Hexachlorocyclohexane toxicity in water bodies of Pakistan: challenges and possible reclamation technologies

Talat Ara, Waqar-Un Nisa, Muzammil Anjum, Luqman Riaz, Aansa Rukya Saleem and Malik Tahir Hayat

ABSTRACT

Pakistan is an agro-economy country where hexachlorocyclohexane (HCH) pesticides are being used to improve 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), and was therefore added to the list of ‘new POPs’ in 2009. This review report revealed that the major rivers of Pakistan such as the 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 a 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 a 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 the environment and their mitigation through proper treatment methods.

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 the agricultural sector has led to irreversible damage to the environment and natural resources due to exposure to 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 as a result, intensified the use of chemicals such as fertilizers, herbicides, and pesticides (Ali et al. 2014a, 2014b). Pakistan is an agricultural country with 25% of total land under cultivation and 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 for environmental degradation (Abrantes et al. 2010). In particular, hexachlorocyclohexane (HCH) has been extensively used since the 1940s as an effective insecticide for preventing vector-borne diseases and in agriculture (Chen et al. 2020). 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 was prohibited many years previously 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). The persistence, toxicity, and bioaccumulation of HCH in the environment 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 is a monocyclic chlorinated hydrocarbon commonly abbreviated to 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) has been used as a pesticide due to its strong insecticidal properties (Jennings & Li 2015). In the Stockholm Convention’s fourth meeting in May, 2009 HCH (lindane, α-HCH, β-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 foci for achieving the UN-Sustainable Development Goals. Pesticides use is one of the leading causes of land degradation and HCH has been widely used as an organochlorine pesticides over the past decades (Tripathi et al. 2019). For Pakistan, HCH in the environment is one of the region’s most pressing environmental issues, and current information is dispersed 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 70 of the most germane and encompassing national and international original research, review articles and newsfeed regarding persistent organic pollutants and HCH were scrutinized and summarized. Case studies of HCH in soil and sediments of Pakistan were collected from years 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 has been reported around the world over many years and up to the present day. Worldwide estimated use of all HCH was 40,000 tons in 1982 and 29,000 tons in 1990, whereas 5,900 tons of γ-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 the 1970s the use of technical HCH was restricted in many countries. The top three squanderers of technical HCH were China, India and the 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 tones until 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 use in public health protection. In the Soviet Union the use of technical HCH for agriculture was banned in 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 382,000 t and 81,000 t respectively (Breivik et al. 1999). Similarly, production of HCH has been stopped in the US since 1976 (Chen 2014). The overall estimated agricultural consumption of lindane reported in various countries amounts to 287.16 (Europe), 75.20 (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 (Kakhkashan et al. 2019). Numerous studies have been conducted in Italy, which reports HCH as the third most dominant OCP (Qu et al. 2018, 2019). Also the concentration in agricultural soil was higher compared with non-agricultural land (Qu et al. 2016). Fang et al. (2017) reported on HCH residues studied in top soil (1,890–205,000 ng/g) and deep soil (50–21,300 ng/g) samples from pesticide-contaminated sites, which is the highest concentration among all studies reported in this review paper, also higher than that observed in the pesticide burial ground in Hyderabad (Alamdar et al. 2014) and pesticide dumping ground in Lahore (Syed et al. 2014a). The occurrence of HCH in various soil and sediments in different regions of world is summarized in Table 1.

Nemr & Sadaawy (2015) investigated OCPs in surface sediments from the Mediterranean Sea and observed higher concentrations of HCH than reported in sediments in the coastal belt of Pakistan (Ali et al. 2014a, 2014b). The ratio of α-HCH/γ-HCH indicated recent input of lindane in the Mediterranean coast (Nemr & Sadaawy 2015). 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 the 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 their special properties such as toxicity, non-degradability, long range atmospheric transport
Table 1 | Global scenario of residual HCH in soil and sediments

<table>
<thead>
<tr>
<th>Country</th>
<th>Sampling site</th>
<th>Concentration ng/g</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>Surface sediment samples from northern Bering Sea, Chukchi Sea and adjacent Arctic Ocean</td>
<td>0.29–3.05</td>
<td>Kahkashan et al. (2009)</td>
</tr>
<tr>
<td>Southern Italy</td>
<td>Surface soil samples from territory of Benevento Province</td>
<td>Nd – 0.72</td>
<td>Qu et al. (2019)</td>
</tr>
<tr>
<td>Southern Italy</td>
<td>Sediment sample from gulf of Nepal and Salerno</td>
<td>0.37–1.10</td>
<td>Qu et al. (2018)</td>
</tr>
<tr>
<td>Mediterranean Sea</td>
<td>Sediment samples from Egyptian Mediterranean coast</td>
<td>0.07–45.79</td>
<td>Nemr &amp; Sadaawy (2013)</td>
</tr>
<tr>
<td>Kent</td>
<td>Top soil from rural and suburban area surrounding Nairobi city</td>
<td>Nd – 7.38</td>
<td>Sun et al. (2016)</td>
</tr>
<tr>
<td>Ningde, China</td>
<td>Paddy and vegetable farmland soil from hill region of Ningde</td>
<td>0.45–151.21</td>
<td>Qu et al. (2014)</td>
</tr>
<tr>
<td>China</td>
<td>Soil sediment from Old Yellow River Estuary</td>
<td>0.0014–14.85</td>
<td>Da et al. (2014)</td>
</tr>
<tr>
<td>South Korea</td>
<td>Soil from agricultural, industrial and urban areas</td>
<td>Nd – 0.385</td>
<td>Kim et al. (2014)</td>
</tr>
</tbody>
</table>

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 are 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 PD. Therefore, a case–control study was conducted by Richardson et al. (2011) at the University of Texas Southwestern Medical Center and Emory University, 283 serum samples of PD patients were investigated to determine any association between β-HCH and PD disease. Samples were collected and studied in two discrete periods from 2001–2003 and 2006–2008. Although the data obtained were consistent with the decrease in β-HCH in the environment from 2001–2008, results showed that higher levels of β-HCH in serum were associated with increased risk of PD disease (Richardson et al. 2011).

Non-degradability

POPs are resistant to degradation either through chemical, physical, biological or microbiological means, hence they are resistant and persistent in the environment for longer period of time (Aftul et al. 2010; Yadav et al. 2015). β-HCHs are less volatile and resistant to microbial degradation and hydrolysis, while α-HCHs and γ-HCHs are the more volatile isomers of HCH and can stay in the air and travel over long distances (Wu et al. 2013). 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 long distances away from the source through air and ocean currents (Hung et al. 2013; Nadal et al. 2015). Even though Antarctica is considered an isolated continent on Earth even there the presence of POPs has been recognized by scientists (Nash 2011). A study was conducted by Zhang et al. (2015) to assess OCPs residue levels, their distribution and potential sources in King George Island, West Antarctica. Samples were collected from different environmental matrices and analyzed using high resolution gas chromatography and high-resolution mass spectrometry techniques. The results indicated the presence
of 23 OCPs, among these DDT and HCHs were the main contaminants in all samples.

Bioaccumulation potential

HCH are lipophilic in nature, thus these can accumulate in fatty tissues, breast milk and blood showing lipid solubility (Williams 2008) and enter the food chain (Yadav et al. 2015). Human exposure to OCPs is mainly through consumption of contaminated food. Therefore, research was conducted to assess levels of OCPs and the potential human health risk associated with OCPs after consumption of edible cattle tissues (Mahmoud et al. 2016). Out of a total 135 random samples, HCHs were highest among OCPs and showed the 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 stay for longer periods on the target crops and later enter the human body through the food chain (Blushan 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 chains, certain limits have been proposed and developed which include maximum residue limits (MRLs), theoretical maximum daily intake (TMDI) and acceptable daily intake (ADI) (Blushan et al. 2015). FAO and the World Health Organization (WHO) have 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 were proposed in a Joint Meeting on Pesticide Residues (JMPR) and the Codex Alimentarius Commission. JMPR recommendations evolved after a thorough review of international data, along with the analysis of presence and cause and effect of pesticides (Fishel 2010; FAO & WHO 2011). The JMPR ADI value for lindane is 0.005 mg kg day$^{-1}$ Whereas, US Agency for Toxic Substances and Agency for Toxic Substances and Disease Registry (ATSDR) sets minimal risk levels that 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’s 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

HCHs cause serious negative effects on the ecosystem due to their properties of biomagnification, long-range transport and non-degradability. HCHs can cause serious health impacts including neurological, reproductive, and immunological disorders 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 a 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 ($\alpha$-HCH) is a structural isomer of HCH that has been used as an insecticide globally. $\alpha$-HCH was classified as a probable human carcinogen by the US 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, 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 proven 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 the reproductive tract. Khan et al. (2010) determine seminal HCH and its isomers in relation to semen quality and Y-chromosome microdeletion in the azoospermic factor (AZF) region. It was found that HCH and its isomers ($\alpha$, $\beta$ and $\gamma$) were observed in infertile groups similarly, major microdeletions were also observed in azoospermic patients. This study concluded that the male germ line is prone to potential mutagenic activity of HCH, which can lead to spermatogenic failure.

A study conducted in the Faroe Islands also reported a small, but compelling relationship between PD and serum levels of $\beta$-HCH (Petersen et al. 2008; Richardson et al.
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 the 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 the 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 were found 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 has ratified the Stockholm Convention (Ali et al. 2014a, 2014b). Apart from the environmental legislation on POPs there is a huge difference between legislation and implementation, despite the official ban in the country these pesticides are available in the market (Bhambro 2004). Pakistan is a signatory to all these conventions and therefore has 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 the population and the environment. Moreover, Pakistan lacks standard quality control criteria for residual concentrations 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 on HCH contamination in the country are limited due to lack of pesticide monitoring. During the year 2011–2018, a few studies were conducted in different parts of the country and 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 is considered the most polluted river in Pakistan (Syed et al. 2014b). 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 Nullah (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 & 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 reported high concentrations of HCH in sediments both in pre-monsoon and post-monsoon sampling which were higher than previous findings except for Lila stream and Nullah Deg (Malik et al. 2014; Syed et al. 2017b). 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. 2014). The values calculated for ratio of α-HCH and γ-HCH in water and sediment matrices were lower than 3 which showed fresh input of lindane (Baqar et al. 2018).

In another study (Syed et al. 2014b), the presence of HCH in River Ravi was reported. In this study, 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 to 43% of total HCH concentration 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, the concentration of γ-HCH was found higher than that of other isomers (Figure 2). The concentrations of HCH found in sediments were comparable with those detected in River Chenab sediments (Eqani et al. 2015). Syed et al. (2015) assessed the presence of OCP in soil along the sides of the River Ravi including industrial zones (Faisalabad, Phool Nagar, Lahore, Shahdara and Sheikhupura) and agricultural zones (Kabirwala, Khanewal, Mianchau, Cheechawatani

<table>
<thead>
<tr>
<th>Location</th>
<th>Concentration range in ng/g</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Ravi and tributaries</td>
<td>2.15–999.18</td>
<td>Baqar et al. (2018)</td>
</tr>
<tr>
<td>Mehmood Booti Drain Lahore</td>
<td>0.00–1.90</td>
<td>Ali et al. (2016)</td>
</tr>
<tr>
<td>Indus River catchment</td>
<td>0.74</td>
<td>Bajwa et al. (2016)</td>
</tr>
<tr>
<td>Nowshera district</td>
<td>1.67–32</td>
<td>Zehra et al. (2015)</td>
</tr>
<tr>
<td>Coastal belt</td>
<td>0.1–7.5</td>
<td>Ali et al. (2014a, 2014b)</td>
</tr>
<tr>
<td>Soan River</td>
<td>1.93–34.4</td>
<td>Malik et al. (2014)</td>
</tr>
<tr>
<td>River Chenab/Gujranwala division</td>
<td>4.54–18.9</td>
<td>Mahmoud et al. (2014)</td>
</tr>
<tr>
<td>Pesticide burial ground Hyderabad</td>
<td>15.5–4.090</td>
<td>Alamdar et al. (2014)</td>
</tr>
<tr>
<td>Indus basin</td>
<td>3.6–36.63</td>
<td>Sultana et al. (2014)</td>
</tr>
<tr>
<td>River Ravi</td>
<td>Nd – 22</td>
<td>Syed et al. (2014b)</td>
</tr>
<tr>
<td>Pesticide dumping site Lahore</td>
<td>Nd – 122</td>
<td>Syed et al. (2014a)</td>
</tr>
<tr>
<td>Punjab province</td>
<td>1.7–20</td>
<td>Syed et al. (2015)</td>
</tr>
<tr>
<td>Ittehad Chemicals Kalashah Kaku</td>
<td>24.71–121.71</td>
<td>Syed &amp; Malik (2011)</td>
</tr>
</tbody>
</table>

Figure 1 | Spatial distribution of organochlorine pesticides (OCPs) levels in sediments samples before and after monsoon (adopted and modified from Baqar et al. 2018).
The overall trend for OCP concentration in soil samples follow the order dichlorodiphenyltrichloroethane (DDT) > chlordane > Endosulfan > HCB. Hexachlorocyclohexane isomers were found in the majority of soil samples, whereas γ-HCH concentration was highest, which indicated use of lindane in Pakistan. The concentration of ΣHCH 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 the Ittehad Chemical Industry near the sample site. Although the factory stopped formulation of pesticide after the ban was imposed on OCP in 1994, it is reported that HCH residues are still found in the soil.

Lila stream near Lahore is one of the major tributaries of the River Ravi and receives water from several chemical manufacturing units. Syed et al. (2014a) found the residual concentration of ΣHCHs ranged from 0.0 to 121.17 ng/g with the highest values obtained for β-HCH as 0.0–120 ng/g residual concentration of HCH. For the source identification, different isomeric and parental ratios were calculated such as the ratio of γ-HCH to ΣHCH 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 historical use of technical HCH and lindane as a pesticide in the area. Also, β-HCH in soil and sediment samples accounted for 88.16% of the total HCHs in the study area and confirmed the persistent nature and low leaching ability of γ-HCH. Pakistan lacks any standard quality control criteria for pesticides, however according to Chinese Environmental Quality Standards the concentration of HCH in this area can be declared less polluted (<50 ng/g) except for the soil surface sample (SO4) 121.71 ng/g collected from a waste dumping site classified as slightly (50 ng/g–500 ng/g) polluted (Syed et al. 2014a).

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**

Mahmoud et al. (2014) reported the 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 \( \Sigma HCH \) (4.54–18.9 ng/g) was higher than those reported by Ali & Jabbar (1991) and Syed et al. (2014a) but lower than that reported by Alamdar et al. (2014) in an obsolete pesticide dumping site in Hyderabad, Pakistan. Eqani et al. (2012) 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, \( \Sigma HCH \) were among the dominant OCPs in the study area. Higher \( \Sigma HCH \) concentrations (1.77 to 7.59 ng/g) were detected in cotton-growing areas in region three which consisted of seven sampling sites, this concentration exceeded the Intrim Sediment Quality Guidelines (0.94 ng/g).

**Indus basin of Pakistan**

Indus Basin is drained by the Indus River that has an approximate length of 3,180 km and its tributaries support the agricultural activities in this region. The Central Indus Basin of Pakistan besides its socio-economic importance serves as a natural habitat for many species of the area; the Indus delta constitutes the 7th largest Mangrove forest in the world. International organizations are working for the conservation of the Indus Basin of Pakistan, such as the central Indus wetland complex and Indus Dolphin reserve designated by the WWF. Although the Indus Basin is of great ecological and agricultural significance but there is no sufficiently comprehensive study regarding the pollutant load on the Indus River due to anthropogenic activities in the area. Sultana et al. (2014) assessed the OCPs in the 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 a dolphin reserve and Taunsa Barrage has been designated by WWF Pakistan as a wildlife sanctuary. The 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 \( \Sigma HCH \) concentrations in these sites were found in order of HPN > DIK > Suk > RYK > TAU > CHA, whereas different isomeric concentrations of HCH detected in the area were \( \alpha \)-HCH (51% \( \Sigma HCH \)), \( \beta \)-HCH (22.8% \( \Sigma HCH \)) and \( \gamma \)-HCH (25% \( \Sigma HCH \)). The Indus River receives pollution due to high agricultural activities in associated areas, for instance DIK after building the Chashma Right Bank Canal led to the higher level of \( \Sigma HCH \) in the catchment. Similarly, higher levels of \( \Sigma HCH \) in Sukkar (downstream) samples were due to the narrowing down of the river, hence reducing the dilution factor and resulting in concentrated upstream contamination. The ratio of \( \alpha \)-HCH/\( \gamma \)-HCH in the study was reported to be 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 included Taunsa a wild life sanctuary and a Ramsar site, Guddu Sukkar a Dolphin reserve and KOT Mithan which is a conjunction point near Head Panjnad. Among all the selected sites the highest residual concentrations were found in soil samples from 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 the Soan River, polluting it with high amounts of hazardous chemicals. Malik et al. (2014) reported OCP contaminants in the sediments of the 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 the study. Highest \( \Sigma HCH \) concentration was detected in the Soan River (7.65–54.44 ng/g), however these concentrations were at relatively safe levels compared with published guidelines.

**Waste dumping site**

In Pakistan, as in other developing countries, municipalities are accountable for proper disposal of waste which consists 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 (Raaz 2013). Mehmood Booti drain located near the legitimate waste burial site of Lahore (Mehmood Booti waste dumping site) drains into the River Ravi. Ali et al. (2016) reported 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 areas. The overall OCPs concentrations were comparable with other studies in Pakistan, but the residue level of \( \Sigma HCH \) was far less than previous findings in Pakistan. The ratio of
α-HCH/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 for various industrial, agricultural, and anthropogenic activities. The district is also known for a famous DDT production unit which was operational until 1994. Soil from Nowshera has been reported for residual contamination of DDT (Khwaja et al. 2006) which is transferred to adjacent water bodies by the torrential rain and floods reported in the 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 organohalogens in surface soil in Pakistan for which about 28 surface soil samples were collected from selected sites based on land use type such as urban, industrial, and agricultural areas in the Nowshera district. HCHs were frequently detected in all soil samples ranging from 0.8–32.6 ng/g. Among the different land use types, the concentration of residual ΣHCH was highest in industrial samples. The order of occurrence of HCH isomers was as β-HCH > α-HCH > γ-HCH > δ-HCH and source of HCH was determined by isomeric ratios, α-HCG/γ-HCH ratio in the study area ranged from 0.14–9.26. Most of the samples showed lower values for the α/γ-HCH ratio, which indicated historic use of lindane, whereas a higher α/γ-HCH ratio was observed in a few samples, which reflected 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, the 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 (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. In total, 20 soil samples were collected from different land use types which included pesticide dumping sites, and residential, industrial, and background soils. The general trend of OCP occurrence was as ΣDDT > ΣHCH > chlordane > HCB and heptachlor. The highest ΣHCH concentration (43–4,090 ng/g) was detected in the pesticide burial ground and α-HCH (50–80%) was the dominant isomer. Residual concentration of HCH detected in this study was higher than others reported in Pakistan (Eqani et al., 2012; Syed & Malik 2011). According to Alamdar et al. (2014) the pesticide burial ground located in densely populated Hyderabad city was loaded with huge concentrations of OCPs which posed a 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 of the Indus River is released into the Arabian sea by two main rivers Malir and Lyari. The Malir river catches industrial waste from Korangi 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 a concentration of ΣHCH ranging from 0.1–7.3 ng/g was detected in Rohri Goth samples from close to civil and industrial settlements and catches sewage and industrial waste.

REMEDIAMTION TECHNOLOGIES FOR HCH

HCH is highly hazardous and has potential health risks, therefore numerous methods have been devised for the treatment of HCH from the 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, the remediation method involves excavation of contaminated soil for treatment on site and treated soil is returned to original location (Caliman et al. 2011). Soil remediation involves physical, chemical, and biological processes 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 pesticides to be eliminated, as well as site characteristics, and identify the 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 the 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) reported 99% removal efficiency of HCH contaminated soil from obsolete pesticide factory. Table 3 shows the results of studies using soil washing techniques, only a 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 involves excavated contaminated soil being 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 constraint (Morillo & Villaverde 2017); advantages and disadvantages of the technique are discussed in Table 4. Rubinos et al. (2007) reported up to 89% removal of HCH from heavily contaminated soils (>5 g/kg) by applying the land farming technique (Table 3).

**Chemical remediation technology**

Chemical remediation is one of the 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 an electron while other loses an electron and this reducing environment is favorable for breakdown of contaminants persistent in aerobic conditions. Zero-valent iron nanoparticles (nZVI) act as a chemical reductant and are used for the degradation of chlorinated compounds in contaminated soils (Morillo & Villaverde 2017). Cong et al. (2010) reported the 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 posed 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 & Villaverde 2017) followed by a series of biological reactions under aerobic or anaerobic conditions and feed modes. The advantages of this technique over other bioremediation techniques 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%) were increased then the degradation efficiency of the bioreactor was reduced (Quintero et al. 2007).

**In-situ remediation techniques**

**Microbial degradation**

In this technique remediation of contaminated sites can be achieved by utilizing native microflora (bacteria, fungi, algae or actinomycetes), the most important parameter in this technique is the microbial diversity of contaminated sites and the nature of the pollutant to be degraded. 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
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days (Table 3). The degradation of HCH by a single species and consortium both have been reported in the literature, but a major drawback of utilizing a single species culture is the lack of a complete degradation pathway therefore, to overcome this limitation, microbial consortia are utilized where catabolic activity of mixed species together enables complete degradation of the pesticide (HCH).

### Biostimulation

In this technique, the microbial activity of native microflora of contaminated soil is enhanced by addition of nutrients such as nitrogen and phosphorous. A commonly accepted formula for biostimulation strategy is the 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 an 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).

### Bioaugmentation

When native microflora fails to degrade target compounds in contaminated soil then acclimatized consortia are inoculated to biodegrade target compounds 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 inoculation of a *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 showed a 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 with 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 include low cost of installation and maintenance, eco-friendliness and enhanced physical, chemical and biological properties of soil and prevention of soil erosion. However, there are some limitations that need to be considered while opting for this technique which include, plant resistance to contaminant, bioavailability of contaminant, suitable climatic condition for growth of plant, concentration of contaminant, duration of remediation and plant root depth (Azbuike 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 OCPs in soil and sediments of Pakistan in light of the 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 the pesticide burial ground in Lahore, and Ittehad chemicals Kalashah Kaku. HCH contamination was identified by various components of the environment such as agricultural runoff, surface runoff from pesticides, dumping grounds, and 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 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, and 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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