexachlorocyclohexane, Lindane, Pakistan, Persistent organic pollutant, pesticides, soil

HIGHLIGHTS
● Hexachlorocyclohexane is most widely pesticide and toxic to environment.
● Hexachlorocyclohexane as persistent organic pollutants contaminates fresh water resources and pose a potential risk to ecosystem.
● Pakistan’s major rivers and tributaries are contaminated Hexachlorocyclohexane.
● Appropriate treatment of water and mitigation policies are required.
INTRODUCTION

The overgrowth of agricultural sector has led to irreversible damage to environment and natural resources due to exposure of chemical contaminants particularly persistent toxic substances (Kanan et al. 2020; Saleh et al. 2020). Agricultural activities were significantly escalated during the first half of the last century to meet growing global food demand (Merrington et al. 2004), which in result, intensified the use of chemicals such as fertilizer, herbicides, and pesticides (Ali et al. 2014a, 2014b). Pakistan is an agriculture country with a 25% of total land is under cultivation with world’s largest irrigation system. The extensive use of illegal pesticides on the pretext of low cost of production and higher yields has raised serious indications of environmental degradation (Abrantes et al. 2010). In particular, hexachlorocyclohexane (HCH) has been extensively used since 1940s as an effective insecticide for preventing vector-borne diseases and in agriculture (Chen et al. 2006). The overall consumption of pesticides including HCH in Pakistan was 7,000 tons per annum in 1960 (Khan et al. 2010; Syed & Malik 2011), which has now increased by about 20 times. In terms of pesticide use, Pakistan is ranked second among South Asian countries (Randhawa et al. 2007). Pakistan is also one of the countries with the largest reservoirs of outdated pesticides (Syed & Malik 2011). Despite the fact that the use of HCH has been prohibited many years before in various parts of world, many former pesticide manufacturing plant sites are still heavily polluted as a result of improper HCH disposal and storage (Phillips et al. 2006). Because of the persistence, toxicity, and bioaccumulation of HCH in the environment, this presents a significant threat to both the environment and public health (Niu et al. 2013; Chen et al. 2020).

Hexachlorocyclohexane was first discovered by Faraday in 1825, it’s a monocyclic chlorinated hydrocarbon commonly abbreviated as HCH. There are eight isomers of HCH out of which alpha (α), beta (β), gamma (γ) and delta (δ) isomers are commercially significant. Lindane which is gamma isomer (γ-HCH) was used as a pesticide due to its strong insecticidal properties (Jennings & Li 2013). In the Stockholm convention’s fourth meeting in May, 2009 HCH (lindane, alpha-HCH, beta-HCH) was added to the list of ‘new POPs’ persistent organic pollutants (Ali et al. 2014a, 2014b). Therefore, it became necessary to analyze the trend of HCH residues in soil and sediments and their sources from different areas of the country.

The United Nations General Assembly has recently declared 2021–2030 as the ‘International Decade on Ecosystem Restoration’. Restoration of ~350 mha of degraded land across the world is one of the prime focus for achieving the UN-Sustainable Development Goals. Pesticides use is one of the leading causes of land degradation and hexachlorocyclohexane is widely used organochlorine pesticides over the past decades (Tripathi et al. 2019). In case of Pakistan, HCH in the environment is one of the region’s most pressing environmental issues, and current information is dispursed and unnoticed. The concern of HCH contamination of various rivers is highlighted in this scientific analysis, as well as...
potential treatment options based on current technologies. The details in the manuscript could also be very useful for the scientific community in developing and enhancing current HCH pollution management practices.

**METHODOLOGY**

This review will help to identify loop holes in pesticide monitoring and release of historic HCH into water bodies. To formulate the current review on HCH presence and status in Pakistan, nearly seventy most germane and encompassed national and international original research, review articles and newsfeed regarding persistent organic pollutants and HCH were opted and summarized. Case studies of HCH in soil and sediments of Pakistan were collected from year 2011–2018 to update the current information. To discuss the feasibility of HCH treatment, a review of various research studies was conducted to observe the efficiency of different technologies used for HCH removal from the environment.

**STATUS OF HCH IN DIFFERENT PARTS OF THE WORLD**

**Global history of HCH usage**

The global usage pattern of HCH has been raised at much higher levels. Presence of HCH have been reported around the world since many years till present. Worldwide estimated use of all HCH was 40,000 tons in 1982 and 29,000 tons in 1990, whereas 5,900 tons of gamma HCH (lindane) was consumed in 1980 and 4,000 tons in 1990 (Li et al. 1996) whereas, estimated consumption of lindane between 1950 and 2000 was approximately 450,000 tons (López et al. 2020). In 1970s the use of technical HCH was restricted in many countries. The top three squanderer of technical HCH in 1980 includes China, India and former Soviet Union, and in these countries the annual consumption of technical HCH was supposed to be more than 90% of the global usage. The total amount of technical HCH produced in China from 1952–1983 was around 4.5 million tonnes further the usage was restricted. In India the maximum annual usage of technical HCH reached 57,000 tons in later 1980s, however in 1991 government of India banned any usage of technical HCH except for public health protection. In Soviet Union the use of technical HCH for agriculture was banned in the 1990 (Li et al. 1998). Although its usage was banned in 1981 in most parts of Europe (Berntssen et al. 2017) the estimated usage of technical HCH and lindane in Europe during 1970–1996 was 382000t and 81000t respectively (Breivik et al. 1999). Similarly, production of HCH was stopped in US since 1976 (Chen 2014). The overall estimated agricultural consumption of lindane reported in various countries amounts 287.16 (Europe), 73.20 (Asia), 63.57 (America), 28.54 (Africa) and 1.03 (Oceania) in thousands of tons (Vijgen et al. 2011).

**Status of HCH in soil and sediments**

In surface soil and sediment samples, HCH as the most dominant organochlorine pesticide (OCP) and source has been identified as historic residues from river runoff (Kkhakshar et al. 2019). Numerous studies have been conducted in Italy reports HCH as the third most dominant OCP (Qu et al. 2018, 2019) Also the concentration in agricultural soil was higher as compared to non-agricultural land (Qu et al. 2016). Fang et al. (2017) reports HCH residues studied in top soil (1,890–205,000 ng/g) and deep soil (50–21,300 ng/g) samples from pesticide contaminated site which is the highest concentration among all studies reported in this review paper, also higher than observed in pesticide burial ground in Hyderabad (Aimdar et al. 2014) and pesticide dumping ground in Lahore (Syed et al. 2013). The occurrence of HCH in various soil and sediments in different regions of world have been summarized in Table 1.

Nemr & Sadaawy (2013) investigated OCPs in surface sediments from the Mediterranean Sea and observed higher concentration of HCH than reported in sediments of costal belt of Pakistan (Ali et al. 2014a, 2014b). The ratio of a-HCH/g-HCH indicated recent input of lindane in the Mediterranean coast (Nemr & Sadaawy 2013). Sediments from four Ramsar wetlands (Lake St Lucia, Mkhuze, Lake Sibaya and Kosi Bay) were investigated for OCPs where HCH was the most dominant pesticide and source was identified as agricultural activities in the catchment site (Kwofie & Humphries 2017).

**PROPERTIES OF HEXACHLOROCYCLOHEXANE**

During the last decades, POPs including HCHs have gained much attention due to its special properties such as toxicity, non-degradability, long range atmospheric transport and
bioaccumulation potential. These are briefly explained as below.

Toxicity

HCH can cause toxic effects on health such as, neurotoxicity, carcinogenicity and immunotoxicity (Chao et al. 2014; Nadal et al. 2015). These pesticides can affect not only human health but also dangerous for aquatic life (Yadav et al. 2015). A study conducted in the Faroe Islands also reported a small, but significant relationship between serum levels of β-HCH and increased risk of Parkinson disease (PD) (Petersen et al. 2008; Richardson et al. 2011). Exposure to pesticides was considered as a possible risk factor for developing Parkinson disease PD. Therefore, a case control study was conducted by Richardson et al. (2011) in University of Texas Southwestern Medical Center and Emory University, 283 serum samples of PD patients were investigated to determine association between β-HCH and PD disease. Samples were collected and study in two discrete periods from 2001–2003 and 2006–2008. Although the data obtained was consistent with decrease of β-HCH in environment from 2001–2008, but results shows higher levels of β-HCH in serum is associated with increased risk of PD disease (Richardson et al. 2011).

Non-degradability

POPs are resistant to degradation either chemical, physical, biological or microbiological, hence they are resistant and persistent in environment for longer period of time (Afful et al. 2010; Yadav et al. 2015). β-HCH are less volatile and resistant to microbial degradation and hydrolysis, while α-HCH and γ-HCH are more volatile isomers of HCH can stay in air and travel over long distance (Wu et al. 2015). By the action of soil microbes or light γ-HCH can be converted to α-HCH (Zhang et al. 2015).

Long range atmospheric transport (LRAT)

HCH can travel to long distances, away from the source through air and ocean currents (Hung et al. 2013; Nadal et al. 2015). As Antarctica considered an isolated continent on Earth even then the presence of POPs has been recognized there by scientists (Nash 2011). A study conducted by Zhang et al. (2015) for the assessment of OCPs residue level, their distribution and potential sources in King George Island, West Antarctica. Samples were collected from different environmental matrices and analyzed through High Resolution Gas Chromatography and High-Resolution Mass Spectrometry technique. The results

| Country          | Sampling site                                                                 | Concentration ng/g | Reference                      |
|------------------|-------------------------------------------------------------------------------|--------------------|--------------------------------|
| China            | Surface sediment samples from northern Bering Sea, Chukchi Sea and adjacent Arctic Ocean | 0.29–3.05          | Kahkashan et al. 2019          |
| Southern Italy   | Surface soil samples from territory of Benevento Province                     | Nd – 0.72          | Qu et al., 2019                |
| Southern Italy   | Sediment sample from gulf of Nepal and Salerno                                | 0.37–1.10          | Qu et al., 2018                |
| Northwest Mexico | Residential soil & agricultural drain sediments                               | 0.49–2.0           | Osorio et al. (2017)           |
| Simangaliso Wetland Park, South Africa | Sediments from lake St. Lucia, Mkhuz wetland, Kosi Bay, Lake Sabaya, at river inlet and drainage line points | 26.3–282           | Kwofie & Humphries (2017)      |
| Southwest China  | Top soil and deep soil from pesticide contaminated site                        | 1,890–205,000 & 50–21,300 | Fang et al. (2017) |
| Nepal            | Surface soil samples from four cities (Kathmandu, Pokhara, Birgunj and Biratnagar) | 5.0                | Yadav et al. (2016)            |
| Southern Italy   | Soil from Campanian Plains                                                    | 0.03–17.3          | Qu et al. (2016)               |
| Mediterranean Sea Egypt | Sediment samples from Egyptian Mediterranean coast                          | 0.07–45.79         | Nemr & Sadaawy (2013)          |
| Kenya            | Top soil from rural and suburban area surrounding Nairobi city                 | Nd – 7.38          | Sun et al. (2016)              |
| Ningde, China    | Paddy and vegetable farmland soil from hill region of Ningde                  | 0.45–151.21        | Qu et al. (2014)               |
| China            | Soil sediment from Old Yellow River Estuary                                   | 0.0014–14.85       | Da et al. (2014)               |
| South Korea      | Soil from agricultural, industrial and urban areas                            | Nd – 0.385         | Kim et al. (2014)              |
indicate presence of twenty-three OCPs, among these DDT and HCHs were main contaminants in all samples.

Bioaccumulation potential

HCH are lipophilic in nature thus these can accumulate in fatty tissues, breast milk and blood show lipid solubility (Williams 2008) and enters in food chain (Yadav et al. 2015). Human exposure to OCPs is mainly through consumption of contaminated food. Therefore, a research was conducted to assess levels of OCPs and potential human health risk associated with OCPs after consumption of edible cattle tissues (Mahmoud et al. 2016). Out of total 135 random samples, HCHs were highest among OCPs and showed highest concentration (448 ng/g lipid weight) in tongue samples from Mansoura (Mahmoud et al. 2016).

Acute and chronic exposure risk

Residues of persistent pesticides stays for longer periods on the target crops and later enters to human body through food chain (Bhushan et al. 2015). The residue level of persistent pesticides should not go beyond maximum limits which may be harmful to human health. To monitor residue levels of these pesticides in food chain certain limits have been proposed and developed which includes, maximum residue limits (MRLs), theoretical maximum daily intake (TMDI) (Bhushan et al. 2015). FAO and WHO recommended a standard acceptable limit of zero tolerance for POPs (FAO/ WHO 2011). The standards and limits recommended by FAO and WHO are widely accepted and adopted. These acceptable standards are proposed in a Joint Meeting on Pesticide Residues (JMPR) and the Codex Alimentarius Commission. JMPR recommendations evolves after a thorough review of international data, along with the analysis of presence and cure and effect of pesticides (Fishel 2010; FAO & WHO 2011). JMPR acceptable daily intake value for lindane is 0.005 mg kg day$^{-1}$ Whereas, US Agency for Toxic Substances and Disease Registry ATSDR sets minimal risk levels range from 0.05 to 0.0001 mg kg day$^{-1}$ for acute to chronic exposures to different HCH isomers. However, no exposure standards have been proposed for technical HCHs (Chen 2014). Pakistan has no regulatory guidelines for pesticide residue limits in soil. However, according to China National Environmental Protection Agency the grade II limit for HCH in agricultural soils is 50 ng/g which is considered less strict (Sun et al. 2016).

**POTENTIAL HEALTH RISKS OF HEXACHLOROCYCLOHEXANE**

HCH cause serious negative impacts on the ecosystem due to its property of biomagnification, long range transport and non-degradability (WHO, 2011). HCH can cause serious health impacts including neurological, reproductive, and immunological disorder both in animals and humans (Kalyoncu et al. 2009). These health disorders result from constant, accruing and long-term exposure to one or more substances, mostly through non-atmospheric pathway. Dietary uptake is the most common exposure pathway for HCH and semi-volatile contaminants (Nadal et al. 2015; Perelló et al. 2015). Alpha-hexachlorocyclohexane (α-HCH) is a structural isomer of HCH that have been used as insecticide globally. Alpha-HCH is classified as a probable human carcinogen by the U.S. Environmental Protection Agency (Bradley et al. 2016) moreover, it may cause liver tumors in rat and mice. The mode of action (MOA) of liver tumors involved rapid cell growth or mitogenesis. The USEPA National Waste Minimization Program had listed lindane among the 31 priority chemicals intended for reduction (USEPA/NWMP 2011). Many organizations have evaluated health risk associated with the HCH isomers for instance, California environmental protection agency (CEPA 2014) in 1987 included technical HCH in the list chemicals recognized to cause reproductive toxicity or cancer added technical HCH to the list of, whereas in 1989 lindane and other isomers were also added to the list. Lindane is the only isomer of HCH identified by American Conference of Governmental Industrial Hygienists (ACGHI) as a proved animal carcinogen with unknown applicability to humans card (WHO/IPCS/ICSC 2009).

HCH has been recognized to cause Deoxyribonucleic acid (DNA) mutation of maturing male germ cells in reproductive tract. Khan et al. (2010) determine the seminal HCH and its isomers in relation to semen quality and Y-chromosome microdeletion in azoospermic factor (AZF) region. It was found that HCH and its isomers (α, β and γ) were observed in infertile group similarly, the major microdeletions were also observed in azoospermic patients. This study concluded that male germ line is prone to potential mutagenic activity of HCH and can lead to spermatogenic failure.

A study conducted in the Faroe Islands also reported a small, but compelling relationship between Parkinson disease (PD) and serum levels of β-HCH (Petersen et al. 2008; Richardson et al. 2011). Exposure to pesticides was
considered as a possible risk factor for developing Parkinson disease.

**PESTICIDES CONSUMPTION IN PAKISTAN**

Pakistan’s biggest natural resource is arable land, where 25% of total land is under cultivation with world’s largest irrigation system. Agriculture has a major share in the economy of Pakistan and like other agro based nations, use of pesticides is a common practice in order to get higher yields to cope with food shortage. The extensive use of illegal pesticides on the pretext of low cost of production and higher yields has raised serious indications of environmental degradation (Abrantes et al. 2010). During green revolution in 1960, Pakistan imported huge amount of pesticides from Europe and USA for eradication of malaria, locust control and for pest control (Ahad et al. 2010). The overall consumption of pesticide in Pakistan was 7,000 tons per annum in 1960 and reached up to 78,132 tons per annum in 2003 (Khan et al. 2010; Syed & Malik 2011). Reportedly about 5,000 tons of obsolete pesticides exist in different provinces of Pakistan of which 128 tons in Balochistan, 2016 tons Sindh, 179 tons in KPK and 3,803 tons in Punjab (Khwaja et al. 2006). Pakistan is ranked second among the South Asian countries based on pesticide consumption (Randhawa et al. 2007). Also, Pakistan stands among the countries that holds the largest reservoirs of outdated pesticides (Syed & Malik 2011).

**PAKISTAN’S’ LEGISLATIVE STANDING**

During past decades Pakistan showed significant interest to be the part of various international legislation and treaties on pesticides. The international convention about POPs include the Rotterdam Convention which addresses the prior informed consent procedures, certain hazardous chemicals and pesticides in international trade. Moreover, the Stockholm convention deals specifically POPs and third is the Basel convention which deals with transboundary movement of hazardous wastes and their disposal (Ali et al. 2014a, 2014b). The rules and regulations for the manufacture, import, sale and monitoring of pesticides were established in 1971 by Agriculture Pesticide Ordinance and in 1973 by Agriculture Pesticide Rules under the guidelines of FAO. In 1999 Pakistan became 67th signatory of Rotterdam Convention at United Nation in New York and in July 2000 Pakistan ratified the Stockholm Convention (Ali et al. 2014a, 2014b). Apart from the environmental legislation on POPs there is a huge difference between the legislation and implementation, despite the official ban in the country these pesticides are available in the market (Bhambhro 2004). Pakistan is a signatory to all these conventions therefore, demolished banned pesticide formulating units in the country but still Pakistan lacks a legal policy to prevent illegal use, storage and safe disposal of obsolete pesticides which are a potential threat to population and environment. Moreover, Pakistan lacks standard quality control criteria for residual concentration of pesticides in different environmental compartments (Syed & Malik 2011).

**RESIDUAL LEVELS OF HEXACHLOROCYCLOHEXANE IN VARIOUS REGIONS OF PAKISTAN**

Pesticide residues have been present and detected in various compartments of environment such as water and soil in different regions of Pakistan. However, data about HCH contamination in the country is limited due to lack of pesticide monitoring. During the year 2011–2018, a few studies have been conducted in different parts of the country which are discussed in the later section and summarized in Table 2.

**River ravi**

The River Ravi is a transboundary river which flows from North West of India and East of Pakistan, and considered as most polluted river in Pakistan (Syed et al. 2014). It is surrounded by significant agricultural area of the country i.e. the Rachna Doab and the Bari. It has four tributaries namely Nullah Deg, Nullah Basanter and Nullah Bein, these tributaries receive surface, subsurface and agricultural runoff areas. By means of unregulated irrigation pumps water from these Nullahs (streams) is used for livestock, agriculture and domestic purposes. The major cultivation of the catchment includes, rice crop (summer season) and wheat crop (winter season). These Nullahs receive huge quantities of untreated, industrial and municipal waste from the surrounding areas which degrade the quality of the streams (Malik and Nadeem, 2011). A study conducted by Baqar et al. (2018) reported the presence of HCH contamination in sediment samples from these tributaries. In this study 54 sediment samples were collected from various locations such as Nullah Deg, Nullah Basanter and Nullah Bein.
located near upper Rachna Doab in district Narowal, Sialkot and Sheikhupura. The results report high concentration of HCH in sediments both in pre-monsoon and post-monsoon sampling which is higher than previous findings except Lila stream and Nullah Deg (Malik et al. 2014; Syed et al. 2017). The concentration of HCH was higher in pre-monsoon sediments than in post monsoon samples (Figure 1) which was due to the lower dilution factor and dehydration conditions during pre-monsoon season (Farooq et al. 2011). The value calculated for ratio of alpha-HCH and gamma-HCH in water and sediment matrixes were lower than 3 which show fresh input of lindane (Baqar et al. 2018).

In another study (Syed et al. 2014), it is reported that the presence of HCH in river Ravi. In this 21 samples from seven sites were collected along the River Ravi from Lahore to Kot Islam based on human activities in the catchment. β-HCH contributed for 43% of total HCH concentration

Table 2 | Residual levels of ΣHCH in soil surface and sediments reported in Pakistan

| Location                                      | Concentration range in ng/g | Reference                  |
|-----------------------------------------------|-----------------------------|----------------------------|
| River Ravi and Tributries                     | 2.15–999.18                 | Baqar et al. (2018)        |
| Mehmood Booti Drain Lahore                    | 0.00–1.90                   | Ali et al. (2016)          |
| Indus River catchment                         | 0.74                        | Bajwa et al. (2016)        |
| Nowshera district                             | 1.67–32                     | Zehra et al. (2015)        |
| Coastal belt                                  | 0.1–7.3                     | Ali et al. (2014a, 2014b)  |
| Soan River                                    | 1.93–34.4                   | Malik et al. (2014)        |
| River Chenab/Gujranwala division              | 4.54–18.9                   | Mehmood et al. (2014)      |
| Pesticide burial ground Hyderabad             | 13.5–4,090                  | Alamdar et al. (2014)      |
| Indus basin                                   | 3.6–36.63                   | Sultana et al. (2014)      |
| River Ravi                                    | Nd – 22                     | Syed et al. (2014)         |
| Pesticide dumping site Lahore                 | Nd – 122                    | Syed et al. (2015a)        |
| Punjab province                               | 1.7–20                      | Syed et al. (2015b)        |
| Ittehad Chemicals Kalashah Kaku               | 24.71–121.71                | Syed & Malik (2011)        |

Figure 1 | Spatial distribution of ΣOCPs levels in sediments samples during pre-monsoon and post-monsoon seasons (Adopted from Baqar et al. 2018).
which indicates lack of fresh input in the area, on the other hand γ-HCH accounts for 38% of total HCH and indicates fresh input of lindane. Higher concentrations of β-HCH were observed in industrial zones whereas in agricultural areas concentration of γ-HCH was found higher than other isomers (Figure 2). The concentration of HCH found in sediments were comparable to those detected in River Chenab sediments (Eqani et al. 2011). Syed et al. (2013b) assessed the presence of OCP in soil along the sides of the River Ravi including industrial zone (Faisalabad, Phool Nagar, Lahore, Shahdara and Shiekhupura) and Agricultural zone (Kabirwala, Khanewal, Mianchanu, Cheechawatani and Sahiwal). The overall trend for OCP concentration in soil samples follow the order as Dichlorodiphenyltrichloroethane (DDT) ∑DDT>∑HCH>∑Chlordane>Endosulfan>HCB. Hexachlorocyclohexane isomers were found in majority of soil samples whereas γ-HCH concentration was highest which indicates use of lindane in Pakistan. The concentration of ∑HCH was lower than reported in other parts of the world. Highest HCH concentration was observed in soil samples from Shahdara (industrial site) and Khanewala (agricultural site). The higher values of HCH in Shahdara were attributed to the presence of Ittehad Chemical Industry near the sample site. Although the factory stopped formulation of pesticide after the ban was imposed on OCP in 1994 but it is reported that HCH residues are still found in the soil.

Lila stream near Lahore is one of the major tributaries of River Ravi and receive water from several chemical manufacturing units. Syed et al. (2013a) found the residual concentration of ∑HCHs ranged from 0.0 to 121.17 ng/g with the highest values obtained for β-HCH as 0.0–120 ng/g the residual concentration of HCH. For the source identification different isomeric and parental ratios were calculated such as the ratio of γ-HCH to ∑HCH ranges from 0.0 to 1, the ratio of α-HCH/γ-HCH ranges from 0.0 to 0.84 and the ratio of β-HCH/γHCH ranges from 0.0 to 10.4 according to these ratios the source of HCH was linked to the historic use of technical HCH and lindane as pesticide in the area. Also, the β-HCH in soil and sediment samples accounts for 88.16% of the total HCHs in the study area which confirms the persistent nature and low leaching ability of than γ-HCH. Pakistan lacks any standard quality control criteria for pesticides however according to Chinese Environmental Quality Standards concentration of HCH in this area can be declared less polluted (<50 ng/g) except soil surface sample (SO4) 121.71 ng/g

Figure 2 | Isomeric concentrations of HCH along the river Ravi (Adopted from Syed et al. 2014).
collected from a waste dumping site classified as slightly (50 ng/g–500 ng/g) polluted (Syed et al. 2013a). In another study Syed & Malik (2011) found the concentration of various contaminants in order of ΣDDT > ΣHCH > dicofol > endrin > heptachlor > dieldrin > endosulfan II. Residual contamination of ΣHCH ranged from 24.71 to 121.71 ng/g and concentration of β-HCH was higher than other isomers with detection frequency 66.7%. According to the Chinese Environmental Quality Standards, 81% soil samples with residual ΣHCH were listed as less polluted (<50 ng/g) whereas six soil sampling sites were identified as slightly polluted (>50 ng/g) in this study. After calculating isomeric ratios for HCH, technical grade HCH and lindane were the identified sources of HCH indicating historic use in the area.

**River Chenab**

Mehmood et al. (2014) reported presence of HCH in a study conducted along the two tributaries of Chenab river. Twenty-eight soil samples were collected from agricultural fields along the Nullah Aik and Nullah Palkhu from Gujranwala division, Punjab, Pakistan. It was found that the concentration of ΣHCH (4.54–18.9 ng/g) higher than those reported by Ali & Jabbar (1991) and Syed et al. (2013) but lower than that reported by Alamdar et al. (2014) in obsolete pesticide dumping site in Hyderabad, Pakistan. Eqani et al. (2011) reported a study for the assessment of organochlorine pesticides in sediment from River Chenab, Pakistan. Based on OCPs residual concentrations, results were divided into three regions, ΣHCH were among the dominant OCPs in study area. Higher ΣHCH concentration (1.77 to 7.59 ng/g) were detected in cotton growing areas in region three which consist of seven sampling sites this concentration was exceeding the Intrin Sediment Quality Guidelines (0.94 ng/g).

**Indus basin of Pakistan**

Indus Basin is drained by Indus River that has approximate length of 3,180 km and its tributaries support the agricultural activities in this region. Central Indus Basin of Pakistan besides socio-economic importance serves as natural habitat for many species of the area the Indus delta constitutes the 7th largest Mangrove forest of the world. International organizations are working for the conservation of Indus Basin of Pakistan, such as central Indus wetland complex and Indus Dolphin reserve designated by WWF. Although the Indus Basin is of great ecological and agricultural significance but there is no enough comprehensive study regarding the pollutant load on the Indus River due the anthropogenic activities in the area. Sultana et al. (2014) assessed the OCPs in Central Indus Basin along River Indus. Out of six sampling sites of high ecological significance, three sites; Chasma, Taunsa and Sukkar were declared as wetlands, Sukkar Barrage has been designated as Dolphin reserve and Taunsa Barrage which WWF Pakistan has declared as a wildlife sanctuary. Fourth site was Head Panjnad (HPN) a point of conjunction of five major rivers of Punjab while the last two sampling sites Dera Ismail Khan (DIK) and Rahim Yar Khan (RYK) were agricultural areas. The ΣHCH concentration in these sites were found in order of HPN > DIK > Suk > RYK > TAU > CHA, whereas different isomeric concentration of HCH detected in the area were as α-HCH (51% ΣHCH), β-HCH (22.8% ΣHCH) and γ-HCH (25% ΣHCH). The Indus river receive pollution due to high agricultural activities in associated areas, for instance; Dera Ismail Khan after building Chashma Right Bank Canal led to the higher level of ΣHCH in the catchment. Similarly, higher levels of ΣHCH in Sukkar (downstream) samples were due to the narrowing down of the river hence reducing dilution factor and resulting in concentrated upstream contamination. The ratio of α/γ-HCH in the study was reported less than 3 which could be linked to the fresh input of lindane in the agricultural soil of the catchment area (Sultana et al. 2014). Another similar, study was conducted by Bajwa et al. (2016) to investigate OCPs in Indus river catchment areas which includes Taunsa a wild life sanctuary and a Ramsar site, Guddu, Sukkar a Dolphin reserves and KOT Mithan which is a conjunction point near Head Panjnad. Among all the selected sites highest residual concentrations were found in soil samples of Taunsa Barrage.

**River soan**

Soan river originates from Patriata and Murree and feeds the residents of adjacent areas. Many small dams are built on the Soan river. Untreated municipal wastewater and local nullahs join Soan river polluting it with loads of hazardous chemicals. Malik et al. (2014) reports OCP contaminants in the sediments of Soan river in Pakistan. Twenty-four surface sediment samples from Soan river and its tributaries the Korang river, Ling stream, and Lai nullah were collected for study. Highest ΣHCH concentration was detected in Soan river (7.65–34.44 ng/g) however these concentrations were relatively in safe levels in comparison to published guidelines.
Waste dumping site

In Pakistan like other developing countries municipalities are accountable for proper disposal of waste which consist of a verity of material however lack of updated technologies is the reason behind poor waste management practices. Mehmood Booti waste burial site is the only authorized landfill site in the Lahore. It was published in The News’ that there are 11 drains which carry industrial and municipal waste from Lahore city to the River Ravi (Raza 2015). Mehmood Booti drain located near the legitimate waste burial site of Lahore (Mehmood Booti waste dumping site) drains into River Ravi. It is reported by Ali et al. (2016) residue levels of different POPs in sedimentary samples of the area for pollution loads. Four sampling sites were selected from upstream and downstream; two sites were close to landfill and two sites were close to agricultural area. The overall OCPs concentration were comparable with other studies in Pakistan but the residue level of ΣHCH was far less than previous findings in Pakistan. The ratio of α/γ-HCH indicates the historic use of technical HCH in the study area (Ali et al. 2016).

Obsolete pesticide formulation unit

Nowshera district in Khyber Pakhtunkhwa has a historic significance and today it is a center of various industrial, agricultural, and anthropogenic activities. The district is also known for a famous DDT production unit which was operational till 1994. Soil of Nowshera has been reported for residual contamination of DDT (Khwaja et al. 2006) which passes-on to adjacent water bodies by torrential rain and floods reported in last decade. This residual movement of hazardous chemicals from soil to water and atmosphere are of concern due to potential health risks associated with it. Zehra et al. (2015) studied organohalogen in surface soil of Pakistan where about 28 surface soil samples were collected from selected sites based on land use type such as, urban, industrial, and agricultural areas of district Nowshera. HCHs were frequently detected in all soil samples ranging from 0.8–32.6 ng/g. Among the different land use types concentration of residual ΣHCH was highest in industrial samples. The order of occurrence of HCH isomers were as β-HCH > α-HCH > γ-HCH > δ-HCH and source of HCH was determined by isomeric ratios, α/γ-HCH ratio in the study area ranged from 0.14–9.26. Most of the samples show lower values of α/γ-HCH ratio which indicates historic use of lindane whereas higher α/γ-HCH ratio was observed in few samples which reflects historic use of technical HCH in the study area (Zehra et al. 2015).

Pesticide dumping ground

Pakistan holds large stockpiles of banned pesticides and due to the lack of any legal framework and policy for safe disposal of these hazardous chemicals situation further worsens and results in dumping of these banned chemicals in landfills and open storage sites (Ahad et al. 2010). These dumped pesticides are a potential source of secondary emissions in tropical regions that promotes long range transport to other parts of the world (Zhong et al. 2008; Dvorska et al. 2012). In Pakistan a pesticide burial site is located near Hyderabad city. Alamdar et al. (2014) assessed OCP residues in surface soil samples from this pesticide burial site. Total of 20 soil samples were collected from different land use types which include pesticide dumping site, residential, industrial and background soils. The general trend of OCP occurrence was as ΣDDT > ΣHCH > Chlordane > HCB and Heptachlor. Highest ΣHCH concentration (43–4,090 ng/g) was detected in pesticide burial ground and α-HCH (50–80%) was dominant isomer. Residual concentration of HCH detected in this study was higher than other reported in Pakistan (Eqani et al., 2011; Syed & Malik 2011; Syed et al., 2013). According to Alamdar et al. (2014) pesticide burial ground located in densely populated Hyderabad city was loaded with huge concentrations of OCPs which poses serious health risk to the residents and environment of the area.

Coastal belt of Pakistan

Due to rapid growth in population and industrial activities coastal areas are discharging huge loads of untreated domestic and industrial effluents into shallow sea water. This sedimentary pollution from upstream Indus river is released into the Arabian sea by two main rivers Malir and Lyari. Malir river catches industrial waste from Korange Industrial Trading Estate (KITE) of Karachi where many tanneries, textile, paint, dyes, detergent, and pharmaceutical industries are operating. Similarly, Lyari river receives domestic sewage waste and industrial effluents from Sindh Industrial Trading Estate (SITE). Ali et al. (2014a, 2014b) conducted a study along the coastal belt of Pakistan to assess the ground situation of the area. They found that the concentration of ΣHCH ranges from 0.1–7.3 ng/g was detected in Rohri Goth samples which is close to civil and industrial settlement and catches sewage and industrial waste.
REMEDIATION TECHNOLOGIES FOR HCH

The HCH is highly hazardous and have potential health risks, therefore, numerous methods have been devised for the treatment of HCH from environment. The remediation of HCH from soil is carried out in three ways (I) ex-situ, method in which contaminated soil is excavated and taken to another location for its treatment, (II) in-situ, treatment involves the remediation of contaminated soil without excavation, instead the contamination is treated on the place it has occurred, (III) on-site, remediation method involves excavation of contaminated soil for treatment on site and treated soil is returned to original location (Calliman et al. 2011). Soil remediation involves physical, chemical, l and biological process to remove, degrade, isolate or stabilize pesticide contaminants (Gavrilescu 2009). However, for the selection of suitable remediation technique, it is necessary to understand the nature and concentration of pesticide to be eliminated, site characteristics, identify source of contamination (point, diffuse) and the use or disposal of contaminated end media. Aggressive remediation technologies are used for industrially polluted soils whereas agricultural soils must be carefully remediated to maintain soil properties (Morillo & Villaverde 2017).

Ex-situ remediation techniques

Soil washing

This is basically a separation technique in which pesticide contaminated soil is excavated, mixed, and agitated with aqueous solution containing extractants (acids, organic compounds etc.) in an extractive unit. Soil particles are allowed to settle, and clean soil is separated from solution. This wash water (solution) is either regenerated for next round of washing or sent to landfill. This technique is suitable for soil containing 50% gravel and sand (Morillo & Villaverde 2017). Ye et al. (2014) reports 99% removal efficiency of HCH contaminated soil from obsolete pesticide factory. Table 3 shows results of studies using soil washing techniques, only few studies are available on regeneration of solution.

Land farming

Land farming can be carried out ex-situ or in-situ depending on the depth of the pollutant in soil. In ex-situ soil treatment technique where excavated contaminated soil is transported to the site of land farming and soil is spread over the ground in a thin layer. Through periodic tilling and incorporating additional nutrients soil microbial activity is enhanced for degradation of contaminants. Although this technique is conveniently applicable and economical however due to the prolonged duration of operation this technique is germane only when there is no time constrain (Morillo & Villaverde 2017) merits and demerits of the technique are discussed in Table 4. Rubinos et al. (2007) reported upto 89% removal of HCH from heavily contaminated soils (>5 g/kg) by applying land farming technique (Table 3).

Chemical remediation technology

Chemical remediation is among destruction technologies where contaminants are degraded chemically (abiotic) by oxidation, reduction, hydrolysis or ionization reactions. Most of the chemical remediation techniques are ex-situ and very few can be applied in-situ or on-site. In a redox reaction one reactant gains electron while other loses electron and this reducing environment is favorable for breakdown of contaminants persistent in aerobic conditions. Zero-valent iron nanoparticles (nZVI,) acts as a chemical reductant and are used for the degradation of chlorinated compounds in contaminated soils (Morillo & Villaverde 2017). Cong et al. (2010) reports successful reductive dechlorination of HCH by nZVI in contaminated soil of a pesticide manufacturing plant (Table 3). However, small size and high reactivity of nZVI pose harm to soil microbes, soil worms (earthworm) and certain plants (El-Temsah et al. 2016).

Bioreactor system

In a slurry bioreactor technique contaminated soil is mixed with wastewater residues to prepare slurry of known consistency (Morillo et al., 2017) followed by a series of biological reactions under aerobic or anaerobic conditions and feed modes. The advantages of this technique over other bioremediation technologies include, high quality control of bioprocess parameters, controlled bioaugmentation and greater bioavailability of pollutant (Azubuike et al. 2016). Isomeric degradation of HCH by a white rot fungus Bjerkandera adusta was observed in a slurry batch bioreactor experiment, where different concentrations of HCH spiked soil were used (25–100 mg/kg) and the removal efficiency was between 30–90%. But, when the concentration of HCH (25–100 mg/kg) and amount of soil (10–30%) was increased then the degradation efficiency of the bioreactor was reduced (Quintero et al. 2007).
| Pollutant | Technique | Scale | Chemical/microbe/plant involved | Matrix | Results | References |
|-----------|-----------|-------|---------------------------------|--------|---------|------------|
| Lindane   | Microbial degradation | Laboratory | *Streptomyces Consortium* | Non-sterile Clay Silt Loam Soil (CSLS) | 11% reduction | Fuentes et al. (2017) |
| Lindane   | Microbial degradation | Laboratory | Kocuria sp. and *Staphylococcus* sp. | Soil | 94 and 98% reduction respectively after 8 days | Kumar et al. (2016) |
| HCH       | Biotostimulation, Bioaugmentation | Laboratory and field trial | Bacterial consortium (S. lucknowense F2 and S. sp. UM1) | Soil | Converts β-tetrachlorocyclohexane-1,4-diol and δ-tetrachlorocyclohexane-1,4-diol, into less toxic forms than parent compound | Garg et al. (2016) |
| Lindane   | Soil washing | Laboratory | Rhamnolipids + citric acid | Soil | 85.4% lindane was desorbed from soil | Wan et al. (2015) |
| HCH       | Soil washing | Laboratory | Methyl-β cyclodextrin (0–150% w/w) + ultrasonication | Soil from Abandoned pesticide factory | 99% removal efficiency was obtained after four successive washing cycles | Ye et al. (2014) |
| Lindane   | rhizoremediation | Greenhouse experiment | *Jatropha curcas* L (plant) | Soil | 89–72% removed from soil for 5–20 mg/kg in 300 days | Abhilash et al. (2013) |
| Lindane   | Biotostimulation, Bioaugmentation | Laboratory | Lindane-acclimated inoculum | Soil slurry | 55-70% biodegradation after 7 days | Varo-Arguello et al. (2012) |
| Lindane & HCH | Soil washing | Laboratory | Rhamnolipid, sophorolipid and Trehalose-containing lipid | Soil | 30–50% enhanced degradation of lindane and HCH isomers. Sophorolipid offered highest degradation | Manickam et al. (2012) |
| Lindane   | Bioaugmentation | Laboratory | *Kocuria rhizophila*, *Microbacterium resistent*, *Staphylococcus equorum* and *Staphylococcus cohnii* | Soil | Upto 41% after 45 days | Abhilash et al. (2011) |
| HCH       | Chemical degradation | Laboratory | ZVI as reducing agent (10% w/w) | Soil from pesticide manufacture plant | Reductive dechlorination of HCHs | Cong et al. (2010) |
| HCH       | Landfarming | Pilot lindane manufacturing site | Endogenous flora | Soil | 89% biodegraded after 21 days | Rubinos et al. (2007) |
| HCH       | Slurry batch bioreactor | Laboratory | *Bjerkandera adusta* (White rot fungus) | Slurry | γ-HCH 94.5% | Quintero et al. (2007) |
|          | | | | | α-HCH 78.5% | |
|          | | | | | β-HCH 66.1% reduction after 30 days | |
| Lindane   | Slurry bioreactor | Laboratory | Sewage sludge | Slurry | 90% degradation after 10 days | Quintero et al. (2006) |
In-situ remediation techniques

Microbial degradation

In this technique remediation of contaminated site can be achieved by utilizing native microflora (bacteria, fungi, algae or actinomycetes) most important parameter in this technique is the microbial diversity of contaminated site and nature of the pollutant to be degraded (Niti et al. 2013). Many species of bacteria are known to degrade HCH isomers such as Clostridium rectum, Pandoraea sp. Kocuria sp. and Staphylococcus sp (Ohisa et al. 1980; Okeke et al. 2002; Kumar et al. 2016) but complete pathway of degradation of lindane is only known for S. paucimboilis UT26 (Nagata & Takagi 1999). Degradation of lindane by Kocuria sp. and Staphylococcus sp achieved 98% removal after 8 days (Table 5). The degradation of HCH by single specie and consortium both have been reported in literature, but a major drawback of utilizing single specie culture is the lack of complete degradation pathway therefore, to overcome this limitation microbial consortia are utilized where catabolic activity of mixed species together enables complete degradation of pesticide (HCH).

Biostimulation

In this technique microbial activity of native microflora of contaminated soil is enhanced by addition of nutrients such as nitrogen and phosphorous. Commonly accepted formula for biostimulation strategy is ratio of C/N/P as 100/10/1 (Shahi et al. 2016; Wu et al. 2019). A study was conducted in UP, India at a HCH dumping site close to a lindane manufacturing unit and results showed that biostimulation by application of soil nutrient, moisture and favorable aeration achieved <30% removal of HCH residues within 24 days (Dadhwal et al. 2009).

| Technology         | Advantages                                                                 | Disadvantages                                                                 |
|--------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Soil washing       | Reduce contaminant concentration hence ease further treatment of soil, it is commercially available. | Although concentration of contaminant is reduced but its toxicity is not altered, less effective for remediation of soil with high level of clay and silt, considerable disposal cost of subsequent waste stream. |
| Landfanning        | Short treatment time (6 month – 2 years), relatively simple and easy implementation | Not applicable for contaminants greater than 95% or lower than 0.1 ppm concentration, Large area required, Generates dust and vapor thus cause air pollution |
| Chemical remediation | Small size and high redox reactivity of zero valent iron nanoparticles are useful for environmental remediation | On the other hand, same properties of zero valent iron nanoparticles may be harmful for microbial population and plants |
| Bioreactor         | Ability to control and alter process operating parameters (temperature, pH, inoculum concentration, substrate and aeration), and increased bioavailability of contaminant, genetically modified microbes can be utilized in a bioreactor which can be destroyed afterwards before the treated soil is returned to its site. | Being an ex-situ treatment it requires more labor, capital and safety measures to transport contaminated soil to treatment site, many operating variables if any one variable is not well controlled or maintained it may become a limiting factor and reduce microbial activity in bioreactor. |
| Biostimulation     | Use native soil microbes for remediation, agro-industrial waste can be used as source of nutrients | Excessive supply of nutrients suppresses microbial activity and diversity |
| Bioaugmentation    | More efficient                                                             | Threat to native species, risk of introducing pathogenic microbes to the environment and risk of survival of inoculated microbes in new environment |
| Rhizoremediation   | Environment friendly, less expensive, conservation of soil, large scale operation | This method is time consuming, depends on pollutant concentration, bioavailability and toxicity to plant, slow growth rate and reduced root length may limit application. Also, plant harvest after remediation may incur additional cost. Plants lack catabolic enzymes to completely mineralize organic pollutants to carbon dioxide and water thus may transfer along food chain |
Biaugmentation

When native microflora fails to degrade target compound in contaminated soil than acclimatized consortia are inoculated to biodegrade target compound in a process called bioaugmentation. Studies reporting bioaugmentation so far have been successful at laboratory level but not at field level which might be due to the competition with native species (Tomei & Daugulis 2012). Saez et al. (2014) conducted a study to observe lindane degradation by inoculum of Streptomyces consortium in concentrated slurry (2:3 soil water ratio) and diluted slurry (1:4 soil water ratio) contaminated with 50 mg/kg lindane concentration. Results show greater removal of lindane in concentrated slurry (35.3 mg/kg) than in diluted slurry (28.7 mg/kg) after an incubation period of 7 and 14 days respectively. Garg et al. (2016) conducted a study at Ummari village in Lucknow, India, where a combination of both biostimulation and bioaugmentation techniques were applied for removal of HCH. A bacterial consortium (lucknowense F2 and S. sp. UM1) was applied which enable the degradation of HCH isomers, although β-HCH and δ-HCH isomers could not be completely degraded however they were converted into β-tetrachlorocyclohexane-1,4-diol and δ-tetrachlorocyclohexane-1,4-diol, which are less toxic forms as compared to the parent compound (Table 3).

Rhizoremediation

In this technique contaminants in the soil are degraded by the microbes present in the rhizosphere. Many advantages of rhizoremediation includes, low cost of installation and maintenance, eco-friendly, enhance physical, chemical and biological properties of soil and prevent soil erosion. However, there are some limitations also which are necessary to be considered while opting for this technique which incudes, plant resistance to contaminant, bioavailability of contaminant, suitable climatic condition for growth of plant, concentration of contaminant, duration of remediation and plant root depth (Azubuike et al. 2016). Abhilash et al. (2015) observed a successful rhizospheric degradation of lindane by Jatropha curcas sp (Table 3).

CONCLUSION AND RECOMMENDATION

It is concluded that HCH is one of the most dominant OCP in soil and sediments of Pakistan in light of above-mentioned literature. According to the Environmental Quality Standards, the current research suggests a high concentration of HCH exceeding (50 ng/g) as reported in the literature. Areas with high HCH levels include pesticide burial ground in Hyderabad, river Ravi and its tributaries, pesticide dumping site in Lahore, and Ittehad chemicals Kalashah Kaku. HCH contamination is identified various components of environment such as agricultural runoff, surface runoff from pesticide, dumping grounds industrial and municipal discharge into the water bodies thereby depleting, water, sediments and the aquatic organism which are consumed by the terrestrial organisms, thus effecting environment on a large scale. In current scenario, the water treatment containing HCH is not in practice in Pakistan. In the current situation, the treatment of wastewater containing HCH is not in practice in Pakistan. As a result, there is an urgent need to consider a more efficient way of handling such water before it reaches rivers.

Due to the persistent nature of HCH, soil is said to be a secondary source of emission for such pollutants. In order to prevent environmental pollution and health risk associated with HCH contamination, it is of utmost importance to control unplanned dumping of pesticides, monitoring of industrial effluents and municipal wastewater prior to the discharge into the major waterbodies. Pesticide regulatory standards should be established by the government for quality control and monitoring of soil and sound remediation strategies (low cost and high efficiency) should be introduced. A time frame must be enforced by the Stockholm convention to achieve HCH elimination by the signatory countries. Legal bodies should ensure proper implementation of existing laws and encourage further studies for risk assessment of contaminated areas.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 18 July 2020; accepted in revised form 17 March 2021. Available online 26 March 2021