Polycyclic Aromatic Hydrocarbons in Food Plants and their Immobilization through Biochar

(PhD Thesis)

Submitted
By
Muhammad Waqas

Department of Environmental Sciences
University of Peshawar
Session 2009-2010

Polycyclic Aromatic Hydrocarbons in Food Plants and their Immobilization through Biochar

Thesis submitted to Department of Environmental Sciences University of Peshawar in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Sciences

Submitted By

Muhammad Waqas

Department of Environmental Sciences

University of Peshawar

Session 2009-2010

بسم الله الرحمن الرحيم

This thesis is dedicated to my Parents&**Wife**

For their love, patience, prayers, and encouragement

Approval Sheet

1. Supervisor: ____________________
   Dr. Sardar Khan
   Associate Professor
   Department of Environmental Sciences,
   University of Peshawar

2. Co-Supervisor: ____________________
   Dr. Yangguan Zhu
   Professor and Director General
   Institute of Urban Environment, CAS, Xiamen, China

3. Examiner: ____________________
   Dr. Abdul Wahid
   Professor and Chairman
   Department of Environmental Sciences
   Bahauddin Zakariya University, Multan

4. Chairperson: ____________________
   Dr. Shahida Nazreen Zakir
   Department of Environmental Sciences,
   University of Peshawar

Special Thanks to

TWAS (Third World Academy of Science)

CAS (Chinese Academy of Science)

for their financial support

and

IUE(Institute of Urban Environment)

Xiamen, China providing me every facility

in their laboratory to complete my research

TABLE OF CONTENTS

ACKNOWLEDGEMENT

First and above All praises to Almighty Allah, for providing me health, capability and strength to complete my research work successfully. Blessing and Peace of Allah be upon last Prophet Muhammad (Peace Be upon Him).

My cordial thanks to my supervisors Dr. Sardar Khan Associate Professor in Department of Environmental Sciences, University of Peshawar and Professor Yangguan ZHU Director General of the Institute of Urban Environment, Xiamen China who mobilize each and every resource to complete my research.

I want to express my deep thanks to my beloved wife for the insightful discussion, offering valuable advice and especially for her patience and guidance during the writing process.

I would like to thank the research scientists, specially: Chai Chao, Huang Qing and students group of Professor Yongguan ZHU, laboratory assistants for excellent technical assistance, who helps us in every step, to provide better guidance in the laboratory work in the Institute of Urban Environment (IUE), Xiamen, China and I am also thankful to Brian J. Reid School of Environmental Sciences, University of East Anglia, Norwich, UK.

I am grateful to the Department of International Cooperation in IUE, Xiamen China especially Lily and Grace for her kind assistance.

I extend my thanks to all of my colleagues and friends for their valuable services and pleasurable company specially Muhsin from my village and Muhammad Hamid Aljabar from Albieda University Yemen and Xien from Huaqiao university (HQU) Xiamen, China I will never forget the time that we were together and discussed about lab work, religion and politics and their continuous cooperation Therefore, I can just say thanks for everything and may Allah give you all the best in return. I am grateful for the financial support of the Chinese Academy of Sciences (CAS) and Third world Academy of Sciences (TWAS) during my PhD last year research work.

I would like to thanks to my family: my parents supporting me morally throughout my life.

Muhammad Waqas, 2014

Institute of Urban Environment
Chinese Academy of Sciences
1799 Jimei Road, Xiamen 361021, China http://www.iue.cas.cn

Post Graduate Research Certificate

Mr. Muhammad Waqas conducted his PhD research work in the key lab of Urban Environment and Health, Institute of Urban Environment under my supervision for one year. His work focused on biochar effects on minimization of bioavailability of PAHs in soil and their subsequent bioaccumulation by food crops. He conducted various greenhouse experiments to investigate the fate of PAHs in soil and plants in the presence of biochar. He has also assessed the PAH load in the soil and vegetable collected from wastewater irrigated farms. I found him a hard working person and satisfied with the work done by him during his stay in IUE.

I wish him best wishes for his future carrier.

Yongguan Zhu

Professor Yong-Guan ZHU, PhD (London)
Director General
Associate editor, Environment International (Elsevier)
1799 Jimei Road, Xiamen 361021, China
Tel: 86 592 6190997; Fax: 86 592 6190977
Email: ygzhu@rcees.ac.cn

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and carcinogenic compounds accumulating in the soil ecosystem and subsequently causing contamination of food chain. The present study was conducted to evaluate the concentrations of 16 PAHs (declared carcinogenic by US-EPA) in the soil and vegetable irrigated with wastewater collected from 11 districts of Khyber Pakhtunkhwa Province, Pakistan. The sum of 16 total PAHs ranged from 222.6-928.6 μg kg^{-1} in the soils. The highest PAH concentration was observed in the soil collected from district Peshawar (high urbanized) while the lowest PAH concentration was observed in the soil collected from district Laki Marwat (less urbanized) which was within the permissible limit (4.23 mg kg^{-1}) set by the Ministry of Environmental Protection, China. PAH concentrations in vegetable were ranged from 51.6-402 μg kg^{-1}. The benzo[b]fluoranthene, chrysene, fluoranthene and pyrene were abundantly present in the soil samples whereas naphthalene, phenanthrene fluoranthene and pyrene were frequently observed in selected vegetable. PAH concentrations were higher in leafy vegetable (*L. sativa* > *S. oleracea)* as compared to underground bulb (*A. cepa* and *A. sativum*). The concentrations of high molecular weight (HMW) PAH compounds were lower in vegetable as compared to low molecular weight (LMW) PAHs. In high urbanized districts, daily intake (DI) of individual PAHs through consumption of vegetable ranged from 2.13E-04 to 4.19E-02 μg $kg^{-1}d^{-1}$ in adults, while ranged from 2.54E-04 to 2.30E-02 μg $kg^{-1}d^{-1}$ in children. In less urbanized districts, DI-PAHs through consumption of vegetable ranged from 1.77E-04 to 5.57E-02 μg kg-$^{1}d^{-1}$ in adults, while ranged from 7.26E-04 to 3.85E-02 μg $kg^{-1}d^{-1}$ in children. The uniqueness of this study is to quantify the concentrations of PAHs in the soil and vegetable collected from a large scale area of Khyber Pakhtunkhwa Province which is rapidly urbanizing. The health risk of the human exposure to PAHs through the consumption of

vegetable was also calculated using different models. Among the high urbanized districts, the highest total toxic BaP equivalent (TEQ) value (7.2) was observed for pyrene followed by naphthalene (4.9) for the vegetable collected from Mardan, while the lowest mean TEQ value (0.12) was observed for acenaphthylene followed by benzo[k]fluoranthene (0.26) in Peshawar. For the whole province, the highest TEQ value (4.1) was observed for flouranthene followed by naphthalene (3.8).

Looking into PAH recalcitrance, availability, bioaccumulation and toxicity, it is necessary to immobilize PAHs present in contaminated soil using different amendment techniques such as biochar, activated carbon etc. In order to compare the effectiveness of biochar towards the minimization of PAH and PTE availability and bioaccumulation, different biochars were prepared from different feedstocks using pyrolysis technique. Biochars changed the soil pH and also increased EC, NO_3-N and NH_4-N, available P, and dissolved organic carbon (DOC) concentrations which reduce the available concentrations of PAHs and PTEs in the soil and their uptake/bioaccumulation in food crops.

During this research, different control environment experiments were conducted to investigate the effectiveness of biochar towards minimization of PAH and PTE availability in soil and their subsequent bioaccumulation in vegetable. In control environment (greenhouse), sewage sludge (SS) and sewage sludge biochar (SSBC) were used to investigate their effects on PAH mobility and their uptake rate in vegetable. However, the presence of contaminants such as PAHs and potentially toxic elements (PTEs), including As, Cd, Cu, Pb and Zn, restrict the direct application of SS in agricultural land. This research established that the conversion of SS to SSBC significantly ($P \leq 0.01$) decreased PAH and available PTE concentrations. Once added to soil both SS and SSBC significantly ($P \leq 0.05$) decreased PAH availability. Bioaccumulation of

PAHs in cucumber (*Cucumis sativa* L) was reduced by both SSBC (44.3-57.1%) and (to a lesser extent 19.9-35.6%) by SS. Following addition to soil SSBC significantly (P≤0.05) reduced available PTEs (except Cd), while SS significantly (P≤0.05) increased PTE availability. Consequently, SSBC significantly (P≤0.05) reduced PTE bioaccumulation (except Cd), while SS increased PTE bioaccumulation. These results suggest SSBC to be a candidate for soil amendment that offers advantages over SS in terms of PAH/PTE bioaccumulation mitigation.

Another control environment experiment was conducted to confirm the effects of SSBC on availability and uptake of PAHs and PTEs using tomato *(Solanum lycopersicum)* plant. This study aimed to investigate the effects of SSBC on mobility and uptake rate of PAHs and PTEs by *S. lycopersicum* plant grown in wastewater irrigated soil contaminated with PAHs and PTEs. SSBC amendments significantly (P≤0.01) reduced PAH and available PTE concentrations in soil. Bioaccumulation of PAHs and PTEs in *S. lycopersicum* was reduced by the amendments of both SSBC (48.0-62.4%) and SS (21.5-39.3%). Available fractions of PTEs were significantly (P≤0.05) decreased in SSBC amended soil, while significantly (P≤0.05) increased in SS amended soil. Thus SSBC amendment significantly (P≤0.05) decreased PTEs bioaccumulation, while SS enhanced PTEs bioaccumulation in *S. lycopersicum*. These findings also confirmed our previous results and suggest that SSBC can be used for soil amendment to reduce the availability and bioaccumulation of both PAHs and PTEs.

The third control environment experiment was conducted to compare the influence of SSBC with other biochars prepared from different feedstocks/biomass such as peanut shell biochar (PNBC), soybean straw biochar (SBBC) and rice straw biochar (RSBC). This research established to verify the bioavailability of PAHs and PTEs using different biochars and their uptake in turnip (*Brasic rapa*). The addition of different biomass biochars significantly (p≤0.05) reduced the PAHs and PTEs. The bioaccumulation of PAHs in the *B. rapa* decreased by 70.77-82.57% ,40.46-69.43%, 10.24-48.51% and 16.91-32.55% by adding PNBC SBBC, SSBC and RSBC respectively. The addition of biochars significantly ($p \leq 0.05$) reduced the accumulation of PTEs such as As, Cd, Cu, Fe, Pb and Zn in *B. rapa*. The effectiveness of PNBC in reduction of PTEs was more significant ($p \leq 0.05$) as compared to SBBC, SSBC and RSBC. The results of this research summarized that PNBC was more effective in the reduction of accumulation of PAHs in food crops, but it should be apply in a proper quantity. The PAH bioaccumulation rate was observed in order of PNBC >SBBC>RSBC>SSBC. Similarly, PTE uptake can be reduced by increasing the application rate of different biochars. The uptake rate of PTE varied because of their characteristics and vegetable types. However, all the biochars showed great effectiveness in reducing the availability and accumulation of of PTEs but depended on the application rates.

On the basis of findings, it is concluded that long term wastewater irrigation leads to contaminate the soil with PAHs and PTEs and then bioaccumulate in vegetable up to the extent of high risks. Furthermore, this study highlights that biochar can help to reduce the availability of these selected contaminants in soil ecosystem and their bioaccumulation in grown vegetable which can protect the consumers along with enhancing the agricultural yields.

Chapter-1

Introduction

1.1 The Issue

Soil contamination with polycyclic aromatic hydrocarbons (PAHs) is increasingly prevalent throughout the world due to numerous manmade activities particularly wastewater irrigation, sludge applications, solid waste disposal and fossil fuel burning and natural activities such as forest fires and volcanic eruption caused very rare soil contamination with PAH in the range of 1-10μg kg^{-1} (Edwards, 1983). The use of wastewater in the agriculture sector is rising due to the water scarcity, population growth and urbanization. However, this wastewater contained substantial amount of nutrients, PAHs, heavy metals (HMs), and pathogenic microorganisms which have both beneficial and adverse impacts on the farming sector (Chen at al., 2005; Singh et al., 2004; Lehmann et al., 2011; Zhang et al., 2011).

Soil is the ultimate repository for PAH either it is originated from natural or anthropogenic activity. PAH levels in the urban soil are 10-100 times higher than in remote soils due to industrialization in the past 100-150 (Wild and Jones, 1995). Jones et al. (1989a) mentioned in his report that total PAH concentration (0.1-55 mg kg^{-1}) in Welsh soils resulting from atmospheric deposition with wind transportation with no direct industrial toxic waste.

Wastewater is considered as both a source and a problem in developing and developed countries. Wastewater irrigation is a common practice to increase cropyield because of its three major macronutrients such as potassium (K), nitrogen (N), and phosphorous (P) which are essential for cropgrowth. The farmers extensively use wastewater for increase in their crop yields near the urban cities without using the fertilizers by saving money. Besides these positive impacts with continuous application of wastewater may cause the excessive accumulation of HMs, persistent organic pollutants (POPs) such as PAHs and pathogens which cause a negative impact on the human health and environment (Scott et al., 2000). In

Asia, the 35% wastewater is treated, 14% in North America and in Africa only a small proportion is treated. The remaining water is discharges into rivers, canals, lakes without treatment (WHO/UNICEF, 2000).This water is used for irrigation near the urban vicinity and downstream areas. Wastewater is used on an estimated 20 million ha or 7% of the total global irrigated area (WHO, 2006).

In Pakistan, sewage water irrigation is very common practice and it is estimated that 32,000 ha land is irrigated with sewage water without prior treatment and 26% of all produce irrigated with wastewater in Pakistan (Ensink et al.,2004).

Polycyclic aromatic hydrocarbons are mainly accumulated in the soil irrigated with wastewater and/or amended with sewage sludge which causes contamination of the food plants. Plants take up these pollutants through various pathways,including root uptake and atmospheric deposition from gaseousor particulate forms (Edwards, 1988).The bioaccumulation of PAH in food plants is considered as one of the serious concerns because of the pervasive and carcinogenic nature (Jian et al., 2004). PAHs are persistence lipophilic organic pollutants formed due to incomplete incineration of organic materials. Translocation rate is greatly altered by the molecular weight, structure, water solubility, and vapor pressure of each PAH compound (Jones et al., 1996; Pierzynski et al., 2000; Reid et al., 2000a). Low molecular weight (LMW) PAHs are rapidly degraded and volatized as compared to high molecular weight (HMW) PAHs. HMW-PAH is recalcitrant, not easily degrade because of their low volatility, resistance to leaching (Jones et al., 1996; Wild and Jones, 1995). PAH compound solubility in water depends on temperature, pH, ionic strength (concentration of soluble salts), and other organic chemicals (i.e. dissolved organic carbon) (Pierzynski et al., 2000). Solubility increases with the decrease in the number of benzene rings (Wilson and Jones, 1993). The octonol-water partition co-coefficients (K_{ow}) are expressed as log K_{ow}. If the PAH has low value of Log K_{ow}, it will have greater solubility (Mackay and Callcott, 1998). PAH compounds of high vapor pressure will be more soluble in water ((Mackay and Callcott, 1998). PAHs accumulate in vegetation mainly through atmospheric wet deposition in soil and transfer through roots in the plants. Concentrations of PAHs in plant tissue in non industrialized regions range from 50-80 μg kg^{-1} (Edwards, 1986). Several mechanisms are involved in bioaccumulation of PAHs (Fryer and Collins, 2003; Wild et al., 2004) which depends upon plant species, type of PAH compounds and environmental conditions (Salanitro et al., 1997).Urban vegetables uptake higher PAHs than rural vegetation (Juhasz and Naidu, 2000) because of the vehicle emissions and sewage irrigation near urban vicinity. Vegetables uptake PAHs in enough quantities from soil and atmospheric wet deposition on plant leaves and their ingestion is the major exposure pathway. Human can be exposed to PAHs through ingestion of contaminated food plants. In human, the PAHs upon ingestion of vegetable are metabolized and stored or excreted. The stored PAH impacts the neurological, respiratory and reproductive systems and cause cancers of lung, urinary bladder and respiratory tract. Therefore it is necessary to monitor and minimize the transportation and bioaccumulation of PAHs in food plants. Different materials such as activated carbon, charcoal, biochar are used as a soil conditioner/amendment to minimize the PAH uptake by food plants.

1.2 Biochar

Biochar is a carbon (C) rich material produced by the process of pyrolysis under continuous flow of nitrogen or anoxic conditions (Cao et al., 2011). Biochar properties are distinguished from the charcoal and can be used as a soil amendment (Lehmann and Joseph, 2009). Biochar has been reported to have considerable agronomic value; this being associated with improved soil fertility, improved ecosystem functioning and sequestration of C (Sohi et al., 2010). The application of biochar brings changes in the soil properties including increase of pH, cation exchange capacity, and bulk density and also improves nutrient retention. Similarly, biochar application can also affect the rooting pattern in soil ecosystem on account of changes to micro-and macropores (Downie et al., 2009), which contribute to air and water storage.

1.3 Biochar as a potential solution

Biochar can reduce the bioavailability of hydrophobic organic compounds including PAHs and toxic HMs due to its sorptive capacity (Wang et al., 2011). Biochar application is, expected to improve the overall sorption capacity of soils and consequently, influence toxicity, transport and fate of trace contaminants, which may be already present or are to be added to soils (Yang and Sheng, 2003).

The application of biochar has attracted the attention of environmental scientists throughout the globe due to its associated environmental, economic and agronomic effects. In the past, numerous papers have been published exclusively documenting the effects of biochar on bioavailability of PAHs and HMs in soils (Beesley et al., 2010; Peng et al., 2010; Chen and Yuan, 2011; Gomez-Eyles et al., 2011). Zhang et al. (2010) indicated the biochar derived *Pinus radiataha* was effective in the sorption of hydrophobic organic compounds such as phenanthrene from soil. Furthermore, the application of biochar into contaminated soils can also minimize the bioavailability of organic contaminants to earthworms, microbes and plants (Yu et al., 2009; Cao et al., 2011). Biochar characteristics such as high porosity and large surface area are responsible for high affinity for organic contaminants. The biochar effectiveness against PAHs and HMs bioavailibity depends on different factors such as source materials of biochar, pyrolysis conditions, concentrations of contaminant, complexation and decomplexation of biochar and contaminants, contact time and soil organic carbon (Chen and Yuan, 2011; Khan et al., 2013).

However, less work has been conducted to determine the effectiveness of sewage sludge biochar on immobilization or sorption of PAHs and HMs, its contribution to PAH and HM level in soil and influence upon their bioaccumulation by plants.

1.4 Study objectives

The aim of the present study was to:

1. Investigate the vegetable uptake of PAHs from contaminated soils and assess the potential risk to public health.
2. Assess the effects of biochar on bioaccumulation of PAHs and HMs using greenhouse experiments
3. Determine the effectiveness of biochar application on PAH and HM immobilization or sorption.

1.5 Study area description

The research area of this study is limited to the province of Khyber Pakhtunkhwa (KPK) occupies the north western part of Pakistan has an area of 74,521 km. The province has total population more than twenty million with the annual growth rate of 2.8%. Geographically province could be divided into two zones.

The districts wise land utilization and population are explained in table 1.1.

Table 1.1 Districts wise population , land utilization of Khyber Pakhtunkhwa Province in 2007-08

Districts	Reported Area (ha)	Population(1998)	Density People/km^2	Total Cultivated Area(ha)	Total uncultivated area(ha)	Not available for cultivation(ha)
Peshawar	126661	2018742	1606	78892	47769	27986
Nowshera	169479	1026095	665	52555	116924	60674
Mardan	162085	1460640	895	112769	49316	38181
Swabi	148689	1026095	665	87046	61643	8508
Charsadda	98646	1021896	1026	73445	25201	16102
Kohat	295054	562445	221	71197	223857	183707
Hangu	132265	314839	287	27251	105014	83035
Karak	265201	431616	128	75429	189772	167402
Lakki Marwat	314990	490420	155	116917	198073	112153
Bannu	118958	676077	551	74129	44829	28541
Tank	165599			49661	115938	7154
DeraIsmail(DI) Khan	730575	849816	116	233077	497498	132576

SOURCE: Agriculture Statistics, NWFP, Peshawar.

1.5.1 Central Khyber Pakhtunkhwa

The central Khyber Pakhtunkhwa is dominated by Peshawar basin, a fertile valley covered with consolidated deposits of silt, sands and gravel of recent geological times. Peshawar, Nowshera, Mardan, Swabi and Charsadda are main districts of central Khyber Pakhtunkhwa.

1.5.2 Southern Khyber Pakhtunkhwa

As one moves further away from the foothills of the Himalaya and Karakoram ranges, the climate changes from humid subtropical to typicallyarid climate. Kohat, Hangu, Karak, Laki Marwat, Bannu and DI Khan are the main districts present in centralKhyber Pakhtunkhwa, where the temperature is extremely hot as compared to central Khyber Pakhtunkhwa.Over population and unplanned growth of the city, transportation and industrialization are the important factors which strongly affect the environmental condition. The major industries such as marble, match, steel, textile, paper, embroidery carpets, wood works and leather industries are present in and around the cities, which are considered as the main source of contamination of food chain with heavy metals and PAHs (Sadia et al., 2006).Geographically the province could be divided into two zones as shown in map Fig.1.1.

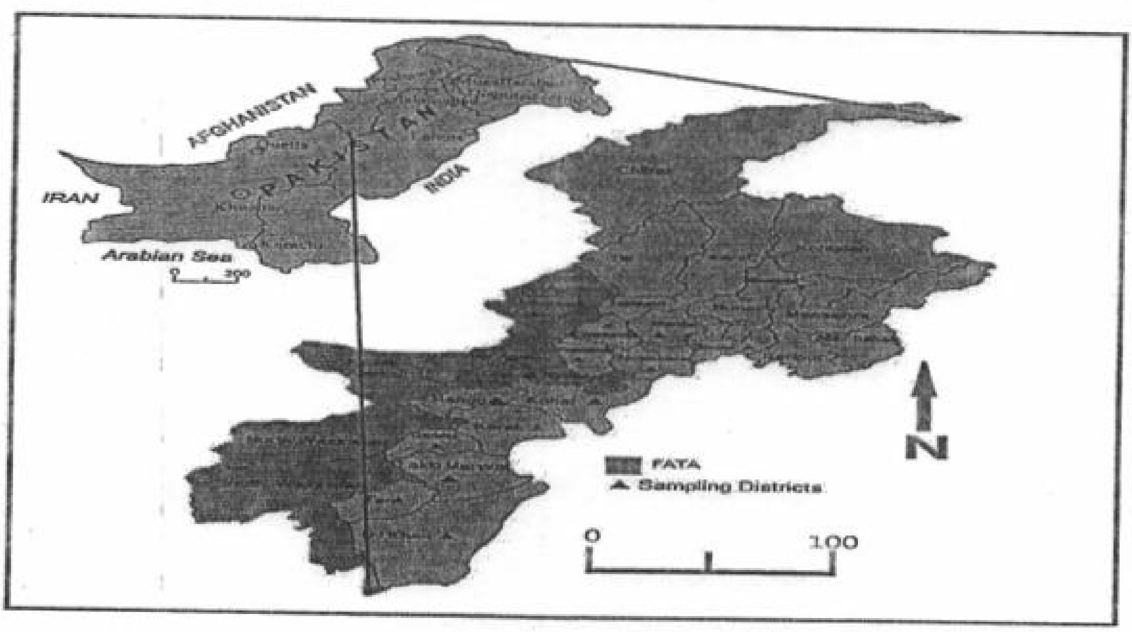


Fig. 1.1Study area map showing the selected cities of the KPK Province.

Chapter-2

Wastewater irrigation, HMs, PAHs, human exposure and their minimization through biochars

A mixture of industrial and municipal wastewater is used for irrigation purposes in some parts of Pakistan, particularly in suburb of major cities. Wastewater contains high level of nutrients such as N, P, K, organic nutrients, salts particularly sodium (Na), chloride (Cl), HMs such as chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni) and zinc (Zn) and organic pollutants such as PAHs. HMs and PAHs are persistent and non biodegradable, therefore, accumulate in food chain through soil and then transfer to human body by ingestion of contaminated food, inhalation of contaminated soil or dermal contacts (Steven et al., 2004). However, long-term irrigation with mixtures of domestic and industrial wastewater has resulted in accumulation of HMs and PAHs in agricultural soil which has brought a potential health risk to human due to consumption of contaminated food (Song et al., 2006).

2.1 Heavy metals

The soil environment is sinking for pollutants such as heavy metals and metaliodsincluding arsenic (As),Cd, iron (Fe),Pb and Zn. These elements show different properties in soil regarding their bioavailability, mobility and uptake in plants. Long term wastewater irrigation causes implications to the agricultural soil including phytotoxicity at a high concentration and effect the soil microbial process and transfer of pollutants to food chain. Different sources including application of SS, agrochemicals and fertilizer, atmospheric deposition and irrigation with wastewater are involved to cause soil contamination with HMs and PAHs (Nicholson et al., 2003).

SomeHMs such as cobalt (Co), Zn and copper (Cu) are essential elements for normal body growth, while the high amounts intake ofthese elements causes health risk in human and aquatic life. There arevarious non essential elementssuch as As,Cd, Pb and Hg which cause health risk even at low concentrations (Caussy et al., 2003).

Table2.1 Toxicological soil quality criteria and cut-off criteria for soil set by different organizations.

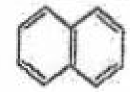
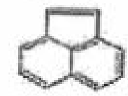
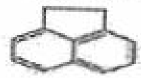
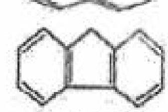
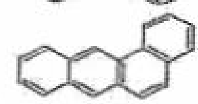
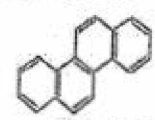
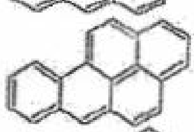
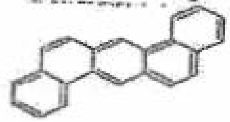
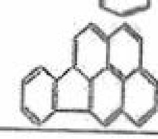
Metals	Dry weight(DW) ($mg\ kg^{-1}$) Danish EPA* Soil quality criterion	Cutoff criterion.	DW ($mg\ kg^{-1}$) SEPA** Soil quality criterion
As	20	20	NG***
Hg	1	3	NG
Cr	500	1000	250
Cd	0.5	5	0.6
Cu	500	500	100
Pb	40	40	350
Zn	500	1000	300
Ni	30	30	60

*Danish EPA(2000) **SEPA (State of Environmental Agency in China, 1995) ***Not given

2.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons are ubiquitous in nature and found in air, water, soil and biological systems, mostly originated from incomplete burning of carbon containing materials (Tang et al., 2005; Tao et al., 2006; Zohair et al., 2006 and Peng et al., 2011). PAHs contain two or more benzene rings arranged in cluster, linear and angular ways. Low temperatures produce more complex PAHs mixtures as compared to high temperature (Gao and Zhou, 2004).PAHscategorized into two groups such as low molecular weight (LMW) containing 2 or 3 benzene rings and high molecular weight (HMW)containing 4,5 or 6 benzene rings (Wenzl et al., 2006). The presence of PAHs in the nature isof great concern because of their health risk. The USEPA has listed 16 possible carcinogenic PAHs in the environment as given in Table 2.2.Due to rapid urbanization the concentrations of PAHs are increasing in the urban streams, canals particularly due to the municipal waste and agriculture wastes (Van Meter et al., 2000).

Table 2.2 PAHs names, benzene rings and structure formulas declared as carcinogens by US-EPA (1993).

PAHs	Abbr.	No rings	Molecular wt.(g $mole^{-1}$)	Benzene Ring structure
Naphthaene $C_{10}H_8$	NA	2	128.17	
Acenaphthene $C_{12}H_{10}$	AC	3	154.21	
Acenaphthylene $C_{12}H_8$	ACN	3	152.2	
Anthracene $C_{14}H_{10}$	AN	3	178.23	
Phenanthrene $C_{14}H_{10}$	PHE	3	23	
Fluorene	Fl	3	166.22	
Fluoranthene $C_{16}H_{10}$	FLA	4	202.26	
Benz(a)anthracene* $C_{18}H_{12}$	B(a)A	4	228.29	
Chrysene* $C_{18}H_{12}$	CHR	4	202.26	
Pyrene $C_{16}H_{10}$	PY	4	228.29	
Benzo(a)pyrene* $C_{20}H_{12}$	B(a)P	5	252.32	
Benzo(b)fluoranthene* $C_{20}H_{12}$	B(b)F	5	252.32	
Benz([k)fluoranthene*$C_{20}H_{12}$	B(k)F	5	252.32	
Dibenz(a,h)anthracene*$C_{22}H_{14}$	D(ah)A	6	278.35	
Benzo(g,h,i)perylene *$C_{22}H_{12}$	B(ghi)P	6	276.34	
Indeno(1,2,3-cd)pyrene*$C_{22}H_{12}$	IP	6	276.34	

2.3 Sources of PAHs

The PAHs created due to the natural and anthropogenic sources ultimately deposits in the soil. The sources andway of affecting human health are explained in Fig.2.1.

There are several anthropogenic sources of PAH these include

1. Wastewater irrigation
2. Sewage sludge application to farmlands
3. Automobiles exhaust
4. Incomplete combustion of fossil fuels
5. Solid waste disposal

The natural sources contribute little PAHs concentration to the nature including the volcanic processes, forest fires and some bacteria and plants formed PAHs (Wild S.R., 1995, Xu et al., 2006).

PAHs come to the water body through wastewater discharge from different industries, houses and leakage of PAHs containing fuel. The PAHs comes to the air coal fired boiler, industrial incinerator, petroleum industries, asphalt production plants and the automobile sources which use petroleum.

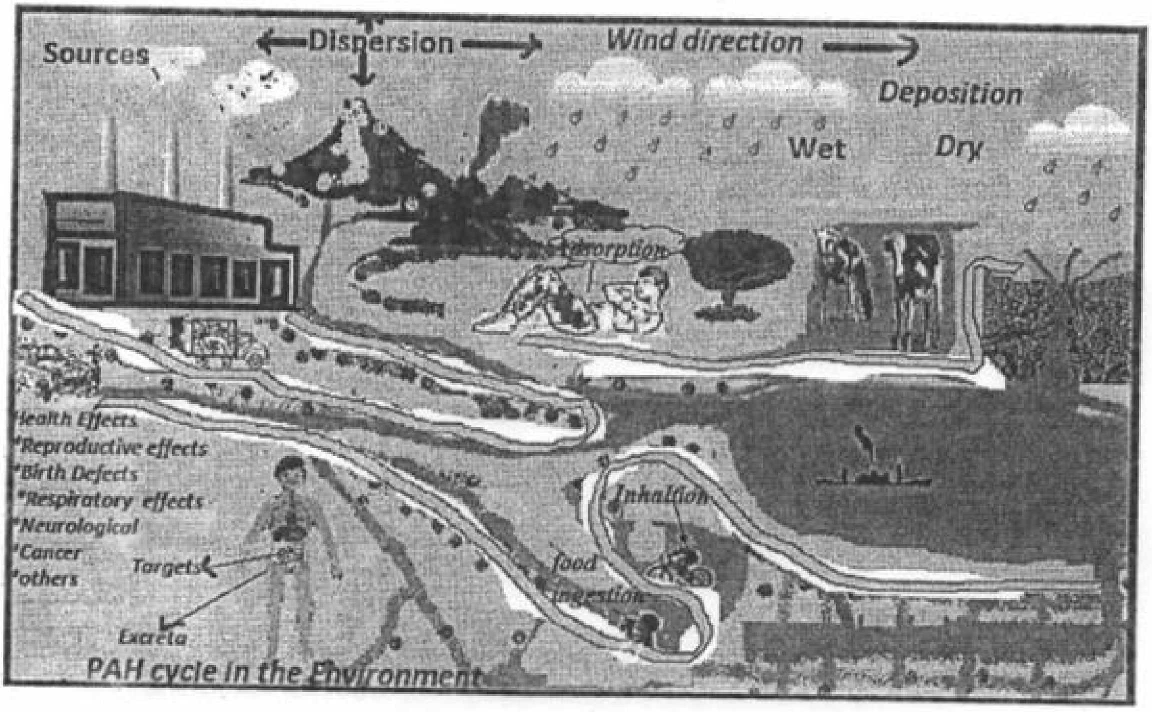


Fig.2.1PAH cycle in the environment

2.4 Human exposure pathways to PAHs and heavy metals

Human exposure pathways of pollutants present in the contaminated soil environment can be summarized as follow:

1. Pathway-1: soil-water-human
2. Pathway-2: soil-plants-human
3. Pathway-3: soil-plants-animals-human

Direct (Ingestion, inhalation and dermal contact)

4. Pathway-4: soil-human (Khan et al.,2008)

2.5 Physio chemical properties of PAHs

The physiochemical properties of PAHs compounds are very important to understand its bioavailability and bioaccumulation in food crops and its transportation and degradation. The PAH transformation rate is altered greatly by the molecular structure, weight, water solubility, and vapor pressure of each PAH compound (Jones et al., 1996; Pierzynski et al., 2000; Reid et al., 2000a).

2.5.1 *ChemicalStructure*

The PAHs chemical structure depends on the number of benzene rings, when number of benzene rings increases, its solubility decreases, not easily degrades in the environment (Wilson and Jones, 1993).

2.5.2*Molecular Weight*

HMW PAHs consists of more than 3 benzene rings shows high persistence in the environment because of less water solubility, high lipophilicity, and low vapor pressure and thus not easily degrade in the environment (Jones et al., 1996).

LMW PAHs consist of 2 or 3 benzene rings easily degrade and evaporate in the nature because of more solubility in the water and high vapor pressure (Harvey, 1997).

2.5.3*Vapor Pressure*

Each PAHs compound have different Vapor pressure at normal temperature if the vapor pressure is high then it will be easily evaporate and degrade in the environment and will shows more solubility in Water (Mackay and Callcott, 1998).Naphthalene (11.866 Pa) is more volatile compared with dibenz (a,h) anthracene (2.80×10^{-9} Pa) at normal temperatures. Vapor pressures of selected PAH are presented in Table (2.3).

2.5.4 *WaterSolubility*

Solubility of PAH compounds in water are dependent upon temperature, pH, ionic strength (concentration of soluble salts), and other organic chemicals (i.e. dissolved organic carbon) (Pierzynski et al., 2000) There is an inverse relationship between octonol-water partition coefficients (K_{ow}) and solubility which is determined with the following equation:

K_{ow}= amount of organic chemical in octanol (mg L^{-1})/amount of organic chemical in water (mg L^{-1}).Acenaphthylene has a log K_{ow} of 3.92,whileBenzo (ghi) perylene has value of 6.5.In this case ACN is more soluble thanB(ghi) P.

Table 2.3PAH solubility in water, vapor pressure and octonol-water partition coefficients (K_{ow})

PAH	SOL. in H_2O(mg L^{-1})	Log K_{ow}	Pressure(Pa)
NA	31	3.37	11.866
ACN	3.8	3.92	0.5
AC	16.1	4	3.866
AN	0.045	4.54	3.40×10^{-3}
PHE	1.1	4.57	9.07×10^{-2}
FL	1.9	4.18	0.432
FLA	0.26	5.22	1.08×10^{-3}
B(a)A*	0.011	5.91	2.05×10^{-5}
CHR*	0.132	5.91	1.04×10^{-6}
PY	0.0015	5.18	5.67×10^{-4}
B(a)P*	0.0038	5.91	6.52×10^{-7}
B(b)F*	0.0015	5.8	1.07×10^{-5}
B(k)F*	0.0008	6	1.28×10^{-8}
D(a,h)A*	0.0005	6.75	2.80×10^{-9}
B(g,h,i)P*	0.00026	6.5	1.33×10^{-8}
IP*	0.026	6.5	1.87×10^{-8}

2.6 PAHs in Soil

PAHs enter to soil environment through anthropogenic activities such as wastewater irrigation,industrial emissions both aerial deposition and effluent discharge, disposal from sewage treatment plant,long range transportation, the application of soil manure and synthetic fertilizer (Maliszewska-Kordybach,1993).

PAHs and other organic pollutants are persistent in nature have low metabolic rate and easily absorbed and soil environment act as long term sink for these contaminates (Nicholson et al., 2003).PAHs concentration is 10-100 times higher in metropolitan soil as compared to the rural area soil (Wild and Jones, 1995). However natural processes such as emission from volcanoes, forest fire but its concentration is very low in the range of 1-10μgg^{-1} in soil (Edwards, 1983).

2.7 PAHs in Plants

Plant can act as pollutant indicator of a particular area. The organic pollutants enter to the plant body through several pathways such as wet and dry deposition of particles-bound on leaves, gaseous diffusion in cuticle and stomata, active and passive uptake from plant root through water phase.PAHs uptake in plants are effecting several factors such as initial soil concentration,plants species composition and soil chemical properties such as Hums contents and soil microbial population (Kapusta, 2004).

In rural areas the PAH concentrations in plant tissues range from 50-80 $\mu g\ kg^{-1}$ (Edwards, 1986), However these depend on plant species and environmental condition and the type of PAH (Salanitro et al., 1997).It is recorded that vegetation in metropolitan areas have 10 times higher PAH concentration then in rural area (Juhasz and Naidu, 2000).

2.8 Human exposure to PAHs

Contamination of food chain is one of the major routesof exposure to these persistent pollutants in to the human body. In mid of 1930s it was identified that PAHs are carcinogenic andMenzie et al., 1992 stated in his work that a human body daily expose to these toxic pollutants,1-5 ng $d^{-1}$from food, absorb 0.0002-0.12 ngd^{-1} PAH from water,0.02-3.0 ngd^{-1} PAH from air. In the ambient environment the PAHs concentration is high in winter season as compared to summer due to the less fossilfuel burning (Harvey, 1997).

Kuo et al., 2003 states in his report that inhaling PAHs range 0.4-1.55ng d^{-1} causedrespiratory tract cancer in human. While, Menzie et al., 1992 studied that leafy vegetables, fates, grains, oils, grilled and smoked meat containing higher amount of PAHs (10ng kg^{-1}) than other food stuff.It has not been established if exposure to PAHs results short-term health effects. However, combined with other chemicals PAHs may be the cause of short-term symptoms such as confusion, diarrhea, eye irritation, nausea, vomiting and PAHs cause possibly long-term health effects including cataracts, jaundice, kidney, liver damage, skin redness and irritation and the breakdown of red blood cells.The International Agency for Research on cancers, US Environmental Protection Agency (EPA), and US department for Health and Human Services (HHS) has classified the 16 PAH as a possible carcinogenicity (See table 2.4).**Table 2.4**Classification of PAHs by IARC, US-EPA& HHS

Agency	PAH	Carcinogenic classification
International Agency for Research on Cancer(IARC)	B(a)F B(k)F I(1,2,3-c,d)P	Possibly causes cancer to human
	AN B(g,h,i)P B(e)P CHR FLA FL PHE PY	Not classifiable as to their carcinogenicity to humans
	B(a)A B(a)P	Probably carcinogenic to human
U.S. Environmental Protection Agency (EPA)	ACN AN B(g,h,i)P FLA FL PHE PY	Not classifiable as to human carcinogenicity
	B(a)A B(a)P B(b)F B(k)F CHR D(a,h)A I(1,2,3-c,d)P	Probable human carcinogens
U.S. Department of Health and Human Services (HHS)	B(a)A B(b)F B(a)P D(a,h)A I(1,2,3-c,d)P	identified as animal carcinogenicity

2.9 Enhancement of soil properties and crop yield using biochar

Biochar added to soil help in sequestration of carbon and reduce the GHG emission .besides these properties biochar amended soil increased pH, EC i in comparison with control soil (Liang et al., 2006).The soil treated with biochar cause reduction in N leaching .The soil treated with biochars can decrease the nitrate leaching (Novak et al., 2010; Deenik et al., 2010). Similarly the soil treated with biochars and bioslids shows less leaching in comparison with control soil (Deluca et al., 2009). The study has been conducted to investigate the bioavailablity of P in soil with the interation b/w P and F(ferrihydrit) in the presence of biochars ,the results shows greater enhancement in the bioavailability of P(Cui et al., 2011) this is due to the changes of soil environment for microorganisms(Atkinson et al., 2010). The further research work is needed to investigate the mechanism of soil fertility.

The biochars added to soil can increase crops yields in sense of nutrients uptake with increase biochars application rate. 15 and 20 tha^{-1} biochar added to maize crop and increased the productivity 98% and 150 % respectively (Uzoma et al., 2011).

The biochar added to soil also enhance plant resistance property against fungal disease, the study has been conducted on tomatoes and pepper plants , the result shows that fungal disease was reduced in pots amended with biochar (Elad et al., 2010) So biochars is served as disease control agent(Harel et al., 2012).

Real-time PCR results indicate that improvement of plant responses to disease can be one of the great advantages gained from biochar application to soil (Elad et al., 2011).

The biomass of rice enhances in biochar amendment soil through increase protein in leaves, the plants rapidly renew to sustain photosynthesis by increasing the degradation rate of protein and synthesis in biochar treated soil (Noguera et al., 2012).

Table 2.5 The effects of different biomass biochars on surface area and porosity at various temperatures.

Biomass	Temp.	SAa (m^2g^{-1})	TPV_b(mgg^{-1}) or MPA_c (m^2g^{-1})	Adsorbate	Effects	Reference
Broiler litter manure	350	59.5±19.7	0c	Ni2+, Cd2+	350BI< 700BI	Uchimiya et al.,2011
	700	94.25.1	41.820c			
Corn stover	350	293		ND_d	ND_d	Nguyen et al., 2009
	600	527				
Cotton seed hulls	350	4.7±0.8	ND_d	Ni2+,Cu2+ Pb2+, Cd2+	Total concentration of soluble metal ions in soil interstitial waters in the order of: CH350<CH500<CH650< CH800	Uchimiya et al.,2011
	500	0	ND_d			
	650	343	0.007±0c			
	800	322±1	274±1c			
Oak wood	350	450	ND_d	ND_d	ND_d	Nguyen et al.,2009
	600	642	ND_d			
Pine needle	100	0.65	ND_d	NAPH, NB, m-DNB	The concentration of adsorption in the order of P100 < P200 < P300 < P400 < P500 < P600 < P700	Chen et al.,2008
	400	112.4	0.0442b			
	500	236.4	0.0952b			
	600	206.7	0.0764b			
	700	490.8	0.186b			
Soybean stalk	300	144.14	ND_d	Methylene Blue	The amount of ethylene blue in order of of BC300< BC400 < BC500 < BC600 < BC700	Kong et al.,2008
	400	138.76				
	500	152.98				
	600	179.03				
	700	250.23				

TPV_b means total pore volume; MPA_c means microspore area; SA_a means BET surface area.
ND_d= Not determined

2.10 Remediation of contaminated environment by biochar

PAH can cause adverse impacts on the environment if these contaminants transfer from soil to plant through roots and degrade the underground water quality through leaching. Biochars can adsorbs these contaminates due to large surface area and special structure(Cao and Harris,2010).The biochars prepared at 500 °C from dry manure removed Pb and atrazine from aqueous solution 100% and 77% respectively. The biochars prepared from different biomass shows great adsorption for dioxins (Chai, et al., 2012), polycyclic aromatic hydrocarbons (Chen et al., 2011), pesticides (Uchimiya et al., 2012), and other contaminants. The biochars shows great effectiveness in terms of reduction in PTEs from aqueous solution and soil. The rice straw biochar reduce on the mobility and bioavailability of Cd (II) Cu (II), and Pb (II) in an Ultisol. The acid extractable Pb (II) and Cu (II) decreased by 19.7-100% and 18.8-77.0% with the increase in biochar amendment dose (Jiang et al., 2012)

The biochars also immobilize the HMs and release with less rate to the environment and exert less impacts on the environment. The study has been conducted on the immobilization of HMs in multi elements contaminated soil, the results shows the biochar can retain As, Cd and Zn and reduced Cd and Zn by 300- and 45- fold respectively but leaching concentration was not reduced (Beesley and Marmiroli, 2011).

Many studies have been conducted on the green waste compost, biochars and SS , that effect the solubility and bioavailability of PTEs. The concentration of Cu and Pb was less in reygrass shoots by adding green waste compost and biochars to soil (Karami et al., 2011). Similarly, in case of the application of SS and SSBC on soil characteristics has been studied, the result shows that SSBC less plant availability of Cd Ni, Pb and, Zn and reduce leaching of Cu, Ni and Zn (Mendez et al., 2012).

Biochars have the ability to remove organic pollutants from soil, water, sediment and also reduce the bioaaviabliy of these organic contaminant to plants and then to organisms, sulfamethoxazole (SMX) leaching from soil reduced by 2-14 % in biochar application while without biochars 60 % leeching occurred(Yao et al., 2012). The free dissolved concentration of PAH in SS can be decreased by adding biochar upto 57 %(PCP leaching can also be reduced by using bamboo biochar in soil upto 42% which prevent the further contamination of surface and groundwater and reduce availability of these contaminants to plant (Oleszczuk et al., 2012). The biochar addition to soil can reduce total PAH concentration (449-306 mg kg -1) bioavailable PAH 276-182 mg kg^{-1} and concentrations of PAH in earthworm Eisenia fetida (up to 45%).(Gomez et al., 2011)The research shows that biochar is a powerful tool to remediate the organic contaminants. it is very important to study the mechanism of biochar adsorption and sorption based on many available research. Sorption incorporate both surface adsorption as well as pollutant molecules resides in the partition of biochar microspores where as adsorption refers to the surface interactions leading to adhesion of contaminant molecules to surface biochar (Chen et al., 2011).

The adsorption of organic pollutants to biochar made at the high temperature whereas the sorption mainly due to partition because partition occurs in the uncarbonized fraction (Kwon et al., 2005).

The pine needle biochars prepared at 100-700°C to study the sorption mechanisms.The resultas shows at low pyrolysis the partition was dominant while at high temperature the adsorption was dominant in needle biochars(Chen et al., 2011).The quantification of organic pollutants adsorption and partition based on relative non carbonized and carboinized fraction biochar surface area and bulk property (Chun et al., 2004) to identify the sorption of PAHs on biochar paticles X-ray microscopy method was used and found that phenanthrene deposits in primary "cracks on biochars particles (Obst et al., 2011).The pep interaction mechanism and pore felling showed contribution less desorption of organic pollutants and increased sorption in the presence of biochar (Qiu etal., 2009). Desorption kinetic indicated that the amended soilthrough biochar contribute to slow desorption and strong binding of PCP in soils(Wen et al.,2009).Biochars application enhance the sorption ability in terms of contaminants such as anionic (Hiller et al., 2007) cationic (Sheng et al., 2005) and diuron (Yang and Sheng, 2003). Previous research work demonstrated that chemical structure and properties (i.e. hydrophobicity molecular weight, planarity) as well as surface ,pore volume influenced adsorption to charcoals (Cornelissen et al., 2004; 2005; Zhu and Pignatello, 2005; Zhu et al., 2005; Wang et al., 2006; Chen et al., 2007).

The biochar introduction to soil can change its adsorption properties due to environmental factors such as the impacts of co-existing.The organic compound with high hydropobicity reduce the adsorption of LMW compounds to biochars. (Sander and Pignatello, 2005; Wang et al., 2006).Biochar can be used to encourage remediation of contaminated soil as microbial carriers (Chen et al., 2012).

Biochars can also be used in immobilization of other contaminant such as nutrient element (Yao et al., 2011) and radionuclide (Kumar et al., 2011). Agriculture activities contribute organic waste to the environment agriculture waste such as crop and animal manure are used to agriculture land to improve soil fertility because of abundant organic components and essential element that plants need (Westeman et al., 2005).

The mechanism of contaminated soil remediation with biochars is shown in Fig. 4.2.

However the use of agriculture waste to soil may cause high risk because of runoff and leaching of P and N which destroy the surface and underground water quality and raw waste addition to soil accumulate excessive amount of element that may contaminate the environment .whereas the conversion of SS to SSBC decrease the risk of HMs leaching and also occur reduction in the bioavailability of HMs to plants (Mendez et al., 2012).

The biochar application account for 80-90% of total uptake of trace HOC in soils and sediment (Cornelissen et al., 2005).So converting of agriculture waste into biochar is a best way of waste management (Fig.4.1;4.2) Composting is one of method for recycling of agriculture waste but direct use of this waste to plant can cause toxicity (Bulter et al., 2001).this process can be promote by converting this waste into biochar which is source for carbon and energy source for micro organisms (Adhikari et al., 2009). Composting of poultry manure reduce the smell and the loss of nitrogen with a balance nutrient composition (Dias et al, 2010). Biochars provide alternative methods for disposing of agriculture wastes.

However biochar is also a matter of great concern for research because of its toxicity, because biochar consist of PAHs. If the level of pollutants in material of biochar surpass the standard, it will cause threaten to environment once they are added to soil or water system but the amount of this PAH concentration depends pyrolysis temperature, the sum of 16 PAH significantly decreased in SSBC in our study as compare to SS, Various research shows that PAHs inside biochar is unavailable to plants, however biochar adsorb the organic contaminants on the surface of biochar and Cause partition of organic contaminats molecule in the micro pore of biochar (Kwon et al., 2005; Chen et al., 2011).

Chapter-3

Quantification of PAHs and Health Risk via Ingestion of Vegetable in Khyber Pakhtunkhwa

3.1 Introduction

Food crop contamination with polycyclic aromatic hydrocarbons (PAHs) is a growing problem in the key metropolitan cities of the world due to wastewater irrigation, sewage sludge application, automobiles exhaust, solid waste disposal, and industrial activities (Shi et al., 2005; Khan et al., 2013). These pollutants accumulate in the soil through irrigation and aerial deposition and then crop uptake these pollutants from the soil through roots (Khan et al., 2007) and atmospheric aerial deposition on plants which affect the food quality (Li et al., 2008).Air and root uptake are considered as the main pathways of PAH accumulation in vegetable but their entry mostly depends upon variety of vegetable, locality and nature of PAH compounds (Wang et al., 2011).

Like many other countries, the wastewater irrigation is a common practice in Pakistan. The farmers prefer to use it because of its beneficial macro and micro nutrients needed for plant growth (Jan et al., 2009 Sing et al., 2010; Khan et al., 2013). Besides these agronomic inputs the wastewater cause contamination of soil and food crops (Hua et al., 2013) through excessive accumulation of persistent organic containinants such as PAHs which cause adverse impacts on environment and human health (Scott et al., 2000). Poor quality of groundwater and lack of fresh surface water for irrigation have resulted in the use of wastewater for irrigation in urban and peri-urban areas in Pakistan. The increase in population and urbanization caused the shortage of fresh water which led to increase the recycling of urban wastewater in agriculture sector. Total discharge of wastewater from 14 main cities of Pakistan was 1.83×10^7 m^3 h^{-1} (FAO, 2002), while the total amount of wastewater produced in Pakistan was 962,335 million gallons (4.369×10^9 m^3y^{-1}). A current countrywide wastewater assessment has shown that total water supply is 4.6 x 106 m^3 day^{-1}, and about 30% of wastewater is used for irrigating an area of 32,500 ha (Ensink et al., 2004). Characteristically, PAHs are persistent hydrophobic contaminants consisting of two or more aromatic benzene rings, present in wastewater. PAHs can travel over a long distance before deposition and may accumulate in the food crops which might cause human health risk (Zohair, 2006). PAH contain both low molecular weight (LMW-PAHs) and high molecular weight (HMW-PAHs) compounds. HMW-PAHs are more stable and toxic than LMW-PAHs (Wenzl et al., 2006), which are more volatile, water soluble and less lipophilic compounds (Ferrarese et al., 2008). US environmental protection agency (US-EPA) has classified the 16 priority pollutants out of more than 100 PAHs and seven of HMW-PAHs consider being possible carcinogenic (Michael et al., 2004).

Soil is the ultimate storehouse for the PAHs either it is instigated by anthropogenic or natural activities. The chemical structure, molecular weight, vapor pressure and water solubility are greatly changed the rate of PAH transformation (Reid et al., 2000a). HMW-PAHs remain stable in the environment due to low volatility, resistance to leaching and recalcitrant nature (Jones et al., 1996). LMW-PAHs persist for short time in the environment due to high volatility, water solubility and less lipophility as compared to HMW-PAHs. Solubility of PAH compounds decreases in water, with increasing the number of benzene rings and also depends upon the pH, ionic strength, temperature, and the presence of organic materials (i.e. dissolved organic carbon) (Pierzynski et al., 2000). PAH bioavailability in the environment affected by soil characteristics (organic matters, texture and aggregation) and physiology of the receptors (Stokes et al., 2006; Harmsen, 2007) which are changing with time and weathering (Uyttebroek et al., 2007).

The food crop uptake these carcinogenic PAHs through roots from the soil and accumulate them in their tissues (Jian et al., 2004). This food chain rout is considered as one of the serious concerns and results in 90% ingestion of contaminants into the human body (Martorell et al., 2011). After exposure, PAHs cause damage to DNA as a result cause mutations, reproductive effects and cancer of lungs, urinary bladder and respiratory tracts (Bosetti et al., 2007). 30% of human cancers are thought to be caused through exposure to these chemical compounds present in the food (Mansour et al., 2009). PAH translocations from soil to plant depend on various factors including plant species, soil microbial community and initial soil concentrations (Kapusta, 2004). Fismes et al. (2002) mentioned that vegetable (carrot, potato and lettuce) can uptake PAHs from contaminated soils. Similarly, Camargo and Toledo (2002) also observed PAHs in grapes, apples, pears, tomatoes, cabbages and lettuces grown in Brazilian rural areas located nearby urban centers.

In Pakistan, a few studies have been conducted regarding PAHs in the environment (Khan et al., 2008; Farooq et al., 2011). However, no work has been undertaken regarding PAH uptake, transportation and bioaccumulation in vegetable grown on wastewater-irrigated soils in Khyber Pakhtunkhwa (KPK) Province, Pakistan. In the study area, agricultural farms are irrigated with wastewater containing a mixture of PAHs and as a result cultivated-vegetables can absorb these pollutants at high level. Therefore, this study aimed to monitor the PAH concentrations in wastewater irrigated soil and vegetable, their uptake, translocation and the human health risk associated with ingestion of contaminated vegetables.

3.2 Material and methods

3.2.1 *Study area description*

This study was conducted in 11 districts (having highly fertile land and extensively cultivated with vegetable) of KPK Province, Pakistan. The main cities (Peshawar Nowshehra, Swabi, Mardan, Charsadda, Kohat, Hangu, Karak, Lakki Marwat, Bannu and DI Khan) of this province (Fig.1.1) were selected for soil and vegetable samplings. The farmlands in these urban/suburb areas irrigated with wastewater (mix of industries, transport, car washing stations and domestic/residential sectors). The farmers prefer to irrigate their crops with wastewater because of containing high nutrient contents. The wastewater irrigation has caused the contamination of soil and vegetable with PAHs which may be caused serious risk for human.

3.3 Soil sampling and characterization

Soil samples (0-20 cm) were randomly collected from agricultural lands, freeze dried, sieved through 2 mm mesh and stored at -20°C in paper envelopes until further analyses. The basic characteristics such as DOC, EC, pH, N, C, S, particle size and bulk density were measured as shown in table 3.1.

3.4 Plant Sampling and preparation

Standing vegetable samples including mustard (*Sinapis alba*), radish (*Raphanus sativus*), coriander (*Coriandrum sativum*), cauliflower (*Brassica oleracea* B.), cabbage (*Brassica oleracea* C.), carrot (*Daucus carota*), turnip (*Brassica rapa*), pea (*Pisum sativum*), lettuce (*Lactuca sativa* L.), garlic (*Allium sativum*), spinach (*Spinacia oleracea*) and onion (*Allium cepa*) were collected from the same sites where soil samples were collected. At harvest, plants were washed with tap water to remove all visible soil particles and finally with double deionized water (DDW). The washed plant samples were freeze-dried, ground and stored at -20°C for further analyses.

Table 3.1 Physicochemical characteristics of field soil in khyber pakhtunkhwaProvince

Parameters		Peshawar (n=32)	Nowshera (n=28)	Mardan (n=27)	Swabi (n=30)	Charsadda (n=27)	Kohat (n=28)	Hangu (n=27)	Karak (n=28)	Lakki marwat (n=27)	Bannu (n=29)	DI Khan (n=26)
pH	Range	7.96-8.16	7.91-8.16	7.22-8.0	7.95-8.16	7.6-8.24	7.03-8.2	6.7-8.1	6.76-7.8	6.5-7.3	7.78-8.1	7.6-8.4
	Mean,±	8.2±0.31	8.0±0.10	7.71±0.3	8.1±0.30	8.02±0.6	7.63±0.74	7.43±0.59	7.22±0.42	7.1±0.30	7.8±0.15	7.8±0.46
EC	Range	116.1-428.9	120.7-428.9	95.07-170.6	120.23-207.02	150.23-224.02	906.54-1100.08	731.43-956.61	1653.41-2351.31	1243-1657	1305.78-1695.53	304.69-397.93
(μScm^{-1})	Mean,±	239.92±103.93	264.6±136.41	139.67 ±28.2	146.4±35.29	168.8±45.634	963.14±76.40	850.54±81.10	2032.93±265.82	1453±250.21	1513.47±116.49	361.76±35.14
TC (%)	Range	2.01-3.67	3.02-3.69	1.21-2.49	3.02-3.69	1.25-1.57	1.32-3.79	1.43-3.21	1.61-2.93	1.21-2.32	0.4-1.5	0.5-0.9
	Mean,±	2.64 ±0.55	3.4 ±0.22	1.95 ±0.6	1.95 ±0.03	1.39 ±0.137	2.39±0.95	2.03±0.46	2.37±0.34	2.13±0.21	0.81±0.41	0.64±0.74
TN (%)	Range	0.10-0.13	0.13-0.18	0.10-0.18	0.08-0.16	0.08-0.11	0.004-0.008	0.09-0.13	0.11-0.14	0.13-0.17	0.06-0.14	0.043-0.083
	Mean,±	0.14 ±0.04	0.2 ±0.02	0.14±.03	0.12 ±0.28	0.10 ±0.016	0.005 ±0.0002	0.07±0.002	0.09±0.001	0.11±0.003	0.10±0.004	0.06±0.02
TS (%)	Range	0.015-0.020	0.02-0.05	0.02-0.04	0.01-0.02	0.014-0.016	0.01-0.03	0.02-0.031	0.029-0.034	0.027-0.037	0.013-0.017	0.1-1.11
	Mean,±	0.02 ±.002	.03±0.01	.03±0.01	.02±.002	0.02 ±0.001	0.019±0.001	0.02±0.003	0.03±0.001	0.02±0.002	0.01±0.003	0.78±0.34
DOC (%)	Range	1.13-2.5	0.63-1.19	1.29-1.47	0.51-0.83	1.13-2.7	0.31-1.67	0.29-1.53	0.37-1.74	0.22-1.84	0.31-2.76	0.47-0.86
	Mean,±	1.43±0.60	0.90±0.24	1.370.07	0.67±0.12	1.76±0.13	0.98±0.52	1.05±0.41	1.01±0.51	1.17±0.31	1.91±0.58	0.71±0.18
Soil Texture (%)	silt	28.21	72	69.21	40	67.1	41.96	7.1	5.1	6.3	37.93	50
	Sand	60.47	17	18.43	47.4	18.6	29.73	61.43	65.3	64.2	17.83	30
	Clay	11.32	11	12.36	12.6	14.3	28.31	31.47	29.6	28.5	44.24	20
Textural class		Sandy loamy	Silt loamy	Silt clay	Sandy loamy	Silt loamy	Silt loamy	Sandy clay	Sand clay Loamy	Sand Clay Loam	Clay loam	Silt loamy
Bulk Density	Range	1.37-1.46	1.19-1.61	1.31-1.52	1.21-1.38	1.36-1.57	1.03-1.68	1.02-1.61	1.07-1.62	1.02-1.57	1.03-1.65	1.39-1.63
	Mean,±	1.41±0.04	1.39±0.18	1.43±0.05	1.37±0.23	1.53±0.18	1.48±0.24	1.47±0.17	1.37±0.25	1.35±0.62	1.44±0.21	1.51±0.19

3.4.1 Chemicals

All solvents were HPLC/spectro grade purchased from Tedia Company Inc, USA. Surrogate standards (PAH-Mix 24 deuterated, LA20950024HE) and reference materials (PAH-Mix 9, XA20950009CY) were purchased from the laboratory of Dr. Ehrenstorfer, Augsburg, Germany.Silica gel, alumina and sodium sulfate were purchased from Sinopharm Chemical Reagent Co Ltd., China.

3.4.2 pH and Electrical conductivity(EC)

pH and EC (n=3) were measured in deionized water suspensions of soil (1:2.5 w/v), using Accumet XL 60 meter equipped withboth pH and EC electrodes.

3.4.3 Nitrogen , carabon and Sulfur

Total C, N and S in soil, theseparameterswere measured (n=3) using amacroelementor(VarioMax CNS, Germany). For this dry combustion process, soil (400 mg) samples were processed using combustion temperature (H1) 1140°C, post combustion (H2) 800°C and reduction (H3) 850°C, while He flow was 680 ml/min as mentioned in the operational manual.

3.4.4 Soil particle size

Soil particle size was measured using the Mastersizer 2000 (Malvern Instruments Ltd, UK.

3.4.5 Dissolved organic carbon (DOC)

The value of DOC was extracted (n=3) with 40 ml of K2SO4 solution (0.5 M) from 4.0 g moist sample by shaking for 1 h at 200 rpm (Feng et al., 2012). After centrifugation, the supernatant was filtered and analyzed for DOC using Total Carbon Analyzer (TOC-VCPH Shimadzu, Japan).

3.4.6 PAH Extraction

The powdered samples of vegetable (1 g) and soil (3 g) were extracted for PAHs with dichloromethane (DCM) and acetone (1:1 ratio) using accelerated solvents extraction (ASE, Dionex-350). After reducing these extracts to 1 ml through a rotary evaporator, silica chromatography columns were used to purify the samples (Khan et al., 2008; Waqas et al., 2013). For columns elution, a 60 ml mixture of hexane and DCM (7:3) was used and the eluted fractions were again evaporated up to 1 ml and transferred to Kuderna-Danish concentrator and rinsed with 10 ml of n-hexane. Thereafter, the eluted fraction was again reduced to 1 ml under nitrogen flow and transferred to a 2 ml vial capped with a Teflon-lined septum for analysis of PAHs.

The final concentrated extracts were analyzed for 16 PAHs such as naphthalene (NA), anthracene (AN), acenaphthylene (ACN), acenathphene (AC), fluorine (FI), benzo(b)fluoranthene (BbF), phenanthrene (PHE), fluoranthene (FLA), pyrene (PY), benzo(b)anthracene (BbA), chrysene (CHR), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-c,d)pyrene (IP), dibenzo(a,h)anthracene (D[ah]A), benzo(g,h,i)perylene (B[ghi]P) using GC-MS (Agilent Technologies 5975C).

3.4.7 Quality *control*

The effectiveness of ASE extraction and silica column purification for PAH recovery from soil and vegetable extracts was checked using surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10). The results showed satisfactory recovery, with the average recovery ranging from 83.7±8.2% to 94.6±5.4%. Similarly, reagent blanks were also included to check the contamination of PAHs during extraction and purification processes.

3.5 Data analysis

3.5.1 Transfer factor

The concentrations of 16 PAHs in the extracts of vegetable and soil were calculated on the basis of dry weight. Plant concentration factor (PCF) was calculated as follows:

$$PCF = \frac{C_{plant}}{C_{soil}}$$ (Cui et al., 2004)

Where C_{plant} and C_{soil} represent the PAH concentrations in plants and soils on dry weight basis, respectively.

3.5.2 Daily intake of PAH

The daily intake (DI) of PAH was determined by the following equation:

$$DI = \frac{C_{PAH} \times C_{factor} \times D_{food\ intake}}{B_{average\ weight}}$$

Where C_{PAH}, C_{factor}, $D_{food\ intake}$ and $B_{average\ weight}$ represent the PAH concentrations in vegetable in micrograms per kilogram, conversion factor, daily intake of vegetable and average body weight, respectively. A conversion factor 0.085 was used to convert fresh green vegetable weight to dry weight, as described by Rattan et al., 2005. Average daily vegetable intake for adults and children were considered of 0.345 and 0.232 kg $person^{-1}$ d^{-1}, respectively, while the average adults and children body weights were considered of70 and 32.7 kg, respectively, as used in the previous studies (Ge, 1992; Wang et al., 2005).

The total toxic BaP equivalent (TEQ) for selected PAHs was calculated using the formula:

TEQ = $\sum C_{PAH} \times TEF$ (Wang et al., 2011) Where C_{PAH} = individual PAH concentrations (μg kg^{-1}) in vegetable, while TEF = toxic equivalency factor for PAHs set by USEPA (2002).

3.5.3 Statistical analysis

The data were statistically analyzed using the statistical package SPSS 11.5. The measurements were expressed in terms of means, while the figures presented the mean values and standard deviation of threereplicates. Treatment differences were tested using analysis of variance (ANOVA), while for mean significance Tukey's test was usedwith a level of $P<0.05$.

3.6 Result and discussion

3.6.1 *PAH in soil*

Table 3.2summarizes the concentrations of PAHs in soil samples collected from the study area. The sum of 16 PAH (Σ16PAH) concentrations in district Peshawar ranged from 586-1345 μg kg^{-1} with a mean concentration of 929 μg kg^{-1}. In Mardan, total PAHs ranged from 383-724 μg kg^{-1} mean of 604 μg kg^{-1}. In Charsadda, the Σ16PAH concentrations ranged from 396-788 μg kg^{-1} with the mean concentration of 565 μg kg^{-1}, while in Nowshera ranged from 536-955 μg $kg^{-1}$with the mean concentration of 751 μg kg^{-1}. In district Swabi, Σ16PAHs ranged from 348-584 μg kg^{-1} with the mean concentration of 499 μg kg^{-1}, while in DI Khan ranged from 477-810 μg kg^{-1} with the mean concentration of 668 μg kg^{-1}. Among these highly urbanized districts, the soil in district Peshawar showed the highest contamination with PAHs followed by Nowshera. Though the concentrations of mutagenic Σ7PAHs were found below the maximum permissible limit (4.23 mg kg^{-1}) set by the Ministry of Environmental Protection, China (MEP, 2007).

Among the less urbanized districts, the concentration of Σ16PAH ranged from 311-506 μg kg^{-1} with the mean concentration of 436 μg kg^{-1}. In districts Hangu, Bannu, Karak, and Lakki Marwat (less urbanized districts), Σ16PAH concentrations ranged from 269-434, 256-396, 202-322 and 135-246 μg kg^{-1}, respectively with mean concentrations of 384, 341, 288 and 213 μg kg^{-1}, respectively. The data indicated that the soils collected from less urbanized districts were contained Σ16PAH concentrations lower than highly urbanized districts. The concentrations of mutagenic Σ7PAHs were also found within maximum permissible limit Long term wastewater irrigation is one of the major anthropogenic sources responsible for soil and vegetable contamination with PAHs in the suburb areas of urbanized cities (Khan et al., 2008; Zhang et al., 2011). The present study focused on 16PAH concentrations in soil and their accumulation in vegetable irrigated with wastewater in the urbanized districts of KPK province, Pakistan. Accumulation of these possible carcinogenic PAHs in vegetable and their subsequent ingestion, inhalation of dust and dermal contact are responsible for major health concern in the local population. Different countries have set different permissible limits of PAHs for soil and vegetable but so far Pakistan EPA has not established the limits for PAHs in soil ecosystem and food crops.

In this study, a great variation was observed in the concentrations of individual PAH compounds in the soils collected from different districts. However, NA, ACN, AC, FI, PHE, PY, CHR, BbF and BkF were detected as predominant compounds in the soils of KPK province. The soil samples of high urbanized districts (Peshawar, Nowshera, Charsadda, Mardan and DI Khan) were more contaminated with 16 PAHs than less urbanized districts (Kohat, Bannu, Karak, Hangu and Lakki Marwat). Though, the concentrations of LMW-PAHs (2-3 rings) were counted for 31.7-35.9%, while HMW-PAHs (4, 5 and 6 rings) were counted for 40.7-49.3, 6.8-9.2 and 4.9-5.7%, respectively. The trend of PAH concentrations in the soil of the study area were observed in order of Peshawar > Nowshera > DI Khan > Mardan > Charsadda > Swabi > Kohat > Hangu > Bannu > Karak > Lakki Marwat (See Fig.3.1).The highest PAH levels were observed in the suburb areas of highly urbanized districts because the farmlands in these areas were mostly irrigated with wastewater from the last thirty years. According to Wang et al. (2002) wastewater irrigation and aerial deposition are the major sources of PAHs in the soil of urbanized areas. PAHs are released to environment through vehicular exhaust emission, industrial activities, power generation, steel work, oil wastes (Arias-Estevez, 2007) wastewater irrigation, sludge applications and solid waste disposal (Tsai et al., 2001; Shi et al., 2005) which can contaminate soil and grown vegetable. In the study area, BKF, BbF and BaP were frequently observed (50% samples) in the urban areas which are more toxic and persistent in nature.

Previously, numerous studies have been shown that the urban soils were contaminated with higher concentrations of PAHs than rural soils (Wilcke, 2000; Mielke et al., 2004; Zhang et al., 2006). In this study, as moving towards less urbanized areas (Kohat, Bannu, Laki Marwat and Hangu) the agricultural fields were not frequently irrigated with wastewater, therefore, it is possible that wet and dry deposition could be the other sources of PAH contamination in the soil. However, the input of air borne PAHs was also considerably lower in these districts as compared to highly urbanized areas and decreased with increasing the distance away from urban sites. According to classification mentioned by Maliszewska-Kordybach (1996) for ΣPAHs in soil, 36% of the soil samples (600-1000 μg kg^{-1}) were considered contaminated, while 64% samples (200-600 μg kg^{-1}) were recorded as weakly contaminated (Table3.2)

It is important to investigate the origin of PAHs present in the soil environment, therefore, the ratios between HMW-PAHs and LMW-PAHs (Solo et al., 2000) and ratios of FLA and PY (Zhu et al., 2008; Budzinski et al., 1997) were used to investigate the origin of PAHs in this study. The concentration of PY is commonly higher in fossil fuels then the concentrations of FLA (Sanders et al., 2002), while most of LMW-PAHs compounds are petrogenic (Berner et al., 1990). The pyrolytic compound contamination is dominant with HMW-PAHs (Muel and Saguem, 1985). The ratio of LMW-PAHs/HMW-PAHs being greater than 1 indicates pyrogenic PAH contamination, while ratios of FLA/PY being less than 1 indicates petrogenic derived PAH contamination (Berner et al., 1990; Budzinski et al.,1997; Solo et al., 2000; Sanders et al., 2002; Zhu et al., 2008). In this study LMW-PAHs/HMW-PAHs were greater than 1 and the ratios of FLA/PY were less than 1, suggesting that the origin of PAHs in agricultural soils were both pyrogenic and petrogenic in nature

Table 3.2 PAH concentrations (µg kg^{-1}) in the soil collected from Khyber Pakhtunkhwa Province.

PAHs		Mardan (n=27)	Charsadda (n=27)	Peshawar (n=32)	Nowshera (n=28)	Swabi (n=30)	DIkhan (n=26)	Kohat (n=28)	Hangu (n=27)	Banuu (n=29)	Karak (n=28)	Lakki marwat (n=27)
NA	Range	54.6-62.6	47.6-71.6	77.2-123	70.7-106.6	41.1-57.3	61.3-92.4	35.6-53.8	30.9-46.6	30.3-40.4	23.20-35.0	17-23.7
	Mean,±	70.6±8.6	60.6±7.1	102.1±14.5	91.1±11.1	53±6	79±9.6	45.9±5.6	39.8±4.8	35.5±2.8	29.9±3.6	21.9±2.7
ACN	Range	16.9-56.5	9.3-35.9	25.1-64.1	21.8-72.9	8.7-31.1	18.9-63.2	11-36.8	9.5-31.9	8.3-27.6	7.2-23.9	5.2-17.6
	Mean,±	33.9±13.8	27.2±13	49.9±21.1	43.8 ±17.9	25.5± 10.3	38±15.4	22.1±8.9	19.1±7.8	16.6±6.7	14.4±5.8	10.5±4.1
AC	Range	10.5-19.8	6.58-16.5	15.71-29.48	13.61-22.91	7.92-17.82	11.86-22.14	6.86-15.45	5.95-13.39	6.15-11.60	4.47-10.05	3.28-7.37
	Mean,±	14.81±3.37	13.70±4.69	21.42±5.15	15.74±4.35	12.64±3.57	16.58±3.77	10.96±3.10	9.50±2.68	8.23± 2.33	7.13± 2.02	5.23± 1.48
Fl	Range	5.2-9.6	5.4-15.5	8.7-19.3	6.8-13.6	17.2-55.7	6.9-25.85	14.9-48.2	12.9-41.8	11.7-36.2	9.7-31.4	7.1-18.9
	Mean,±	6.8± 1.3	13.9± 13.02	12.4±4.1	8.1±2.7	30.9±13.3	10.5±3.7	26.8±11.6	23.3±10.02	20.2± 8.7	18.6± 6.9	13.6± 5.1
PHE	Range	51.4-81.9	53.7-74.6	76.6-123.6	66.4-107.1	49.5-66.6	57.5-92.8	42.9-57.7	39.2-49.9	35.7-43.3	27.9-37.6	20.5-26.8
	Mean,±	68.5±10.7	59.4± 20.1	106.7±17.2	91.7±14.4	59.6±6.3	79.5±12.5	51.4±5.5	44.8±4.7	38.7±4.1	33.5±3.7	24.6±2.8
AN	Range	7.4-19.9	32.6-69.2	11-32.4	11.4-29.6	9.7-15	9.9-22.3	8.4-14.7	7.3-12.8	6.3-11.1	5.5-9.6	4.02-6.3
	Mean,±	15.4±5.1	55.4±3.2	23.97±8.65	22.4±5.8	14.5±2.1	19.4±5.1	12.5±1.8	10.9±1.6	9.4±1.4	8.17±1.18	5.9±0.9
FLA	Range	38.8-79.9	133-369	56.9-119	48.9-103.1	28.5-54.03	42.4-89.9	25.2-51.9	21.4-45.1	18.5-48.2	24.4-33.8	11.9-24.8
	Mean,±	62.8±16.7	162±18	92.3±25.1	81.1± 21.5	46.62±12.6	70.3±18.6	41.7±9.3	35.01±9.6	32.9±10.2	26.3±7.1	19.3±5.2
PY	Range	123-286	53.5-146	63-635	72.6-273.04	145.6-237.7	62.9-177.6	36.8-205.9	87.1-178.5	75.4-208.7	1.9-112.6	Nd-132.6
	Mean,±	202±49	40.7±127	297±183	261±92	152.18±112.1	180.8±88.9	120.3±61.9	110.3±54.9	99.1±72.9	63.8±37.6	62.9±46.4
BbA	Range	14.9-168.9	2.5-24.9	22.3-61.1	19.3-218.1	12.8-126.9	16.8-189	11.1-109	9.5-65.3	7.3-82.6	7.2-71.6	5.3-52.4
	Mean,±	41.5±52.2	16.1± 41.9	61.8±81.6	53.6±67.4	33.3±38.4	46.4±58.4	28.9±33.3	25±28.8	22.±24.7	18.8± 21.7	13.8±15.9
CHR	Range	11.5-26.8	8.9-29.4	17.1-42.9	19.4-37.2	11.3-22.02	16.8-32.2	9.8-18.8	8.5-16.3	7.3-17.3	6.35-12.1	4.7-8.4
	Mean,±	20.3±6.1	20.4±7.2	29.8±9.4	27.4±6.5	17.7±3.6	23.8± 5.7	15.3±3.2	13.3±2.7	11.9± 3.1	9.9±2.1	7.3±1.5
BbF	Range	15.2-31.1	3.2-10.1	22.9-50.5	19.7-40.2	11.4-25.5	17.03-34.8	9.9-20.3	8.6-19.1	7.4-16.6	6.5-14.4	4.7-10.5
	Mean,±	25.2±5.9	7.9± 6.4	38.9±7.1	32.6± 7.5	19.2± 4.3	28.2± 6.5	16.6±3.7	14.4±3.2	12.5± 2.8	10.8±2.4	7.9±1.5
BkF	Range	3.7-12.1	7.4-16.8	5.5-19.3	9.4-16.7	2.8-9.1	8.1-14.5	2.4-7.6	3.9-8.1	1.8-7.03	2.9-5.6	2.1-4.1
	Mean,±	9.01± 2.9	13.2± 2.4	14.8±4.9	13.9± 2.5	6.8± 2.2	12.9± 2.3	5.9±1.9	5.8±1.5	4.8± 1.7	4.3± 1.01	3.5±1.1
BaP	Range	10.4-19.5	5.9-94.5	17.00-29.1	17.9-34.73	2.4-18.6	20.3-24.3	9.02-16.1	4.1-8.1	6.8-12.1	5.9-11.12	4.3-8.15
	Mean,±	16.03± 3.7	25.7± 3.7	24.4±6	24.7± 3.2	14.5± 2.7	22.4± 1.6	12.6±2.3	5.8±2.4	9.5± 1.8	8.6±1.6	6.3±1.3
IP	Range	ND-48.04	ND-10.9	ND-71.6	8.7-27.3	2.4-36.1	7.6-53.8	7.3-31.3	6.3-27.1	5.5-23.5	4.8-20.5	3.5-14.9
	Mean,±	17.9± 13.4	7.5± 27.9	28.8±20.3	8.62±17.3	14.9±9.7	20.02± 15.00	12.9±8.4	11.2±7.3	10.3±5.8	9.3± 4.9	6.8±3.7
D[ah]A	Range	4.6-10.2	ND-56.8	6.8-16.8	5.9-25.9	4.9-8.5	6.4-15.1	3.7-7.4	3.2-7.1	3.2-9.8	2.8-6.4	2.9-3.1
	Mean	8.4±2.5	12.6 ±2.8	12.1±4.03	13.4± 3.2	7.1±1.7	10.7±2.8	6.1±1.5	5.3±1.3	5.5± 1.9	4.5±1.1	3.3±0.8
B[ghi]P	Range	ND-9.9	ND-56.8	ND-18.8	ND-12.8	4.2-7.4	6.9-11.1	4.04-7.7	3.5-5.5	3.04-9.5	2.4-4.2	1.8-3.04
	Mean	8.2± 1.6	12.6±16.6	12.9±3.8	10.6±2.01	6.4±1.2	9.52± 1.3	5.8±1.1	4.9±0.7	5.4±2.4	3.48± 0.66	2.5±0.5
∑Sum of 16 PAHs		604±113	565±114	928±252	751±144	499±94.	667±124	435±71	383±70	341±46	288±47	222±33
Classification		Contaminated	Weakly Contaminated	Contaminated	Contaminated	Weakly Contaminated	Contaminated	Weakly Contaminated	Weakly Contaminated	Weakly Contaminated	Weakly Contaminated	Weakly Contaminated

Maliszewska-Kordybach (1996) Classification for ∑PAHs in soil non-contaminated soil (<200 µg kg^{-1}), weakly contaminated soil (200-600 µg kg^{-1}), contaminated soil (600-1000 µg kg^{-1}), and heavily contaminated soil (>1000 µg kg^{-1})

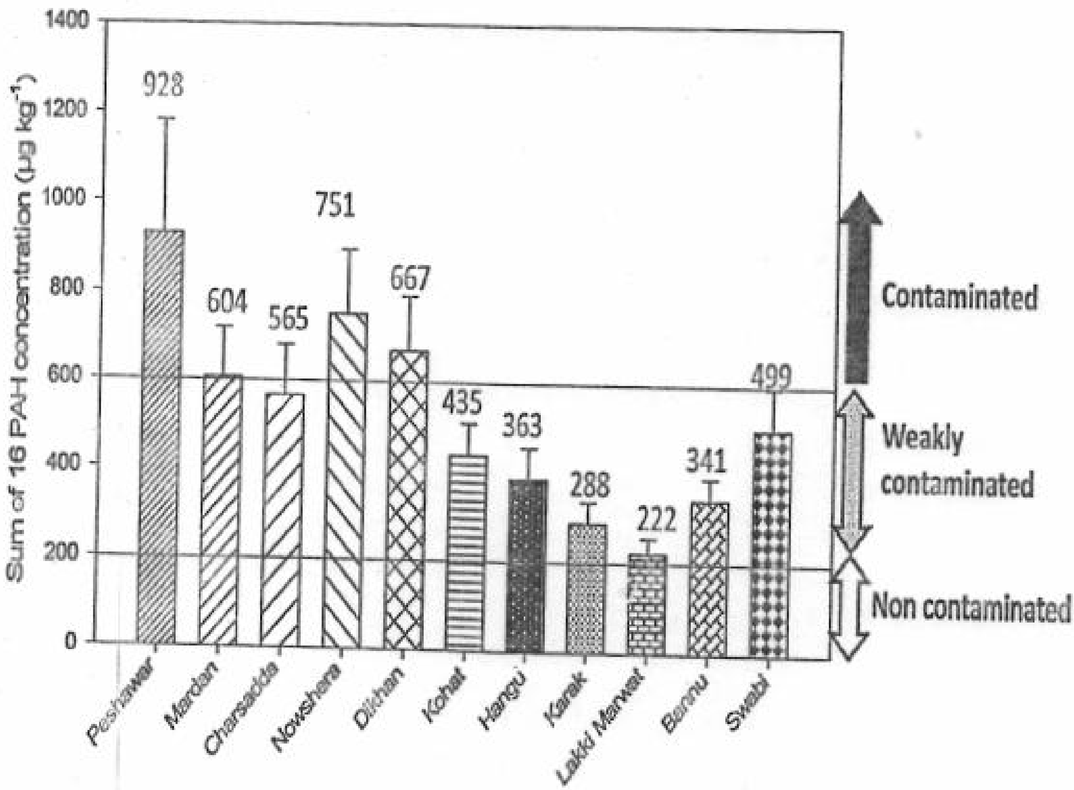


Fig. 3.1 PAH concentration in soil in Khyber Pakhtunkhwa Province, Pakistan

3.6.2 *PAHs in Vegetable*

Table 3.3 summarizes the concentrations of PAHs in vegetable samples collected from wastewater irrigated fields. PAH concentrations in vegetable were strongly depended on growing sites e.g. the samples collected from highly urbanized districts such as Peshawar, Nowshera, Charsadda, Mardan, Swabi and DI Khan were highly contaminated with PAHs as compared to less urbanized districts including Kohat, Karak, Lakki Marwat, Hangu and Bannu.

The bioaccumulation of different PAH compounds (based on benzene rings) was greatly varied among the selected vegetable and districts. LMW-PAH compounds were higher than HMW-PAH compound concentrations. In district Mardan, the highest concentration (402 µg kg^{-1}) of ∑16 PAHs was observed in *R. sativus* followed by *S. alba* (393 µg kg^{-1}) in district Nowshera (Fig. 3.2). In Peshawar,*L. satuva* L. was observed highly contaminated vegetable with PAH concentration of 333 µg kg^{-1} whereas in district Charsadda the highly contaminated vegetable was *B. oleracea* B. with PAH concentration of 276 µg kg^{-1}. *S. Alba* accumulated the highest concentration of PAHs (276 µg kg^{-1}) in DI Khan followed by the same vegetable (152 µg kg^{-1}) in district Swabi (Fig. 3.2).

The vegetable grown in high urbanized areas were highly contaminated with PAHs as compared to less urbanized areas. In Kohat, *B. rapa* accumulated the highest concentration of PAH (149 µg kg^{-1}) as compared to other selected vegetable from the same district. *R. Sativus* was observed as the most contaminated vegetable in districts Hangu, Karak and Laki Marwat with mean PAH concentrations of 218,138 and 90 µg kg^{-1} respectively. In district Bannu, *B. Oleracea* C. was observed highly contaminated vegetable with PAHs of 177 µg kg^{-1} (Fig. 3.2).

In leafy vegetable, NA (2 ring PAH) was ranged from 35-55 µg kg^{-1}, while ACN, Fl, PHE and AN (3 ring PAHs) ranged from 9 -86 µg kg^{-1}. HMW-PAH including FlA, BaP, CHR and PY

(4 ring PAHs) ranged from 2-70 μg kg^{-1}, while BaP, BbF and BkF (5 ring PAHs) ranged from 2-42 μg kg^{-1} and D[ah]A, B[ghi]P and IP (6 ring PAHs) ranged from below detection limit to7.2 μg kg^{-1} (Table 3.3).

In underground bulb vegetable, such as *A. sativum* and *A. cepa*, the concentrations of 2 ring PAHs (NA) ranged from 30-45 μg kg^{-1}, 3 ring PAHs (ACN, Fl, PHE and AN) ranged from 1-6 μg kg^{-1}, 4 ring PAHs (FLA, BaA CHR and PY) ranged from 4-47 μg kg^{-1}, 5 ring PAHs (BaP, BbF and BkF) ranged from 3-18 μg kg^{-1} and 6 ring PAHs (D[ah]A, B[ghi]P and IP) ranged from below the detection limits to 6.2 μg kg^{-1} (Table 3.3).

In the samples of *D. carota*, R. *sativus* and *B. rapa*, 2 ring PAH compound (NA) ranged from 51-66 μg kg^{-1}, 3 ring PAHs (ACN, Fl, PHE and AN) ranged from 2.29-61.73 μg kg^{-1}, 4 ring PAHs (FLA, BaA CHR and PY) ranged from 1-140.30μg kg^{-1}, 5 ring PAHs (BaP BbF and BkF) ranged from 1-26.4μg kg^{-1} and 6 ring PAHs (D[ah]A, B[ghi]P and IP) ranged from below the detection limits to 9.5 μg kg^{-1} The concentrations of PAHs in leafy vegetable (*L. sativa* L., *S. oleracea*, and *B. oleracea* C.) ranged from 91.3 to 333 followed by underground bulb (*A. cepa,*and *A. sativum*) which were ranged from 149 to 264.3

Bioaccumulation of PAH compounds in vegetable were greatly varied and LMW-PAHs (2-3 rings) were counted for 60% of the ∑16 PAHs, while the potentially toxic carcinogenic PAHs (5-6 rings) were counted for 6%. NA is the most degradable and volatile compound due to its low ring number (Bossert and Bartha, 1984), but comparatively higher concentration of NA was calculated in vegetable because of its higher solubility in water and its entrance into soil profile and then uptake through plant roots. Plants can also absorb PAH compounds from contaminated air and this mode of transport could be more considerable for LMW-PAHs such as NA (Li et al., 2010). PAH accumulation in vegetable depends on soil characteristics such as organic matters, concentrations of PAHs, soil texture and type of vegetable and transfer pathways. In this study, total and dissolved C concentrations were higher in the soil of high urbanized districts than less urbanized districts which could be the reason to reduce the accumulation of PAHs in vegetable grown in high urbanized districts. Vegetable grown on PAH contaminated soil have shown elevated levels of PAHs (Samsoe et al., 2002). PAH concentrations were higher in highly urbanized districts as compared to less urbanized districts. This could be attributed with high PAH concentrations in soil caused by frequent irrigation of farmland with wastewater.

Table 3.3 Concentration of 16 PAH in vegetable collected from Khyber Pakhtunkhwa Province (µg kg^{-1})

Vegetable	2rings	3 rings					4-rings				5-rings			6-rings		
	NA	ACN	AC	Fl	PHE	AN	FLA	PY	BbA	CHR	BbF	BkF	BaP	IP	D[ah]A	B[ghi]P
Peshawar																
A. cepa (n=4)	45.01±3.2	4.4±0.1	2.6±0.004	6.9±0.7	21.6±13.3	14.8±0.04	47.8±5.3	37.7±4.3	13.9±2.3	13.9±2.3	13.03±1.04	13.03±1.02	18.1±3.2	1.8±0.5	6.2±0.2	4.4±0.3
A. sativum (n=4)	32.6±4.3	1.2±0.002	6.7±0.03	6.7±0.06	11.3±1.8	11.3±1.03	37.5±4.8	32.3±3.4	10.3±1.7	10.3±1.3	10.9±1.2	0.7±0.04	12.6±2.6	1.1±0.003	2.3±0.1	2.2±0.1
S. alba (n=3)	39.4±4.4	1.4±0.003	9.9±1.3	7.6±0.4	11.5±1.3	11.5±2.5	58.3±6.5	61.2±5.2	12.5±1.02	12.5±2.34	5.2±0.8	0.6±0.03	6.4±0.6	1.01±0.001	0.8±0.004	ND
B. oleracea C. (n=3)	35.9±4.7	1.2±0.004	8.5±0.2	6.3±0.2	86.3±7.6	11.02±3.04	34±2.3	27.5±3.7	4.1±0.1	4.1±0.04	6.3±0.3	1.3±0.003	23.6±2.3	1.7±0.1	1.00±0.002	0.6±0.03
B. oleracea B. (n=3)	30.8±4.3	1.01±0.001	7.6±0.34	10.97±2.1	7.2±1.04	7.2±1.02	11.8±3.6	11.6±1.2	0.79±0.004	0.8±0.004	3.5±0.04	0.6±0.002	16.2±3.5	ND	1.2±0.04	ND
B.rapa(n=3)	37.3±3.7	1.1±0.004	6.95±0.4	8.02±0.96	42.5±4.4	9.04±1.03	25.3±4.4	16.84±1.9	4.5±0.002	4.5±0.003	3.3±0.004	0.4±0.005	14.9±2.45	ND	1.3±0.1	ND
L. sativa L. (n=4)	55.6±6.3	9.1±1.03	5.4±0.02	2.2±0.03	23.98±2.7	23.98±3.8	70.7±5.7	12.4±1.4	32.9±3.5	13.9±1.7	42.6±3.8	2.9±0.23	18.96±2.7	1.01±0.034	7.2±0.3	1?.7±1.6
P. sativum (n=4)	29.8±3.2	1.03±0.03	5.3±0.23	13.5±2.4	7.8±0.9	7.8±1.2	16.5±3.2	13.6±1.02	2.3±0.23	2.3±0.03	11.2±1.5	11.15±1.3	17.4±1.9	ND	3.9±0.2	ND
S. oleracea (n=4)	35.8±4.6	1.3±0.02	12.5±1.33	29.7±3.21	13.1±1.3	13.2±3.3	65.9±4.4	57.5±6.5	19.9±2.9	8.9±0.3	18.8±3.4	2.02±0.5	8.6±0.34	4.5±0.6	3.1±0.1	4.7±0.03
Mardan																
S. alba (n=3)	54.03±4.4	1.4±0.03	4.4±0.2	1.5±0.1	22.3±3.2	4.3±0.98	10.5±1.8	40.3±4.4	18.4±1.5	17.4±1.4	24.8±3.3	5.8±0.4	13.8±1.1	8.6±0.6	2.7±0.7	3.3±0.2
B. oleracea B.(n=3)	40.3±4.7	1.2±0.05	10.1±1.2	2.9±0.2	79.8±6.8	11.5±1.34	73.4±6.4	47.8±4.3	29.8±2.7	19.9±2.3	18.5±1.9	2.5±0.1	8.5±3.4	10.8±1.4	6.4±0.7	6.3±0.3
B.oleracea C.(n=3)	57.3±5.7	1.5±0.03	10.3±2.3	3.6±0.4	10.9±3.5	11.03±1.9	56.7±5.4	74.7±8.8	14.7±1.9	14.8±0.9	17.04±2.5	7.03±0.2	14.9±3.6	5.8±0.5	2.3±0.3	6.3±0.8
B. rapa (n=3)	66.9±6.8	2.3±0.34	13.4±2.98	3.2±0.7	11.7±2.6	11.7±1.5	72.6±7.3	53.4±5.6	11.3±0.9	3.6±0.2	10.4±1.7	10.4±1.9	23.4±1.9	5.9±0.2	9.6±1.3	5.6±0.2
C. sativum (n=4)	5.8±0.6	1.4±.02	11.60±1.5	3.8±0.4	41.4±4.5	14.8±2.5	64.5±6.8	119.3±7.9	30.4±3.9	5.4±0.2	3.4±0.5	8.4±01	12.3±3.5	0.4±0.06	6.7±0.7	7.6±0.7
D. carota (n=4)	51.2±7.7	1.6±0.03	2.8±0.7	2.1±0.1	44.9±3.9	7.96±1.03	51.7±4.9	52.5±4.7	13.9±1.8	0.7±0.1	0.44±0.001	0.4±0.03	13.2±1.9	ND	3.6±0.2	ND
P. sativum (n=4)	52.1±6.6	1.3±0.02	2.1±0.2	4.3±0.98	13.6±1.2	13.6±2.03	52.2±4.2	44.2±4.9	13.9±1.6	13.9±2.3	24.6±2.7	1.3±0.04	14.3±0.98	7.3±0.4	3.6±0.1	3.3±0.2
R. sativus(n=3)	62.6±6.9	1.7±0.02	3.8±0.3	3.2±0.5	61.7±5.7	11.7±1.8	25.6±2.7	140.30±12.3	16.4±2.7	16.3±2.3	26.4±3.7	1.9±0.23	7.1±0.8	8.4±0.9	9.1±1.2	6.3±0.6
Nowshera																
B. oleracea B.(n=3)	30.8±4.3	0.9±0.2	15.4±2.3	0.7±0.01	14.5±3.8	8.8±1.03	56.2±4.22	36.7±3.4	21.0±3.4	22.9±3.5	12.5±1.2	5.5±0.3	15.9±2.6	8.3±1.2	6.03±0.4	4.8±0.2
B. oleracea C.(n=3)	43.9±3.9	1.1±0.1	7.9±0.5	2.8±0.2	8.4±1.2	8.5±1.4	43.5±4.2	57.2±5.3	11.3±3.4	11.3±2.4	13.1±2.5	7.1±0.4	19.7±2.3	6.8±1.3	1.8±0.4	4.8±0.4
S. alba (n=3)	41.4±4.3	1.01±0.1	3.5±0.03	1.1±0.2	35.8±5.3	10.9±2.4	42.9±3.7	107.6±11.2	14.7±1.2	24.7±4.6	31.3±3.7	4.3±0.2	26.6±3.5	18.9±3.7	21.2±0.1	7.9±0.8
B. rapa (n=4)	51.3±5.6	1.8±0.4	10.3±1.2	3.9±0.5	8.9±1.2	8.9±1.2	55.7±4.3	40.9±3.2	8.7±1.7	2.8±0.5	7.9±0.9	7.9±0.9	14.9±1.3	4.6±0.7	7.4±0.92	4.3±0.8
C. sativum (n=4)	4.5±0.5	1.1±0.2	8.9±1.02	5.2±1.2	15.1±1.6	11.3±1.5	56.9±6.3	110.5±7.9	38.7±3.9	18.7±3.72	2.6±0.3	2.6±0.7	23.1±2.5	0.34±0.01	12.8±1.2	5.8±0.3
D. carota (n=5)	39.2±6.6	1.2±0.1	2.1±0.3	2.8±0.7	41.8±3.8	6.10±0.8	70.3±6.9	40.2±3.80	10.7±1.2	0.5±0.1	0.3±0.02	0.33±0.1	23.1±3.5	0.6±0.02	6.23±0.7	ND

P. sativum (n=3)	39.9±5.2	0.9±0.1	1.6±0.02	2.3±0.4	10.4±1.03	10.4±1.1	39.9±3.01	33.9±3.1	10.6±0.9	10.5±1.5	18.9±3.6	1.01±0.04	13.3±3.3	5.6±0.5	2.8±0.2	2.5±0.5
R. sativus (n=3)	48.01±3.6	1.3±0.1	2.9±0.3	28.5±4.2	23.7±3.2	18.2±5.7	49.6±7.4	84.2±9.7	21.6±2.2	18.6±3.3	35.9±3.5	1.5±0.2	19.9±4.2	20.3±1.9	4.5±0.8	8.7±1.3
Charsadda																
A. cepa (n=4)	44.5±5.5	1.2±0.2	15.6±2.7	11.9±3.2	11.7±1.2	11.7±2.5	18.6±3.2	30.9±3.6	3.8±0.4	3.9±0.2	17.9±3.2	0.7±0.02	9.9±1.2	ND	ND	ND
A. sativum (n=4)	28.6±3.3	1.2±0.1	8.8±1.4	7.7±1.2	48.9±4.3	9.2±1.2	40.8±4.7	31.8±5.6	7.04±0.8	7.1±0.9	10.1±1.2	0.6±0.04	8.4±2.03	ND	ND	1.3±0.2
S. alba (n=3)	31.8±4.3	1.2±0.2	7.9±1.03	6.7±0.9	46.3±6.5	14.7±2.3	19.2±3.7	38.6±3.5	10.9±1.2	13.9±2.3	13.7±1.3	0.9±0.1	11.5±2.3	4.4±0.4	8.6±0.9	2.3±0.2
B.oleracea B. (n=3)	37.6±4.4	1.2±0.2	14.7±2.3	8.8±1.7	32.4±3.2	10±3.5	66.4±7.4	54.6±5.7	14.5±1.5	14.5±3.2	3.5±0.7	7.5±0.8	8.9±1.1	ND	1.08±0.02	1.02±0.01
C. sativum (n=3)	28.2±1.9	1.3±0.3	8.04±1.2	9.9±0.9	29.9±2.9	7.9±1.02	27.7±4.7	75.4±8.7	16.4±2.3	6.5±0.2	1.8±0.7	1.8±0.04	6.6±0.8	ND	ND	0.3±0.01
L. Sativa L.(n=6)	42.6±4.9	0.8±0.1	2.9±0.2	5.2±0.2	62.3±7.9	15.00±2.31	26.6±3.8	14.4±3.8	14.04±3.2	13.9±2.3	16.3±3.2	5.2±0.7	15.9±2.3	8.7±0.8	1.8±0.5	1.3±0.5
S.Oleracea (n=4)	33.9±3.7	1.1±0.2	11.3±3.8	7.6±0.2	15.1±3.2	8.7±1.2	27.7±4.3	53.4±5.6	16.3±3.2	16.3±2.3	10.1±1.5	0.8±0.03	3.6±0.5	1.9±0.5	1.8±0.7	1.8±0.5
Swabi																
A. cepa (n=4)	29.8±3.7	0.8±0.05	11.1±1.2	20.7±2.3	7.9±1.2	7.9±0.2	25.88±2.34	20.7±2.6	2.6±0.1	2.6±0.3	11.99±2.1	0.5±0.05	8.8±1.2	ND	ND	ND
A. sativum (n=4)	19.2±3.4	0.8±0.1	5.9±0.7	13.9±3.1	39.8±3.7	6.2±0.3	27.36±4.65	21.4±3.43	4.7±0.54	4.7±0.2	6.8±0.2	0.4±0.02	12.9±1.9	ND	ND	0.9±0.2
S. alba (n=3)	22.8±2.3	0.8±0.03	7.6±1.5	14.5±1.2	40.6±4.2	5.8±0.1	45.33±4.25	35.6±3.2	10.9±1.3	10.9±2.3	6.8±02	0.5±0.03	4.6±0.2	1.2±0.2	1.2±0.1	1.2±0.3
B. oleracea C. (n=3)	38.6±3.8	0.8±0.1	7.9±1.2	30±3.7	62.9±5.3	11.5±1.2	31.03±3.5	52.5±6.8	10.72±1.7	13.7±2.3	2.9±0.1	2.9±0.2	6.9±0.2	2.8±0.1	2.5±0.2	3.6±0.3
B. oleracea B.(n=3)	25.2±2.4	0.7±0.1	9.9±1.7	9.4±1.7	45.3±3.2	7.03±3.2	44.8±4.7	36.6±2.9	9.7±2.3	9.7±1.3	9.3±1.2	4.3±0.3	5.8±0.3	ND	0.7±0.05	0.7±0.1
C. sativum (n=4)	18.9±3.6	0.8±0.05	5±0.6	13.±4.2	40.7±6.4	7.32±1.7	25.5±5.3	50.5±4.3	11.01±3.2	11.02±1.2	1.2±0.7	1.2±0.5	11.1±1.3	ND	ND	0.22±.003
L.sativa L.(n=5)	28.53±4.6	16.6±2.3	1.9±0.7	34.3±4.3	43.7±4.7	2.45±0.9	53.9±7.4	41.7±3.5	2.5±0.2	4.4±0.22	7.8±0.9	3.5±0.8	9.1±0.9	2.1±0.5	1.2±0.2	0.9±0.04
S. oleracea(n=4)	21.3±3.4	0.8±0.2	12.02±2.3	24.6±2.7	35.04±3.4	9.88±1.7	23.4±3.5	86.1±70.1	10.7±1.3	20.8±2.3	9.3±1.3	0.7±0.02	2.7±0.8	2.9±0.2	5.8±0.11	1.5±0.1
DI Khan																
S. alba (n=3)	34.2±3.7	0.9±0.02	2.9±0.2	0.9±0.1	14.4±1.5	9.03±1.2	26.3±2.4	88.9±10.2	6.9±0.9	16.9±2.1	25.9±2.3	3.6±0.4	16.8±2.6	15.6±1.2	6.5±1.2	6.5±0.2
B. oleracea B. (n=3)	25.5±2.5	0.8±0.11	12.7±2.3	0.6±0.05	12.2±2.3	7.3±0.2	46.5±4.3	30.3±3.2	18.9±2.7	18.9±3.2	4.4±0.8	3.4±0.1	15.3±1.3	6.9±1.1	8.8±3.2	4.00±0.7
B. oleracea C. (n=3)	36.3±4.7	0.9±0.23	6.5±0.8	19.4±1.2	6.9±0.5	6.9±0.5	35.9±3.6	47.3±5.3	9.3±1.2	9.3±1.2	10.8±0.9	10.8±1.2	21.1±3.2	5.6±0.2	1.5±0.1	4.00±0.6
B. rapa (n=3)	42.3±4.3	1.5±0.6	8.5±0.9	9.8±1.2	7.4±0.1	7.4±0.9	46.01±3.2	33.9±4.7	7.2±0.2	2.3±0.5	6.6±0.2	6.6±0.5	19.3±2.7	3.8±0.2	6.1±0.3	3.5±0.3
C. sativum (n=4)	3.7±0.9	0.9±0.2	7.3±1.2	4.3±0.3	12.9±2.3	9.3±1.1	29.5±2.8	32.6±3.7	31.9±3.5	11.9±1.5	2.1±0.2	2.1±0.2	13.1±1.3	0.3±0.03	10.6±1.5	4.8±0.2
D. carota (n=4)	32.4±3.7	1.00±0.1	1.7±0.4	7.2±1.	13.1±3.2	5.04±0.02	58.1±4.6	23.7±3.	8.8±1.2	0.4±0.05	0.3±0.05	0.3±0.02	17.4±2.7	ND	4.8±0.3	ND
P. sativum (n=3)	33.02±2.9	0.8±0.1	1.3±0.2	2.7±0.2	8.6±1.2	8.6±0.34	33.04±3.5	27.9±2.5	8.8±1.2	8.8±1.2	15.6±1.2	0.8±0.04	12.7±4.6	4.7±0.1	2.3±0.1	2.1±0.1
R. sativus (n=3)	39.7±3.4	1.03±0.05	2.4±0.9	3.5±0.8	18.8±2.3	15.01±1.2	26.2±4.4	52.3±5.3	7.4±1.5	27.4±2.5	19.4±1.2	1.3±0.11	19.8±3.6	2.7±0.1	3.8±0.2	7.2±1.2

Kohat																
B.oleracea B.(n=3)	18.4±3.2	0.55 ±0.11	9.20± 1.21	0.43±0.04	6.79±0.02	5.25 ±0.23	33.55±3.65	21.88 ±2.35	13.61 ±.21	13.7± 1.21	3.33±0.23	2.33±0.22	7.06±0.32	4.95±0.34	2.06±0.11	2.89±0.12
B.oleracea C.(n=3)	26.2±4.5	0.68 ±0.05	4.70±0.23	14.01±3.21	5.03±0.23	5.04±0.98	25.94±2.75	34.15±4.35	6.73±0.23	6.74 ±0.21	7.79±1.21	3.79±0.22	4.12±0. 12	4.04±0.22	1.07±0.13	2.89±0.98
S. alba (n=3)	24.7±1.8	0.64± 0.02	2.09±0.24	0.67±0.01	11.82±1.23	6.52±0.97	21.24±2.72	34.17±3.45	2.69±0.11	1.69 ±0.32	18.67±3.21	2.58±0.32	7.99±1.43	11.23±2.34	2.67±0.11	4.72±0.21
B. rapa (n=3)	30.6±3.7	1.05±0.31	6.14±0.21	14.26±2.31	5.34±0.98	5.34±1.12	33.23± 3.54	24.44±2.43	5.19± 0.68	1.64± 0.04	4.75±0.73	4.74± 0.53	2.30± 0.03	2.72 ±0.01	4.38± 0.93	2.54± 0.087
C. sativum (n=4)	2.7 ±0.2	0.64±0.03	5.30±0.98	3.08±0.78	10.42±1.21	6.74±0.98	23.54±2.35	35.74±3.21	23.06 ±2.03	2.06±0.76	1.55±0.11	1.55±0.11	7.66± 0.23	0.2± 0.002	3.63± 0.04	3.47±0.67
D. carota (n=4)	23.4±2.3	0.72±0.12	1.26 ±0.76	12.40 ±2.3	4.58±0.23	3.64±0.21	21.93±1.98	24.00 ±2.08	6.37±0.24	0.31±0.05	0.20±0.03	0.20±0.01	9.75±1.21	ND	3.38±0.76	ND
P.sativum (n=3)	23.9±1.2	0.59±0.11	0.96±0.05	15.68±3.45	6.22±0.11	6.22±0.11	23.86±2.01	20.22±2.43	6.34±0.32	6.35±0.24	11.25±2.34	0.60±0.02	3.62±0.78	3.35±0.23	1.66±0.11	1.49±0.12
R. sativus (n=5)	28.64±3.45	0.74±0.05	1.71±0.11	16.99±4.52	13.44±1.21	10.84±1.21	18.92±3.21	109.92±10.21	9.67±1.21	8.66±0.65	6.23±0.34	0.91±0.11	7.22±1.23	12.1± 2.34	2.57±0.23	5.17±0.24
Hangu																
B. oleracea C.(n=3)	19.7 ±2.9	0.5± 0.2	3.5±0.2	10.5±1.3	3.8±0.2	3.8±0.1	19.5±1.4	25.7±2.3	5.1±1.2	5.1±1.2	5.9±0.8	3.8±0.3	10.4 ±1.2	3.03 ±0.2	0.8±0.03	2.2±0.2
B. oleracca B. (n=3)	13.8±1.2	0.4±0.03	6.9±0.5	0.3±0.03	6.2±0.9	3.9 ±0.3	25.2±2.3	16.4±2.5	10.2±2.3	10.3±2.3	5.5±0.2	4.5 ±0.2	5.2 ±0.2	3.7±0.2	2.1±0.1	2.1±0.3
B. rapa (n=3)	22.9±2.3	0.8±0.02	4.6±0.6	10.7±2.1	4.01±0.7	4.01±0.4	24.9±3.5	18.5±2.5	3.9 ±0.3	1.9±0.2	3.6±0.1	3.6 ±0.3	4.9±0.4	2.04±0.4	3.3 ±0.2	1.9±0.3
S. alba (n=3)	18.6±3.5	0.5±0.03	1.6±0.3	0.5±0.03	23.2±2.5	4.9±0.5	38.5±3.4	18.2±2.3	20.1±2.3	10.1±3.2	14.02 ±2.5	1.9±0.2	11.4±0.3	8.4±1.2	3.5±0.1	3.5±0.8
C. sativum (n=4)	1.9±0.1	0.6±0.03	3.5±0.3	2.3±0.2	32.9±2.2	5.1±0.4	26.3±3.5	19.9±3.4	17.3±2.3	7.2±0.6	1.2±0.1	1.2±0.1	8.3±1.2	0.2±0.03	5.7 ±0.2	2.6±0.6
D. carota (n=3)	17.6±2.3	0.5±0.11	0.9±0.02	9.3±0.9	6.5±0.6	2.7±0.1	31.5 ±2.3	15.03±2.7	4.8 ±0.5	0.2±0.01	0.2±0.02	0.2±0.01	4.9±0.2	ND	2.6±0.03	ND
P. Sativum (n=4)	17.9± 1.5	0.4±0.13	0.7±0.1	11.8±1.2	4.7±0.5	4.7±0.3	17.9 ±3.4	15.2±1.2	4.8 ±0.4	4.8±0.5	8.5±0.9	0.5±0.1	10.7±2.3	2.6 ±0.2	1.3±0.02	1.1±0.05
R. sativus (n=4)	21.5±2.3	0.6±0.14	1.3±0.1	12.8±1.01	21.2±2.1	8.1±1.4	19.8±2.3	42.6±4.2	6.6±1.2	13.6±1.1	15.9± 1.21	0.7±0.02	11.8 ±2.3	9.1 ±1.2	4.9±0.2	3.9±0.2
Karak																
B. oleracca B. (n=3)	15.9±1.6	0.5±0.02	7.9±0.8	0.4± 0.02	7.2±1.3	4.6±0.3	29.1± 2.8	18.9±2.3	11.8±1.2	6.9± 1.2	7.2±0.8	2.2± 0.1	5.6 ±0.3	4.3±0.7	2.3±0.2	2.5±0.1
B. oleracea C. (n=3)	22.7±22.3	0.6± 0.03	4.1±0.5	12.1±2.1	4.4±0.2	4.6±0.4	22.5±1.9	29.6±3.7	5.8± 1.2	5.8±1.3	6.8±0.3	2.8 ±0.1	7.6±0.9	3.5±0.4	0.9±0.03	2.5 ±0.1
S. alba (n=3)	20.7±2.5	0.5±0.03	0.8±0.2	13.6±3.7	5.9±0.9	5.4±0.6	20.7±2.1	17.5±1.8	5.5±0.2	5.5±1.5	9.8±1.02	0.5±0.04	3.5 ±0.5	2.9±0.1	1.4±0.01	1.3±0.01
B. rapa (n=3)	26.5±2.9	0.9±0.01	5.3±0.7	12.96±3.02	4.6±0.7	4.6±0.4	28.8±2.7	21.2±1.2	4.5±0.3	1.4±0.8	4.1±0.3	4.1 ±0.1	3.9 ±0.2	2.4 ±0.2	3.8±0.2	2.2±0.04
C.sativum (n=5)	2.3±0.3	0.6±0.01	4.6±0.3	2.7±0.2	9.7±1.02	5.8±0.3	8.1±0.02	22.7±2.4	0.6±0.1	1.9±0.5	1.3±0.03	1.3±0.04	3.7±0.5	0.2±0.001	0.6±0.003	3.01±0.3
D. carota (n=4)	20.9±2.4	0.6± 0.3	1.1±0.2	10.8±1.2	7.3±1.03	3.2 ±0.2	16.3±3.3	20.8±3.4	5.5±0.9	0.3±0.02	0.2±0.01	0.2±0.001	2.1 ±0.1	ND	5.9±0.9	ND
P. sativum (n=3)	21.4±0.2	0.6±0.04	1.8±0.2	0.9±0.02	6.04±0.3	5.7±0.8	7.1± 0.2	15.6±2.2	2.1±0.2	3.1±0.03	6.2±0.5	2.2±0.02	8.2 ±1.2	1.7 ±0.003	3.9±0.02	2.1±0.6
R.sativus (n=4)	24.8±2.5	0.8±0.1	1.49± 0.7	14.7±1.3	11.7±1.3	2.4±0.3	12.1±1.2	35.3 ±3.5	4.2±0.9	4.2 ±0.54	8.4 ±0.9	0.8 ±0.05	9.9 ±1.2	2.5 ±0.01	3.03±0.2	2.5 ±0.2

Lakki Marwat																
B. oleracea B. (n=3)	11.1±2.3	0.3±0.003	5.5±0.2	0.3±0.003	2.2±0.02	3.2±0.3	20.2±2.5	13.2±1.01	8.2±0.2	8.2± 0.3	1.8±0.2	0.8±0.1	4.3 ±.4	2.7± 0.2	1.86±0.14	1.74±0.02
B. oleracea C. (n=3)	15.8 ±3.5	0.4±0.003	2.9±0.11	8.4±0.9	3.02±0.03	3.03±0.22	15.6±1.2	20.6±2.2	4.1±0.02	4.1±0.1	3.7±0.5	4.7±0.3	0.5±0.02	2.4 ±0.04	0.54±0.02	1.7±0.02
S. alba (n=3)	14.3 ±3.6	0.4±0.002	0.9±0.02	9.4±1.01	3.7±0.2	3.7±0.3	14.3±0.9	12.2±0.8	3.8±0.1	3.8±0.2	6.8±0.8	0.4±0.03	2.5±0.3	2.01 ±0.2	1.1± 0.003	0.9 ±0.1
B. rapa (n=3)	18.4±4.2	0.7±0.001	3.7 ±0.7	8.6±0.3	3.2±0.3	3.2±0.1	19.9±2.3	14.7±1.2	3.1±0.1	0.9±0.1	2.9±0.5	2.9 ±0.02	3.7±0.3	1.6±0.04	2.6±0.9	1.5±0.01
C. sativum (n=3)	1.6±0.5	0.9±0.02	3.2±0.5	1.9±0.02	6.4±0.2	4.1±0.8	6.3±1.2	7.5±0.4	7.9±0.2	1.9±0.1	0.9±0.3	0.9±0.11	2.7±0.4	0.1±0.004	3.5±0.8	2.1±0.3
D. carota (n=4)	14.1±2.3	0.4 ±0.01	0.8±0.1	7.5±0.7	5.8±0.04	2.2±0.1	8.2±1.3	14.4±0.9	3.8±0.8	0.2±0.02	0.1±0.02	0.1 ±0.003	0.9 ±0.04	ND	3.1±0.9	ND
P. sativum (n=4)	14.9±1.5	0.4±0.02	1.3 ±0.02	0.40±0.01	6.6±0.2	3.9±0.2	5.8±0.4	8.6±0.7	1.04 ±0.05	1.04±0.03	1.2±0.03	1.6±0.2	1.8±0.1	0.8±0.003	1.6 ±0.5	0.8±0.001
R.sativus (n=4)	17.2±3.4	0.5±0.01	1.03 ±0.03	10.2±1.4	8.2±0.9	3.5±0.2	9.5±1.8	26.1±2.5	2.25±0.31	2.24 ±0.31	2.76±0.11	0.55±0.003	1.64±0.34	1.3±0.12	1.5± 0.1	1.1±0.002
Bannu																
B. oleracea B. (n=3)	21.3 ±2.5	0.6±0.02	8.6±1.2	0.5±0.03	10.2± 2.3	6.1±0.1	38.7±4.2	25.2 ±3.2	15.8 ±2.3	5.8±0.3	6.2 ±0.2	1.2±0.1	3.8 ±0.2	5.7± 0.4	3.4±0.3	3.3± 0.02
B. oleracea C. (n=3)	30.3±3.5	0.8±0.03	5.4 ±0.7	16.2±2.3	5.8±0.9	5.8±0.2	29.9±3.5	39.4±3.6	7.7 ±0.9	7.8±0.9	8.9±1.2	4.9 ±0.5	5.9 ±0.5	3.6±0.3	1.2±0.2	3.3±0.3
S. alba (n=3)	27.6 ±3.3	0.7±0.01	1.1±0.9	18.1±2.3	7.2±1.02	7.2±2.3	27.5 ±2.5	23.3±2.4	7.3 ±0.7	7.3±0.3	2.8 ±0.2	0.7 ±0.03	4.3 ±0.2	3.9±0.7	1.9±0.1	1.7±0.7
B. rapa (n=3)	35.3±3.1	1.2±0.1	7.1 ±0.8	16.5±2.4	6.2±0.9	6.2 ±2.3	18.3±3.2	28.2±4.2	5.9 ±0.3	1.9±0.1	3.5±0.3	2.5 ±0.1	4.1±0.6	3.1±0.1	2.1 ±0.4	2.9 ±0.3
C. sativum (n=6)	3.1 ±0.02	0.7±0.03	6.1±0.3	3.6±0.2	7.4±0.9	7.8±3.1	17.9±3.2	40.5±4.2	6.6±0.3	6.6±0.5	1.8±0.03	1.8 ±0.8	3.5 ±0.3	0.3±0.001	3.8 ±0.1	4.00±0.4
D.carota (n=4)	27.3 ±2.3	0.8±0.04	1.5 ±0.3	14.3±2.3	9.6±1.21	4.2±0.1	18.4±2.3	27.7±3.2	7.4±0.8	0.4 ±0.03	0.2 ±0.002	0.2 ±0.001	1.8±0.02	ND	7.1±0.9	ND
P.sativum (n=3)	28.5±3.2	0.7±0.03	2.4±0.1	0.8±0.02	12.9 ±1.3	7.5±1.3	15.3±2.4	24.04±4.3	3.8 ±0.3	3.8±0.3	2.5±0.2	2.8 ±0.003	1.3±0.001	2.9±0.5	1.6±0.2	3.5±0.1
R. sativus (n=4)	33.1±3.2	0.8±0.03	1.8±0.4	19.6±3.6	13.9±1.8	4.5±0.6	11.8±1.2	26.8±3.2	15.2±2.3	16.1 ±2.8	2.5±0.3	1.1±0.11	4.6±0.3	1.9±0.2	3.7 ±0.3	5.9±0.8

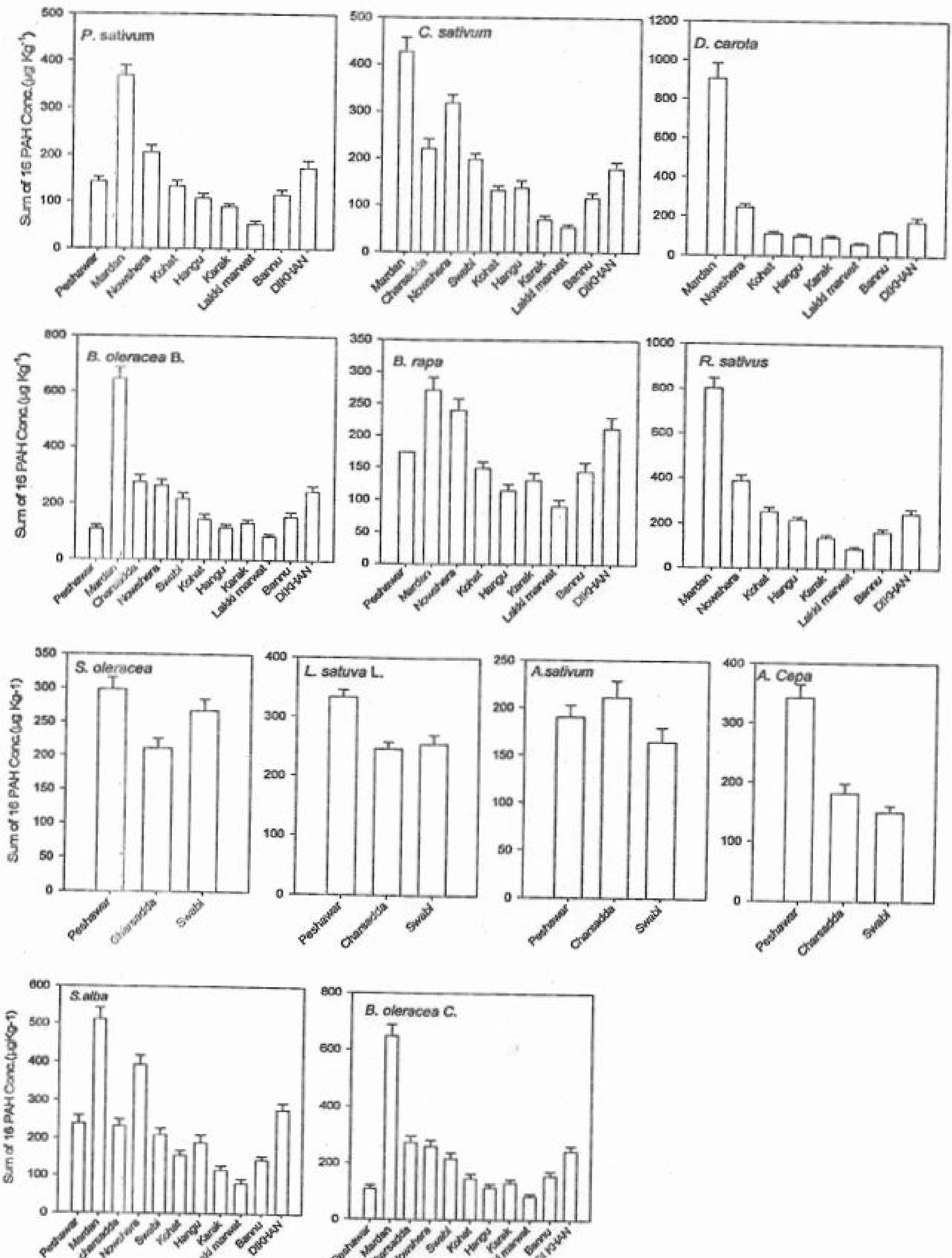


Fig. 3.2 PAH concentration in vegetable in Khyber Pakhtunkhwa Province, Pakistan.

3.6.3 PAH Transfer Factor

Table 3.4 smmarize the values of TF for different vegetable collected from the study areas. Among the vegetable collected from district Peshawar, the highest TF value (0.40) for ∑16PAH was observed in *S. oleracea* whereas the lowest value (0.13) for *P.sativum.* Among the individual PAHs, the highest TF (0.84) was observed for AN in *B. oleracea* B following by *L.satuva* L (0.83). In district Mardan, TF value for ∑16 PAHs was highest (0.64) in *D. carota*, while the lowest value (0.13) was observed in *P. sativum.* Among individual PAH compounds, the highest TF (0.96) was recorded for PHE in *B. oleracea* B followed by *R. sativus* (0.94). The highest TF value (0.59) of ∑16 PAHs was observed in *S. alba* followed by *P. sativum* in Nowshera, while among the individual PAH compounds FLA showed the highest TF value (0.93) in *D. carota*, a rooted vegetable. In other urbanized districts such as Charsadda, Swabi and DI Khan highest TF value of ∑16 PAHs were observed in *L.satuva* L (0.62), *S. oleracea* (0.57) and *S. alba* (0.48), respectively (Table 3.4).

In less urbanized districts the TF values were slightly different than those observed in the vegetable collected from high urbanized districts (Table 3). The highest TF values of ∑16 PAHs were observed in *R. sativus* (0.49), *B. oleracea* C.(0.46), *B. oleracea* C (0.42) in districts Hangu, Lakki Marwat and Bannu (Table 3.4)

The values of TF were calculated for vegetable collected from the study areas as shown in Table 3 and S3. The vegetable collected from Peshawar district, in which the highest TF value for 16 PAH was recorded 0.40 in *S. oleracea* whereas the lowest value 0.13 for P. *sativum.*The individual highest TF for AN recorded 0.84 in *B. oleracea* B following by 0.83 in *L.satuva* L.

In district Mardan TF 0.96 was recorded in *B. oleracea* B for PHE following by *R. sativus* which has TF value 0.94.The sum of 16 PAH TF value was highest 0.64 in *D. carota* whereas the lowest TF value 0.13 in *P. sativum.*

In district Nowshera the highest TF value 0.93 for FLA was recorded in root vegetable *D. carota* following by leafy vegetable *S alba*. The Sum of 16 PAH TF was recorded in S alba.Which has highest TF value 0.59 following by *P. sativum* 0.25.

In district Charsadda the individual PAH BaP transfer factor from soil to *L.satuva* L. was 0.96 following by *A. cepa* which has TF value was 0.86. The highest TF for the sum of 16 PAH was recorded for leafy vegetable *L.satuva* L which was 0.62 whereas the lowest TF value was recorded for underground vegetable *A.sativum* which was 0.32.

In district Swabi the the AC, FI, TF from soil to *S. oleracea* was 0.94 following by *L.satuva* L which has TF value for FLA was 0.93. The sum of 16 PAH TF value 0.57 was highest in *S. oleracea* following by *A.sativum* which has TF value 0.26.

In less urbanized area such as DIkhan the highest TF value for *B. oleracca* B. was 0.89 for FLA following by D[ah]A which has TF value 0.86. Whereas the sum of 16 PAH TF value 0.48 from soil *to S. alba* was highest .following by lowest value 0.24 for *P. sativum.*

In Kohat the highest TF for FLA (0.93) in *C.sativum* following by B[b]F (0.87) in *S.alba*. The sum of 16 PAH TF was highest in *R. sativus* (0.49) following by the lowest value in *P. sativum* (0.28).

In Hangu *B. oleracea* C. and *B. oleracca* B. has TF value 0.92, 0.85 for AC and FLA respectively. Whereas the sum of 16 PAH the highest TF value recorded in the *B. oleracea* C.(0.46) following by the lowest TF value in *S.alba*(0.37).

In Lakki Marwat district the highest TF value in *B. oleracea* B (0.92) and *B. oleracea* C (0.87).was recorded for CHR and FLA respectively. The sum of 16 PAH TF value from soil to *B. oleracea* C (0.42) was recorded highest whereas the lowest TF value recorded in *C.Sativum* (0.19).

In Bannu district highest TF value for AN (0.97) following by NA (0.91) was recorded in *B. rapa* and *S.alba*.The sum of 16 PAH TF value was highest 0.49 in *B. oleracea* C. following by the *C.sativum* which has the lowest TF value 0.33. Soil to plant transfer factor of these PAH in edible parts of vegetable is the key component of human exposure. However no permissible limits and guidelines for PAHs in soil and vegetable have not been recognized in many countries, including China. The trend of the TF for the vegetable in urbanized area of central KPK was

Mardan (0.52).>Swabi (0.42)>Charsadda (0.41)>Nowshera (0.38)>Peshawar (0.25). Whereas the trend of the TF for vegetable in less urbanized area of southern KPK was in order of Banuu (0.41)>Karak (0.39)>Hangu (0.36)>DI Khan (0.32)>Kohat=Lakki Marwat (0.34).

Soil to plant transfer factor of PAH compounds in vegetable is one of the key components of human exposure, therefore, it is necessary to find out TF of PAHs in different vegetable grown in the study area. TF trend of PAHs for vegetable in high urbanized areas present in central part of KPK was observed in order of Mardan > Swabi> Charsadda> Nowshera> Peshawar, while in less urbanized areas in southern KPK was Banuu > Karak > Hangu > DI Khan > Kohat = Lakki Marwat. The values of PAH-TF depend on plant characteristics, nature of PAH compounds and their concentrations in soil. TF values of all individual PAHs (both LMW and HMW) for selected vegetable were observed less than one. It means that no vegetable was shown the hyperaccumulating capacity towards PAH accumulation.

Table 3.4 Transfer factor (TF) for vegetable collected from Khyber Pakhtunkhwa Province

Vegetable(TF)	2rings	3 rings					4-rings				5-rings			6-rings		
	NA	ACN	AC	Fl	PHE	AN	FLA	PY	BbA	CHR	BbF	BkF	BaP	IP	D[ah]A	B[ghi]P
							Peshawar									
A. cepa (n=4)	0.42	0.12	0.1	0.79	0.18	0.55	0.51	0.12	0.53	0.53	0.38	0.64	0.62	0.17	0.64	0.35
A. sativum (n=4)	0.26	0.02	0.27	0.56	0.1	0.58	0.32	0.07	0.28	0.28	0.48	0.04	0.61	0.07	0.15	0.18
S. alba (n=3)	0.36	0.05	0.34	0.53	0.09	0.39	0.49	0.27	0.05	0.29	0.15	0.1	0.41	0.04	0.06	ND
B. oleracea C. *(n=3)*	0.33	0.02	0.48	0.57	0.06	0.29	0.11	0.03	0.03	0.03	0.09	0.03	0.51	ND	0.09	ND
B. oleracea B. *(n=3)*	0.44	0.03	0.5	0.69	0.71	0.84	0.39	0.3	0.18	0.18	0.2	0.07	0.88	0.1	0.12	0.07
B.rapa (n=3)	0.35	0.04	0.28	0.51	0.56	0.82	0.45	0.2	0.15	0.15	0.07	0.03	0.52	ND	0.19	ND
L. satuva L. *(n=4)*	0.52	0.15	0.27	0.14	0.23	0.83	0.66	0.02	0.53	0.81	1.03	0.15	0.78	0.01	0.46	0.73
Pisum sativum (n=4)	0.26	0.01	0.29	0.73	0.08	0.26	0.29	0.03	0.06	0.06	0.24	0.67	0.64	ND	0.23	ND
S. oleracea (n=4)	0.46	0.03	0.77	0.77	0.11	0.4	0.8	0.67	0.31	0.34	0.38	0.12	0.34	0.1	0.39	0.25
							Mardan									
S. alba (n=3)	0.75	0.08	0.27	0.21	0.43	0.57	0.51	0.3	0.92	0.87	0.73	0.78	0.72	0.45	0.59	0.59
B. oleracea B.(n=3)	0.55	0.07	0.51	0.31	0.96	0.58	0.92	0.31	0.18	0.69	0.78	0.67	0.81	0.7	0.69	0.79
B.oleracea C.(n=3)	0.79	0.06	0.58	0.69	0.18	0.61	0.9	0.35	0.83	0.83	0.75	0.85	0.76	0.86	0.36	0.76
B. rapa (n=3)	0.81	0.06	0.8	0.53	0.16	0.9	0.94	0.25	0.46	0.14	0.68	0.92	0.96	0.53	0.94	0.66
C. sativum (n=4)	0.08	0.03	0.88	0.48	0.6	0.76	0.9	0.53	0.74	0.47	0.12	0.5	0.76	0.01	0.64	0.77
D. carota (n=4)	0.93	0.05	0.24	0.34	0.55	0.9	0.88	0.42	0.93	0.04	0.02	0.04	0.73	0.01	0.62	ND
P. sativum (n=4)	0.84	0.03	0.2	0.69	0.22	0.83	0.7	0.2	0.81	0.81	0.94	0.11	0.73	0.91	0.38	0.33
R. sativus (n=3)	0.82	0.03	0.31	0.5	0.94	0.58	0.66	0.49	0.61	0.6	0.85	0.18	0.62	0.36	0.8	0.69
							Nowshera									
B. oleracea B.(n=3)	0.33	0.04	0.6	0.06	0.14	0.34	0.55	0.18	0.1	0.62	0.41	0.37	0.68	0.42	0.51	0.47
B. oleracea C.(n=3)	0.47	0.04	0.34	0.41	0.11	0.36	0.53	0.21	0.49	0.49	0.44	0.66	0.78	0.78	0.21	0.45

S. alba (n=3)	0.44	0.05	0.16	0.08	0.54	0.37	0.88	0.62	0.57	0.96	0.71	0.46	0.77	0.77	0.82	0.77
B. rapa (n=4)	0.48	0.03	0.48	0.38	0.1	0.54	0.56	0.2	0.27	0.09	0.4	0.55	0.84	0.31	0.56	0.39
C. sativum (n=4)	0.05	0.02	0.52	0.38	0.17	0.45	0.61	0.44	0.72	0.75	0.07	0.16	0.82	0.01	0.95	0.46
D. carota (n=5)	0.55	0.03	0.14	0.35	0.4	0.54	0.93	0.51	0.55	0.03	0.01	0.02	0.71	ND	0.84	0.42
P. sativum (n=3)	0.5	0.02	0.12	0.28	0.1	0.49	0.42	0.12	0.48	0.48	0.56	0.06	0.48	0.54	0.23	0.2
R. sativus (n=3)	0.49	0.02	0.18	0.65	0.28	0.7	0.50	0.4	0.62	0.53	0.89	0.11	0.75	0.67	0.31	0.73
Charsadda																
A. cepa (n=4)	0.71	0.06	0.86	0.40	0.22	0.6	0.53	0.17	0.25	0.25	0.91	0.1	0.58	ND	ND	ND
A. Sativum (n=4)	0.4	0.03	0.47	0.83	0.77	0.82	0.61	0.12	0.33	0.33	0.77	0.06	0.7	ND	ND	0.18
S. alba (n=3)	0.5	0.08	0.46	0.81	0.64	0.85	0.28	0.29	0.17	0.56	0.67	0.29	0.72	ND	ND	0.33
B.oleracea B. (n=3)	0.7	0.03	0.77	0.25	0.44	0.61	0.83	0.28	0.	0.58	0.16	0.72	0.53	ND	0.12	0.12
C. Sativum (n=3)	0.59	0.05	0.81	0.65	0.42	0.45	0.54	0.81	0.5	0.5	0.09	0.17	0.42	ND	0.35	0.06
L. Sativa L.(n=6)	0.68	0.05	0.2	0.72	0.84	0.91	0.81	0.27	0.81	0.8	0.55	0.82	0.96	0.53	0.17	0.27
S.Oleracea(n=4)	0.51	0.02	0.55	0.49	0.26	0.5	0.82	0.21	0.69	0.69	0.38	0.08	0.38	0.09	ND	ND
Swabi																
A. cepa (n=4)	0.52	0.02	0.62	0.62	0.16	0.52	0.89	0.1	0.13	0.13	0.51	0.06	0.47	ND	ND	ND
A. sativum (n=4)	0.35	0.06	0.39	0.81	0.64	0.41	0.46	0.18	0.04	0.22	0.38	0.14	0.73	ND	ND	0.14
S. alba (n=3)	0.42	0.02	0.76	0.49	0.78	0.4	0.84	0.3	0.35	0.63	0.33	0.1	0.38	0.03	0.15	0.16
B. oleracea C. (n=3)	0.71	0.04	0.59	0.54	0.94	0.68	0.72	0.33	0.8	0.89	0.15	0.47	0.47	0.56	0.52	0.57
B. oleracea B.(n=3)	0.41	0.02	0.61	0.52	0.83	0.72	0.76	0.15	0.52	0.52	0.81	0.51	0.56	ND	0.09	0.11
C. sativum (n=4)	0.46	0.04	0.62	0.57	0.66	0.48	0.58	0.29	0.39	0.57	0.07	0.13	0.82	ND	0.76	0.05
L.sativa L.(n=5)	0.61	0.53	0.24	0.77	0.69	0.16	0.93	0.25	0.2	0.2	0.4	0.38	0.62	0.12	0.17	0.12
S. oleracea (n=4)	0.39	0.06	0.95	0.94	0.54	0.69	0.82	0.59	0.72	0.94	0.36	0.11	0.19	0.21	0.61	0.36
DI Khan																
S. alba (n=3)	0.42	0.05	0.15	0.08	0.25	0.35	0.62	0.55	0.31	0.76	0.68	0.44	0.78	0.73	0.43	0.7
B. oleracea B. (n=3)	0.31	0.04	0.58	0.06	0.13	0.33	0.52	0.18	0.1	0.59	0.17	0.26	0.75	0.4	0.86	0.45
B. oleracea C. (n=3)	0.45	0.03	0.33	0.75	0.1	0.35	0.51	0.2	0.47	0.47	0.42	0.66	0.88	0.74	0.2	0.43
B. rapa (n=3)	0.46	0.03	0.45	0.75	0.09	0.51	0.53	0.22	0.26	0.08	0.39	0.52	0.82	0.3	0.53	0.37
C. sativum (n=4)	0.05	0.02	0.5	0.36	0.17	0.43	0.37	0.18	0.69	0.56	0.07	0.15	0.54	0.01	0.72	0.43

D. carota (n=4)	0.53	0.03	0.13	0.42	0.14	0.51	0.89	0.35	0.53	0.03	0.01	0.02	0.85	ND	0.74	ND
P. sativum (n=3)	0.48	0.02	0.11	0.39	0.09	0.47	0.4	0.11	0.46	0.46	0.53	0.06	0.53	0.51	0.22	0.19
R. sativus (n=3)	0.46	0.02	0.17	0.5	0.25	0.67	0.6	0.16	0.24	0.9	0.56	0.1	0.85	0.1	0.3	0.7
Kohat																
B.oleracea B. (n=3)	0.39	0.05	0.84	0.02	0.12	0.43	0.66	0.6	0.16	0.76	0.15	0.49	0.57	0.4	0.25	0.38
B.oleracea C. (n=3)	0.49	0.03	0.34	0.9	0.11	0.6	0.51	0.17	0.42	0.41	0.79	0.52	0.46	0.55	0.16	0.53
S. alba (n=3)	0.53	0.04	0.18	0.01	0.2	0.44	0.57	0.25	0.23	0.13	0.87	0.48	0.63	0.78	0.63	0.87
B. rapa (n=3)	0.66	0.04	0.71	0.56	0.12	0.42	0.71	0.23	0.19	0.11	0.26	0.64	0.22	0.09	0.65	0.4
C. sativum (n=4)	0.07	0.03	0.71	0.15	0.2	0.51	0.62	0.9	0.93	0.21	0.11	0.2	0.65	0.03	0.97	0.89
D. carota (n=4)	0.58	0.03	0.18	0.32	0.08	0.28	0.45	0.16	0.57	0.02	0.01	0.03	0.77	ND	0.55	ND
P.Sativum (n=3)	0.48	0.02	0.06	0.54	0.14	0.48	0.68	0.11	0.36	0.36	0.56	0.08	0.22	0.22	0.23	0.25
R. sativus (n=5)	0.62	0.03	0.16	0.63	0.26	0.86	0.45	0.91	0.33	0.57	0.37	0.15	0.57	0.94	0.42	0.89
Hangu																
B. oleracea C.(n=3)	0.48	0.04	0.35	0.25	0.08	0.3	0.6	0.22	0.51	0.44	0.41	0.83	0.95	0.8	0.22	0.46
B. oleracea B. (n=3)	0.34	0.04	0.62	0.02	0.13	0.35	0.56	0.19	0.11	0.63	0.42	0.56	0.39	0.43	0.4	0.48
B. rapa (n=3)	0.49	0.03	0.38	0.79	0.1	0.55	0.57	0.1	0.28	0.09	0.42	0.56	0.62	0.32	0.57	0.4
S. alba (n=3)	0.46	0.05	0.17	0.03	0.48	0.46	0.93	0.57	0.49	0.89	0.73	0.47	0.77	0.79	0.49	0.69
C. sativum (n=4)	0.05	0.02	0.54	0.11	0.84	0.46	0.65	0.14	0.74	0.56	0.07	0.3	0.9	0.01	0.83	0.47
D. carota (n=3)	0.57	0.03	0.15	0.53	0.14	0.24	0.95	0.53	0.22	0.03	0.01	0.02	0.47	ND	0.81	ND
P. Sativum (n=4)	0.51	0.02	0.12	0.35	0.1	0.42	0.43	0.12	0.49	0.29	0.58	0.07	0.97	0.19	0.23	0.2
R. sativus (n=4)	0.5	0.02	0.1	0.51	0.57	0.72	0.91	0.26	0.43	0.89	0.91	0.11	0.85	0.69	3.13	0.75
Karak																
B. oleracca B. (n=3)	0.52	0.07	0.95	0.04	0.21	0.54	0.86	0.29	0.16	0.56	0.72	0.39	0.56	0.66	0.59	0.74
B. oleracea C. (n=3)	0.74	0.06	0.54	0.39	0.12	0.46	0.92	0.33	0.78	0.68	0.63	0.79	0.91	0.36	0.33	0.71
S. alba (n=3)	0.79	0.03	0.19	0.54	0.15	0.64	0.66	0.18	0.76	0.45	0.88	0.1	0.42	0.3	0.36	0.31
B. rapa (n=3)	0.76	0.05	0.59	0.65	0.15	0.84	0.87	0.16	0.43	0.13	0.64	0.86	0.68	0.49	0.88	0.62
C.sativum (n=5)	0.08	0.03	0.82	0.16	0.33	0.71	0.26	0.28	0.03	0.2	0.11	0.46	0.53	0.01	0.14	0.72
D. carota (n=4)	0.87	0.05	0.22	0.82	0.21	0.37	0.66	0.59	0.34	0.04	0.02	0.04	0.28	ND	0.92	ND

P. Sativum (n=3)	0.7	0.08	0.26	0.04	0.17	0.7	0.44	0.77	0.25	0.37	0.43	0.73	0.74	0.22	0.74	0.88
R.sativus(n=4)	0.77	0.03	0.15	0.78	0.42	0.28	0.73	0.29	0.36	0.36	0.64	0.17	0.95	0.25	0.63	0.64
Lakki Marwat																
B. oleracea B. (n=3)	0.49	0.06	0.9	0.04	0.08	0.51	0.81	0.27	0.16	0.92	0.25	0.2	0.59	0.62	0.65	0.7
B. oleracea C. (n=3)	0.7	0.05	0.51	0.37	0.11	0.43	0.87	0.31	0.73	0.64	0.47	0.85	0.08	0.34	0.32	0.67
S. alba (n=3)	0.74	0.03	0.18	0.51	0.14	0.6	0.62	0.17	0.72	0.43	0.84	0.1	0.42	0.28	0.34	0.29
B. rapa (n=3)	0.72	0.05	0.56	0.61	0.14	0.8	0.83	0.15	0.4	0.13	0.6	0.82	0.81	0.47	0.83	0.58
C. sativum (n=3)	0.07	0.03	0.78	0.15	0.29	0.67	0.28	0.07	0.61	0.26	0.11	0.44	0.52	0.01	0.85	0.68
D. carota (n=4)	0.83	0.04	0.21	0.77	0.23	0.35	0.45	0.6	0.32	0.04	0.02	0.03	0.15	ND	0.65	ND
P. Sativum (n=4)	0.66	0.07	0.24	0.04	0.25	0.67	0.49	0.12	0.17	0.17	0.12	0.69	0.21	0.13	0.41	0.48
R.sativus (n=4)	0.73	0.03	0.14	0.74	0.4	0.57	0.79	0.29	0.27	0.27	0.29	0.16	0.21	0.17	0.56	0.39
Bannu																
B. oleracea B. (n=3)	0.59	0.08	0.89	0.04	0.25	0.62	0.82	0.33	0.19	0.41	0.53	0.63	0.32	0.76	0.75	0.86
B. oleracea C. (n=3)	0.86	0.07	0.62	0.45	0.13	0.53	0.62	0.38	0.9	0.78	0.72	0.71	0.62	0.44	0.39	0.82
S. alba (n=3)	0.91	0.03	0.21	0.62	0.17	0.74	0.76	0.21	0.88	0.52	0.23	0.12	0.45	0.34	0.41	0.36
B. rapa (n=3)	0.87	0.06	0.68	0.52	0.17	0.97	0.48	0.18	0.49	0.16	0.47	0.45	0.61	0.57	0.41	0.71
C. sativum (n=6)	0.09	0.04	0.95	0.19	0.22	0.82	0.51	0.41	0.33	0.59	0.13	0.54	0.43	0.01	0.75	0.83
D.carota (n=4)	0.78	0.05	0.26	0.94	0.24	0.42	0.64	0.74	0.4	0.05	0.02	0.04	0.2	ND	0.73	ND
P.sativum (n=3)	0.81	0.09	0.29	0.05	0.31	0.81	0.82	0.4	0.39	0.39	0.15	0.84	0.14	0.32	0.26	0.39
R. sativus (n=4)	0.89	0.03	0.17	0.9	0.43	0.46	0.62	0.19	0.75	0.94	0.16	0.19	0.38	0.17	0.66	0.63

3.6.3 Daily intake (DI) of PAHs

Table 3.6 summarizes the DI-PAHs (LMW-PAHs and HMW-PAHs) via consumption of vegetable in both adult and children in different districts of the study area, In high urbanized districts, DI of individual PAHs though consumption of vegetable ranged from 2.13E-04 to 4.19E-02 μg $kg^{-1}d^{-1}$ in adults, while ranged from 2.54E-04 to 2.30E-02 μg $kg^{-1}d^{-1}$ in children. In less urbanized districts, DI-PAHs though consumption of vegetable ranged from 1.77E-04 to 5.57E-02 μg $kg^{-1}d^{-1}$ in adults, while ranged from 7.26E-04 to 3.85E-02 μg kg-1d^{-1} in children. In urbanized areas including Peshawar, Mardan, Nowshera, Charsadda, Swabi and DI Khan the highest intake of PAHs observed (both adults and children) through consumption of *C. sativum*, *B. oleracea* B. and *S. oleracea*, while the lowest intake estimated through consumption S. alba, and A. cepa. In less urbanized districts such as Kohat Hangu, Karak, Lakki Marwat and Bannu the highest intake was calculated though ingestion of R. sativus, and C.sativum, while the lowest intake observed though ingestion of D. carota, and B. rapa in both adult and children. Daily intake of PAHs depends on the types of vegetable, ingestion rate, and concentration of PAHs. In this study, DI values were not constant for all PAHs and varied among the districts selected for this study. In high urbanized districts greater DI of PAHs was noted as compared to less urbanized districts. In both children and adults, the highest DI of ∑16PAH was observed in Mardan followed by Nowshera. In KPK province, the lowest DI value of ∑16PAH was observed for adults (3.15E-02 μg kg^{-1} body weight d^{-1}) and children (1.39E-01 μg kg^{-1} body weight d^{-1}) in district Lakki Marwat

To calculate the carcinogenicity of the 16PAHs, the TEQ value of BaP was used as suggested by US EPA (2002). Table 3.5 summarizes the toxicological potency of all selected possible 16 carcinogenic PAHs. The values of TEQ for selected PAHs were greatly varied in the study area.

Except Swabi, the high urbanized districts showed slightly greater TEQ values (ranged from 0.18-7.16) for individual PAHs than less urbanized districts (0.12-2.57).

Table 3.5. The total toxic BaP equivalent (TEQ) concentration for selected PAHs in Khyber Pakhtunkhwa Province

	2rings	3 rings					4-rings				5-rings			6-rings		
TEQ	NA	ACN	AC	Fl	PHE	AN	FLA	PY	BbA	CHR	BbF	BkF	BaP	IP	D[ah]A	B[ghi]P
Peshawar (n=32)	3.80	0.24	0.73	1.01	2.50	1.22	4.09	3.01	1.11	0.79	1.28	0.36	1.52	0.09	0.30	0.22
Mardan (n=27)	4.88	0.15	0.73	0.31	3.58	1.08	5.20	7.16	1.86	1.15	1.57	0.41	1.22	0.58	0.55	0.48
Nowshera (n=28)	3.74	0.12	0.66	0.59	1.98	1.04	5.19	6.39	1.74	1.38	1.53	0.38	1.96	0.82	0.78	0.48
Charsadda (n=27)	3.81	0.11	1.02	0.79	3.62	1.19	3.04	4.72	1.67	1.02	0.97	0.27	0.89	0.19	0.20	0.18
Swabi (n=30)	2.55	0.28	0.77	2.01	3.95	0.73	3.46	4.32	0.79	0.97	0.70	0.17	0.78	0.09	0.14	0.11
DI Khan (n=26)	3.09	0.10	0.54	0.60	1.18	0.86	3.77	4.21	1.24	1.20	1.06	0.36	1.69	0.49	0.55	0.40
Kohat (n=28)	2.23	0.07	0.39	0.97	0.80	0.62	2.53	3.81	0.92	0.51	0.67	0.21	0.62	0.48	0.27	0.29
Hangu (n=27)	1.68	0.05	0.29	0.73	1.28	0.47	2.55	2.18	0.91	0.78	0.68	0.20	0.84	0.36	0.49	0.22
Karak (n=28)	1.93	0.06	0.34	0.84	0.70	0.45	1.81	2.27	0.50	0.36	0.55	0.18	0.56	0.21	0.28	0.20
Lakki Marwat (n=27)	1.34	0.04	0.24	0.58	0.49	0.34	1.25	1.46	0.43	0.28	0.25	0.15	0.22	0.14	0.20	0.12
Bannu (n=29)	2.57	0.08	0.43	1.12	0.91	0.62	2.22	2.94	0.87	0.62	0.36	0.19	0.36	0.26	0.31	0.31

Table 3.6 DI-PAH μg $kg^{-1}d^{-1}$ by adults and children through vegetable consumption in Khyber Pakhtunkhwa Province

	2 rings	3 rings					4 rings				5 rings			6-rings		
	NA	ACN	AC	Fl	PHE	AN	FLA	PY	BbA	CHR	BbF	BkF	BaP	IP	D[ah]A	B[ghi]P
Adults						Peshawar										
A. cepa (n=4)	1.89E-02	1.83E-03	1.08E-03	2.59E-03	9.05E-03	6.20E-03	2.00E-02	1.58E-02	5.84E-03	5.85E-03	5.46E-03	5.46E-03	7.57E-03	7.33E-04	2.60E-03	1.82E-03
A. sativum (n=4)	1.36E-02	5.08E-04	2.82E-03	2.81E-03	4.72E-03	4.72E-03	1.57E-02	1.35E-02	4.31E-03	4.32E-03	4.58E-03	3.04E-04	5.28E-03	4.73E-04	9.41E-04	9.27E-04
S. alba (n=3)	1.65E-02	5.76E-04	4.16E-03	3.17E-03	4.80E-03	4.80E-03	2.44E-02	2.56E-02	5.22E-03	5.23E-03	2.18E-03	2.37E-04	2.68E-03	4.23E-04	3.47E-04	ND
B. oleracea C. *(n=3)*	1.51E-02	5.13E-04	3.57E-03	2.65E-03	3.62E-02	4.62E-03	1.42E-02	1.15E-02	1.70E-03	1.71E-03	2.66E-03	5.22E-04	9.90E-03	7.11E-04	4.21E-04	2.69E-04
B. oleracea B. *(n=3)*	1.29E-02	4.22E-04	3.17E-03	4.60E-03	3.02E-03	3.03E-03	4.95E-03	4.87E-03	3.30E-04	3.42E-04	1.47E-03	2.31E-04	6.79E-03	ND	5.17E-04	ND
B.rapa (n=3)	1.56E-02	4.63E-04	2.91E-03	3.36E-03	1.78E-02	3.79E-03	1.06E-02	7.06E-03	1.87E-03	1.89E-03	1.38E-03	1.58E-04	6.24E-03	ND	5.41E-04	ND
L. satuva L. *(n=4)*	2.33E-02	3.82E-03	2.24E-03	9.05E-04	1.00E-02	1.00E-02	2.96E-02	5.21E-03	1.38E-02	5.81E-03	1.78E-02	1.21E-03	7.94E-03	4.23E-04	3.02E-03	4.49E-03
P. sativum (n=4)	1.25E-02	4.32E-04	2.22E-03	5.64E-03	3.27E-03	3.27E-03	6.91E-03	5.71E-03	9.70E-04	9.82E-04	4.67E-03	4.67E-03	7.27E-03	ND	1.65E-03	ND
S. oleracea (n=4)	1.50E-02	5.29E-04	5.22E-03	1.25E-02	5.49E-03	5.49E-03	2.76E-02	2.41E-02	7.90E-03	3.72E-03	7.88E-03	8.48E-04	3.63E-03	1.90E-03	1.32E-03	1.95E-03
Children																
A. cepa(n=4)	2.71E-02	2.63E-03	1.56E-03	3.73E-03	5.95E-02	8.92E-03	2.88E-02	2.27E-02	8.41E-03	8.42E-03	7.86E-03	7.86E-03	1.09E-02	1.05E-03	3.75E-03	2.62E-03
A. sativum(n=4)	1.96E-02	7.31E-04	4.06E-03	4.05E-03	6.79E-03	6.79E-03	2.26E-02	1.95E-02	6.20E-03	6.21E-03	6.59E-03	4.37E-04	7.59E-03	6.82E-04	1.35E-03	1.33E-03
S. alba (n=3)	2.37E-02	8.30E-04	6.00E-03	4.56E-03	6.91E-03	6.91E-03	3.51E-02	3.69E-02	7.51E-03	7.52E-03	3.14E-03	3.41E-04	3.86E-03	6.09E-04	4.99E-04	ND
B. oleraceaC. (n=3)	1.86E-02	6.08E-04	4.56E-03	6.61E-03	4.35E-03	4.36E-03	7.13E-03	7.02E-03	4.75E-04	4.93E-04	2.11E-03	3.32E-04	9.77E-03	ND	7.44E-04	ND
B. oleraceaB. (n=3)	2.17E-02	7.38E-04	5.14E-03	3.82E-03	5.21E-02	6.65E-03	2.05E-02	1.66E-02	2.44E-03	2.46E-03	3.83E-03	7.52E-04	1.43E-02	1.02E-03	6.06E-04	3.88E-04
B.rapa(n=3)	2.25E-02	6.66E-04	4.19E-03	4.83E-03	2.56E-02	5.45E-03	1.52E-02	1.02E-02	2.70E-03	2.71E-03	1.98E-03	2.28E-04	8.98E-03	ND	7.79E-04	ND
L. satuva L*(n=4)*	3.35E-02	5.50E-03	3.23E-03	1.30E-03	1.45E-02	1.45E-02	4.26E-02	7.50E-03	1.98E-02	8.36E-03	2.57E-02	1.74E-03	1.14E-02	6.08E-04	4.34E-03	6.46E-03
P. sativum(n=4)	1.80E-02	6.22E-04	3.20E-03	8.12E-03	4.70E-03	4.70E-03	9.94E-03	8.22E-03	1.40E-03	1.41E-03	6.73E-03	6.72E-03	1.05E-02	ND	2.37E-03	ND
S. oleracea(n=4)	2.16E-02	7.61E-04	7.51E-03	1.79E-02	7.91E-03	7.91E-03	3.98E-02	3.46E-02	1.14E-02	5.36E-03	1.13E-02	1.22E-03	5.22E-03	2.71E-03	1.90E-03	2.81E-03
Adults						Mardan										
S. alba (n=3)	2.26E-02	5.89E-04	1.92E-03	6.16E-04	9.33E-03	1.78E-03	8.16E-03	1.69E-02	7.69E-03	7.27E-03	1.04E-02	2.36E-03	5.79E-03	3.58E-03	1.13E-03	1.39E-03
B. oleracca B.*(n=3)*	1.69E-02	5.02E-04	4.23E-03	1.23E-03	3.34E-02	4.80E-03	3.07E-02	2.00E-02	1.25E-02	8.33E-03	7.75E-03	1.04E-03	3.54E-03	4.53E-03	2.66E-03	2.65E-03
B.oleraceaC.(n=3)	2.40E-02	6.26E-04	4.30E-03	1.51E-03	4.60E-03	4.62E-03	2.38E-02	3.13E-02	6.17E-03	6.17E-03	7.14E-03	2.95E-03	6.23E-03	2.44E-03	9.77E-04	2.65E-03
B. rapa(n=3)	2.80E-02	9.57E-04	5.62E-03	1.33E-03	4.89E-03	4.89E-03	3.04E-02	2.24E-02	4.75E-03	1.50E-03	4.35E-03	4.35E-03	5.60E-03	2.49E-03	4.01E-03	2.33E-03

C. sativum(n=4)	2.43E-03	5.85E-04	4.86E-03	1.56E-03	1.73E-02	6.18E-03	2.70E-02	5.00E-02	1.27E-02	2.27E-03	1.42E-03	1.42E-03	5.17E-03	1.86E-04	2.80E-03	3.18E-03
D. carota(n=4)	2.14E-02	6.62E-04	1.15E-03	8.85E-04	1.88E-02	3.33E-03	2.16E-02	2.20E-02	5.84E-03	2.79E-04	1.84E-04	1.82E-04	5.52E-03	ND	1.49E-03	ND
P. sativum(n=4)	2.18E-02	5.38E-04	8.77E-04	1.79E-03	5.69E-03	5.69E-03	2.19E-02	1.85E-02	5.80E-03	5.81E-03	1.03E-02	5.53E-04	5.97E-03	3.07E-03	1.52E-03	1.36E-03
R. sativus(n=3)	2.62E-02	6.82E-04	1.57E-03	1.32E-03	2.59E-02	4.90E-03	1.07E-02	5.88E-02	6.87E-03	6.86E-03	1.11E-02	8.31E-04	2.97E-03	3.53E-03	3.81E-03	2.64E-03
Children																
S. alba (n=3)	3.26E-02	8.48E-04	2.76E-03	8.87E-04	1.34E-02	2.56E-03	1.17E-02	2.43E-02	1.11E-02	1.05E-02	1.50E-02	3.40E-03	8.34E-03	5.16E-03	1.63E-03	2.00E-03
B. oleracca B.*(n=3)*	2.43E-02	7.22E-04	6.09E-03	1.77E-03	4.81E-02	6.92E-03	4.42E-02	2.88E-02	1.79E-02	1.20E-02	1.12E-02	1.50E-03	5.10E-03	6.52E-03	3.82E-03	3.81E-03
*B.oleracea*C.*(n=3)*	3.46E-02	9.00E-04	6.19E-03	2.18E-03	6.63E-03	6.65E-03	3.42E-02	4.50E-02	8.88E-03	8.89E-03	1.03E-02	4.24E-03	8.97E-03	3.52E-03	1.41E-03	3.81E-03
B. rapa(n=3)	4.03E-02	1.38E-03	8.09E-03	1.92E-03	7.04E-03	7.04E-03	4.38E-02	3.22E-02	6.84E-03	2.16E-03	6.26E-03	6.26E-03	8.06E-03	3.59E-03	5.78E-03	3.35E-03
C. sativum(n=4)	3.50E-03	8.42E-04	6.99E-03	2.23E-03	2.50E-02	8.89E-03	3.89E-02	7.19E-02	1.83E-02	3.26E-03	2.04E-03	2.04E-03	7.44E-03	2.67E-04	4.03E-03	4.57E-03
D. carota(n=4)	3.08E-02	9.53E-04	1.66E-03	1.27E-03	2.71E-02	4.80E-03	3.12E-02	3.16E-02	8.40E-03	4.02E-04	2.64E-04	2.63E-04	7.95E-03	BL	2.15E-03	BL
P. sativum(n=4)	3.14E-02	7.75E-04	1.26E-03	2.58E-03	8.19E-03	8.20E-03	3.15E-02	2.67E-02	8.35E-03	8.37E-03	1.48E-02	7.96E-04	8.60E-03	4.42E-03	2.19E-03	1.96E-03
R. sativus(n=3)	3.78E-02	9.81E-04	2.26E-03	1.90E-03	3.72E-02	7.05E-03	1.54E-02	8.46E-02	9.90E-03	9.88E-03	1.59E-02	1.20E-03	4.28E-03	5.08E-03	5.48E-03	3.80E-02
Adults																
Nowshera																
B. oleracea B.*(n=3)*	1.29E-02	3.85E-04	6.46E-03	2.99E-04	6.06E-03	3.68E-03	2.36E-02	1.54E-02	9.56E-03	9.60E-03	5.24E-03	2.31E-03	6.67E-03	3.47E-03	2.52E-03	2.03E-03
B. oleracea C.*(n=3)*	1.84E-02	4.80E-04	3.30E-03	1.15E-03	3.53E-03	3.54E-03	1.82E-02	2.40E-02	4.73E-03	4.73E-03	5.47E-03	2.96E-03	8.27E-03	2.84E-03	7.49E-04	2.03E-03
S. alba (n=3)	1.74E-02	4.52E-04	1.47E-03	4.72E-04	1.50E-02	4.58E-03	1.80E-02	4.51E-02	6.17E-03	1.04E-02	1.31E-02	1.81E-03	1.11E-02	7.89E-03	8.90E-03	3.31E-03
B. rapa(n=4)	2.15E-02	7.34E-04	4.31E-03	1.64E-03	3.75E-03	3.75E-03	2.33E-02	1.72E-02	3.65E-03	1.15E-03	3.33E-03	3.33E-03	6.28E-03	1.91E-03	3.08E-03	1.79E-03
C. sativum(n=4)	1.86E-03	4.49E-04	3.72E-03	2.16E-03	6.31E-03	4.73E-03	2.38E-02	4.63E-02	1.62E-02	7.81E-03	1.09E-03	1.09E-03	9.69E-03	1.42E-04	5.36E-03	2.44E-03
D. carota(n=5)	1.64E-02	5.07E-04	8.82E-04	1.17E-03	1.75E-02	2.56E-03	2.94E-02	1.69E-02	4.47E-03	2.14E-04	1.41E-04	1.40E-04	9.68E-03	2.63E-04	2.61E-03	ND
P. sativum(n=3)	1.67E-02	4.13E-04	6.72E-04	9.56E-04	4.36E-03	4.37E-03	1.68E-02	1.42E-02	4.45E-03	4.46E-03	7.90E-03	4.24E-04	5.56E-03	2.35E-03	1.17E-03	1.04E-03
R. sativus(n=3)	2.01E-02	5.23E-04	1.20E-03	3.55E-03	9.91E-03	7.61E-03	2.08E-02	3.53E-02	9.04E-03	7.77E-03	1.49E-02	6.37E-04	8.37E-03	8.49E-03	1.90E-03	3.63E-03
Children																
*B. oleracea*B.*(n=3)*	1.86E-02	5.54E-04	9.30E-03	4.31E-04	8.73E-03	5.30E-03	3.39E-02	2.21E-02	1.38E-02	1.38E-02	7.54E-03	3.32E-03	9.60E-03	5.00E-03	3.63E-03	2.92E-03
*B. oleracea*C.*(n=3)*	2.65E-02	6.90E-04	4.75E-03	1.66E-03	5.08E-03	5.10E-03	2.62E-02	3.45E-02	6.81E-03	6.81E-03	7.88E-03	4.26E-03	1.19E-02	4.08E-03	1.08E-03	2.92E-03
S. alba (n=3)	2.50E-02	6.50E-04	2.12E-03	6.80E-04	2.16E-02	6.59E-03	2.59E-02	6.49E-02	8.89E-03	1.49E-02	1.89E-02	2.61E-03	1.60E-02	1.14E-02	1.28E-02	4.77E-03
B. rapa(n=4)	3.09E-02	1.06E-03	6.21E-03	2.35E-03	5.40E-03	5.40E-03	3.34E-02	2.47E-02	5.25E-03	1.66E-03	4.80E-03	4.80E-03	9.03E-03	2.75E-03	4.43E-03	2.57E-03
C. sativum(n=4)	2.68E-03	6.46E-04	5.36E-03	3.11E-03	9.09E-03	6.82E-03	3.42E-02	6.66E-02	2.33E-02	1.12E-02	1.57E-03	1.56E-03	1.39E-02	2.05E-04	7.72E-03	3.51E-03
D. carota(n=5)	2.37E-02	7.30E-04	1.27E-03	1.68E-03	2.52E-02	3.68E-03	4.24E-02	2.43E-02	6.44E-03	3.08E-04	2.03E-04	2.01E-04	1.39E-02	3.79E-04	3.76E-03	ND
P. sativum(n=3)	2.41E-02	5.94E-04	9.68E-04	1.38E-03	6.28E-03	6.28E-03	2.41E-02	2.04E-02	6.41E-03	6.41E-03	1.14E-02	6.10E-04	8.00E-03	3.39E-03	1.68E-03	1.50E-03
R. sativus(n=3)	2.90E-02	7.52E-04	1.73E-03	1.72E-02	1.43E-02	1.10E-02	2.99E-02	5.08E-02	1.30E-02	1.12E-02	2.15E-02	9.17E-04	1.20E-02	1.22E-02	2.73E-03	5.23E-03

Adults								**Charsadda**								
A. cepa(n=4)	1.86E-02	5.04E-04	6.96E-03	4.96E-03	4.92E-03	4.92E-03	7.80E-03	1.29E-02	1.60E-03	1.61E-03	7.49E-03	3.09E-04	4.13E-03	ND	ND	ND
A. sativum(n=4)	1.20E-02	5.15E-04	3.66E-03	3.22E-03	2.05E-02	3.87E-03	1.71E-02	1.34E-02	2.95E-03	2.96E-03	4.23E-03	2.37E-04	3.53E-03	ND	0.00E+00	5.36E-04
S. alba (n=3)	1.33E-02	5.13E-04	3.33E-03	2.79E-03	1.94E-02	6.18E-03	8.05E-03	1.62E-02	4.59E-03	5.85E-03	5.76E-03	3.89E-04	4.81E-03	1.84E-03	3.60E-03	9.44E-04
B.oleracea B. *(n=3)*	1.57E-02	4.85E-04	6.15E-03	3.67E-03	1.36E-02	4.40E-03	2.78E-02	2.29E-02	6.07E-03	6.08E-03	1.48E-03	3.16E-03	3.75E-03	-2.85E-04	4.20E-04	4.27E-04
C. Sativum(n=3)	1.18E-02	5.46E-04	3.37E-03	4.18E-03	1.26E-02	3.32E-03	1.16E-02	3.16E-02	6.88E-03	2.70E-03	7.33E-04	7.32E-04	2.77E-03	ND	ND	1.40E-04
L. Ssatuva L. *(n=6)*	1.78E-02	3.33E-04	1.21E-03	2.18E-03	2.61E-02	6.29E-03	1.12E-02	6.02E-03	5.88E-03	5.81E-03	6.82E-03	2.17E-03	6.68E-03	3.63E-03	7.58E-04	5.43E-04
S.Oleracea(n=4)	1.42E-02	4.69E-04	4.72E-03	3.18E-03	6.32E-03	3.63E-03	1.16E-02	2.24E-02	6.83E-03	6.83E-03	4.24E-03	3.09E-04	1.57E-03	7.76E-04	7.56E-04	7.48E-04
Children																
A. cepa(n=4)	2.68E-02	7.26E-04	1.00E-02	7.15E-03	7.08E-03	7.08E-03	1.12E-02	1.86E-02	2.30E-03	2.32E-03	1.08E-02	4.45E-04	5.95E-03	ND	ND	ND
A. sativum(n=4)	1.73E-02	7.41E-04	5.26E-03	4.63E-03	2.95E-02	5.56E-03	2.46E-02	1.92E-02	4.24E-03	4.26E-03	6.09E-03	3.41E-04	5.09E-03	ND	0.00E+00	7.71E-04
S. alba (n=3)	1.92E-02	7.38E-04	4.79E-03	4.01E-03	2.79E-02	8.89E-03	1.16E-02	2.33E-02	6.61E-03	8.43E-03	8.28E-03	5.59E-04	6.93E-03	2.64E-03	5.19E-03	1.36E-03
B.oleracea B. *(n=3)*	2.26E-02	6.99E-04	8.85E-03	5.29E-03	1.95E-02	6.33E-03	4.00E-02	3.29E-02	8.74E-03	8.75E-03	2.13E-03	4.55E-03	5.40E-03	ND	6.04E-04	6.15E-04
C. sativum(n=3)	1.70E-02	7.85E-04	4.85E-03	6.02E-03	1.81E-02	4.78E-03	1.67E-02	4.55E-02	9.91E-03	3.89E-03	1.05E-03	1.05E-03	3.99E-03	ND	ND	2.02E-04
L. satuva L. *(n=6)*	2.57E-02	4.79E-04	1.74E-03	3.13E-03	3.76E-02	9.05E-03	1.61E-02	8.66E-03	8.46E-03	8.36E-03	9.82E-03	3.13E-03	9.62E-03	5.23E-03	1.09E-03	7.81E-04
S.oleracea(n=4)	2.05E-02	6.75E-04	6.79E-03	4.58E-03	9.10E-03	5.22E-03	1.67E-02	3.22E-02	9.83E-03	9.84E-03	6.11E-03	4.44E-04	2.26E-03	1.12E-03	1.09E-03	1.08E-03
Adults								**Swabi**								
A. cepa(n=4)	1.25E-02	3.38E-04	4.66E-03	8.66E-03	3.29E-03	3.29E-03	1.08E-02	8.66E-03	1.07E-03	1.08E-03	5.02E-03	2.07E-04	3.69E-03	ND	ND	ND
A. sativum(n=4)	8.04E-03	3.45E-04	2.45E-03	5.81E-03	1.67E-02	2.59E-03	1.14E-02	8.95E-03	1.98E-03	1.98E-03	2.83E-03	1.59E-04	5.43E-03	ND	0.00E+00	3.59E-04
S. alba (n=3)	9.53E-03	3.14E-04	3.16E-03	6.06E-03	1.70E-02	2.43E-03	1.90E-02	1.50E-02	4.57E-03	4.58E-03	2.84E-03	2.07E-04	1.93E-03	5.20E-04	5.07E-04	5.01E-04
*B. oleracea*C. *(n=3)*	1.62E-02	3.22E-04	3.32E-03	1.27E-02	2.63E-02	4.83E-03	1.30E-02	2.20E-02	4.49E-03	5.75E-03	1.23E-03	1.23E-03	2.90E-03	1.19E-03	1.06E-03	1.49E-03
*B. oleracea*B. *(n=3)*	1.05E-02	3.25E-04	4.12E-03	3.89E-03	1.90E-02	2.95E-03	1.86E-02	1.53E-02	4.07E-03	4.07E-03	3.88E-03	1.79E-03	2.43E-03	ND	2.81E-04	2.81E-04
C. sativum(n=4)	7.90E-03	3.65E-04	2.26E-03	5.61E-03	1.70E-02	3.07E-03	1.07E-02	2.12E-02	4.61E-03	4.62E-03	4.91E-04	4.90E-04	4.67E-03	ND	ND	9.39E-05
L.satuva L. *(n=5)*	1.20E-02	6.96E-03	8.08E-04	1.44E-02	1.83E-02	1.03E-03	2.26E-02	1.75E-02	1.05E-03	1.84E-03	3.27E-03	1.45E-03	3.81E-03	8.84E-04	5.08E-04	3.64E-04
S. oleracea(n=4)	8.92E-03	3.44E-04	5.04E-03	1.03E-02	1.47E-02	4.14E-03	9.79E-03	3.61E-02	4.50E-03	8.69E-03	3.86E-03	2.60E-04	1.13E-03	1.23E-03	2.41E-03	6.33E-04
Children																
A. cepa(n=4)	1.80E-02	4.86E-04	6.71E-03	1.25E-02	4.74E-03	4.74E-03	1.56E-02	1.25E-02	1.54E-03	1.55E-03	7.23E-03	2.98E-04	5.31E-03	ND	ND	ND
A. sativum(n=4)	1.16E-02	4.97E-04	3.53E-03	8.36E-03	2.40E-02	3.73E-03	1.65E-02	1.29E-02	2.84E-03	2.85E-03	4.08E-03	2.29E-04	7.81E-03	ND	0.00E+00	5.17E-04
S. alba (n=3)	1.37E-02	4.52E-04	4.55E-03	8.72E-03	2.45E-02	3.50E-03	2.73E-02	2.16E-02	6.58E-03	6.59E-03	4.09E-03	2.98E-04	2.78E-03	7.49E-04	7.29E-04	7.22E-04
*B. oleracea*C. *(n=3)*	2.33E-02	4.64E-04	4.78E-03	1.83E-02	3.79E-02	6.96E-03	1.87E-02	3.16E-02	6.46E-03	8.27E-03	1.77E-03	1.76E-03	4.17E-03	1.71E-03	1.52E-03	2.14E-03
*B. oleracea*B. *(n=3)*	1.52E-02	4.68E-04	5.93E-03	5.59E-03	2.73E-02	4.24E-03	2.68E-02	2.20E-02	5.86E-03	5.87E-03	5.59E-03	2.57E-03	3.50E-03	ND	4.01E-04	4.04E-04

C. sativum(n=4)	1.14E-02	5.26E-04	3.25E-03	8.07E-03	2.45E-02	4.41E-03	1.54E-02	3.05E-02	6.64E-03	6.65E-03	7.07E-04	7.06E-04	6.72E-03	ND	ND	1.35E-04
L.satuva L.(n=5)	1.72E-02	1.00E-02	1.16E-03	2.07E-02	2.64E-02	1.48E-03	3.25E-02	2.52E-02	1.51E-03	2.65E-03	4.71E-03	2.09E-03	5.48E-03	1.27E-03	7.32E-04	5.23E-04
S. oleracea(n=4)	1.28E-02	4.95E-04	7.25E-03	1.48E-02	2.11E-02	5.96E-03	1.41E-02	5.19E-02	6.48E-03	1.25E-02	5.55E-03	3.75E-04	1.63E-03	1.77E-03	3.47E-03	9.11E-04
Adults						DI Khan										
S. alba (n=3)	1.43E-02	3.73E-04	1.22E-03	3.90E-04	6.05E-03	3.78E-03	1.10E-02	3.72E-02	2.91E-03	7.10E-03	1.08E-02	1.50E-03	7.02E-03	6.51E-03	2.74E-03	2.74E-03
*B. oleracea*B. *(n=3)*	1.07E-02	3.18E-04	5.33E-03	2.47E-04	5.10E-03	3.04E-03	1.95E-02	1.27E-02	7.90E-03	7.93E-03	1.83E-03	1.42E-03	6.42E-03	2.87E-03	3.71E-03	1.68E-03
*B. oleracea*C. *(n=3)*	1.52E-02	3.96E-04	2.72E-03	8.12E-03	2.92E-03	2.93E-03	1.50E-02	1.98E-02	3.91E-03	3.91E-03	4.52E-03	4.52E-03	8.83E-03	2.34E-03	6.19E-04	1.68E-03
B. rapa(n=3)	1.77E-02	6.06E-04	3.56E-03	4.08E-03	3.10E-03	3.10E-03	1.93E-02	1.42E-02	3.01E-03	9.51E-04	2.75E-03	2.75E-03	8.10E-03	1.58E-03	2.54E-03	1.47E-03
C. sativum(n=4)	1.54E-03	3.71E-04	3.08E-03	1.79E-03	5.40E-03	3.91E-03	1.24E-02	1.36E-02	1.34E-02	5.00E-03	8.98E-04	8.98E-04	5.51E-03	1.18E-04	4.43E-03	2.01E-03
D. carota(n=4)	1.36E-02	4.15E-04	7.28E-04	3.00E-03	5.49E-03	2.11E-03	2.43E-02	9.93E-03	3.70E-03	1.77E-04	1.16E-04	1.16E-04	7.27E-03	ND	1.99E-03	ND
P. sativum(n=3)	1.38E-02	3.41E-04	5.55E-04	1.14E-03	3.61E-03	3.61E-03	1.38E-02	1.17E-02	3.68E-03	3.68E-03	6.52E-03	3.50E-04	5.32E-03	1.94E-03	9.65E-04	8.62E-04
R. sativus(n=3)	1.66E-02	4.32E-04	9.94E-04	1.48E-03	7.86E-03	6.29E-03	1.10E-02	2.19E-02	3.10E-03	1.15E-02	8.12E-03	5.26E-04	8.28E-03	1.14E-03	1.59E-03	3.00E-03
Children																
S. alba(n=3)	[illegible]	5.37E-04	1.75E-03	5.62E-04	8.71E-03	5.44E-03	1.59E-02	5.36E-02	4.20E-03	1.02E-02	1.56E-02	2.15E-03	1.01E-02	9.38E-03	3.95E-03	3.94E-03
*B. oleracea*B. *(n=3)*	1.54E-02	4.57E-04	7.68E-03	3.56E-04	7.34E-03	4.38E-03	2.80E-02	1.83E-02	1.14E-02	1.14E-02	2.64E-03	2.04E-03	9.24E-03	4.13E-03	2.34E-02	2.41E-03
*B. oleracea*C. *(n=3)*	2.19E-02	5.70E-04	3.92E-03	1.17E-02	4.20E-03	4.21E-03	2.17E-02	2.85E-02	5.62E-03	5.63E-03	6.51E-03	6.51E-03	1.27E-02	3.37E-03	8.90E-04	2.41E-03
B. rapa(n=3)	2.55E-02	8.73E-04	5.13E-03	5.88E-03	4.46E-03	4.46E-03	2.77E-02	2.04E-02	4.33E-03	1.37E-03	3.96E-03	3.96E-03	1.17E-02	2.27E-03	3.66E-03	2.12E-03
C. sativum(n=4)	2.22E-03	5.34E-04	4.43E-03	2.57E-03	7.77E-03	5.63E-03	1.78E-02	1.96E-02	1.93E-02	7.19E-03	1.29E-03	1.29E-03	7.93E-03	1.69E-04	6.37E-03	2.90E-03
D. carota(n=4)	1.95E-02	6.03E-04	1.05E-03	4.32E-03	7.91E-03	3.04E-03	3.50E-02	1.43E-02	5.32E-03	2.55E-04	1.67E-04	1.66E-04	1.05E-02	ND	2.87E-03	ND
P. sativum(n=3)	1.99E-02	4.91E-04	7.99E-04	1.64E-03	5.19E-03	5.19E-03	1.99E-02	1.69E-02	5.29E-03	5.30E-03	9.39E-03	5.04E-04	7.66E-03	2.80E-03	1.39E-03	1.24E-03
R. sativus(n=3)	2.39E-02	6.22E-04	1.43E-03	2.13E-03	1.13E-02	9.05E-03	1.58E-02	3.15E-02	4.46E-03	1.65E-02	1.17E-02	7.58E-04	1.19E-02	1.65E-03	2.28E-03	4.32E-03
Adults						Kohat										
B.oleracea B.*(n=3)*	7.72E-03	2.29E-04	3.85E-03	1.79E-04	2.85E-03	2.20E-03	1.41E-02	9.17E-03	5.70E-03	5.73E-03	1.39E-03	9.76E-04	2.96E-03	2.07E-03	8.62E-04	1.21E-03
B.oleracea C.*(n=3)*	1.10E-02	2.86E-04	1.97E-03	5.87E-03	2.11E-03	2.11E-03	1.09E-02	1.43E-02	2.82E-03	2.82E-03	3.26E-03	1.59E-03	1.72E-03	1.69E-03	4.47E-04	1.21E-03
S. alba (n=3)	1.04E-02	2.70E-04	8.78E-04	2.82E-04	4.95E-03	2.73E-03	8.90E-03	1.43E-02	1.13E-03	7.08E-04	7.82E-03	1.08E-03	3.35E-03	4.70E-03	1.12E-03	1.98E-03
B. rapa(n=3)	1.28E-02	4.38E-04	2.57E-03	5.97E-03	2.24E-03	2.24E-03	1.39E-02	1.02E-02	2.17E-03	6.97E-04	1.09E-03	1.09E-03	9.62E-04	1.14E-03	1.84E-03	1.07E-03
C. sativum(n=4)	1.11E-03	2.68E-04	2.22E-03	1.29E-03	4.36E-03	2.82E-03	9.86E-03	1.50E-02	9.66E-03	8.63E-04	6.49E-04	6.48E-04	3.21E-03	8.49E-05	1.52E-03	1.45E-03
D. carota(n=4)	9.80E-03	3.03E-04	5.26E-04	5.20E-03	1.92E-03	1.52E-03	9.19E-03	1.01E-02	2.67E-03	1.28E-04	8.40E-05	8.35E-05	4.08E-03	ND	1.42E-03	ND
P.sativum(n=3)	9.99E-03	2.46E-04	4.01E-04	6.57E-03	2.60E-03	2.60E-03	1.00E-02	8.47E-03	2.65E-03	2.66E-03	4.71E-03	2.53E-04	1.51E-03	1.40E-03	6.97E-04	6.22E-04
R. sativus(n=5)	1.20E-02	3.12E-04	7.18E-04	7.12E-03	5.63E-03	4.54E-03	7.93E-03	4.60E-02	4.05E-03	3.63E-03	2.61E-03	3.80E-04	3.02E-03	5.06E-03	1.08E-03	2.17E-03

Children

B.oleracea B.(n=3)	1.11E-02	3.30E-04	5.55E-03	2.57E-04	4.10E-03	3.16E-03	2.02E-02	1.32E-02	8.21E-03	8.24E-03	2.01E-03	1.40E-03	4.26E-03	2.98E-03	1.24E-03	1.74E-03
B.oleracea C.(n=3)	1.58E-02	4.12E-04	2.83E-03	8.45E-03	3.03E-03	3.04E-03	1.56E-02	2.06E-02	4.06E-03	4.07E-03	4.70E-03	2.29E-03	2.48E-03	2.44E-03	6.43E-04	1.74E-03
S. alba (n=3)	1.49E-02	3.88E-04	1.26E-03	4.06E-04	7.13E-03	3.93E-03	1.28E-02	2.06E-02	1.62E-03	1.02E-03	1.13E-02	1.56E-03	4.82E-03	6.77E-03	1.61E-03	2.85E-03
B. rapa(n=3)	1.84E-02	6.30E-04	3.70E-03	8.60E-03	3.22E-03	3.22E-03	2.00E-02	1.47E-02	3.13E-03	9.89E-04	2.86E-03	2.86E-03	1.38E-03	1.64E-03	2.64E-03	1.53E-03
C. sativum(n=4)	1.60E-03	3.85E-04	3.20E-03	1.86E-03	6.28E-03	4.07E-03	1.42E-02	2.16E-02	1.39E-02	1.24E-03	9.34E-04	9.33E-04	4.62E-03	1.22E-04	2.19E-03	2.09E-03
D. carota(n=4)	1.41E-02	4.36E-04	7.57E-04	7.48E-03	2.76E-03	2.19E-03	1.32E-02	1.45E-02	3.84E-03	1.84E-04	1.21E-04	1.20E-04	5.88E-03	ND	2.04E-03	ND
P.sativum(n=3)	1.44E-02	3.54E-04	5.77E-04	9.46E-03	3.75E-03	3.75E-03	1.44E-02	1.22E-02	3.82E-03	3.83E-03	6.78E-03	3.64E-04	2.18E-03	2.02E-03	1.00E-03	8.96E-04
R. sativus(n=5)	1.73E-02	4.49E-04	1.03E-03	1.02E-02	8.10E-03	6.54E-03	1.14E-02	6.63E-02	5.83E-03	5.22E-03	3.76E-03	5.47E-04	4.35E-03	7.29E-03	1.55E-03	3.12E-03

Adults

Hangu

B. oleracea C.(n=3)	8.25E-03	2.15E-04	1.48E-03	4.41E-03	1.58E-03	1.59E-03	8.16E-03	1.07E-02	2.12E-03	2.12E-03	2.45E-03	1.61E-03	4.37E-03	1.27E-03	3.36E-04	9.09E-04
B. oleracca B. (n=3)	5.80E-03	1.72E-04	2.89E-03	1.34E-04	2.59E-03	1.65E-03	1.06E-02	6.88E-03	4.28E-03	4.30E-03	2.32E-03	1.90E-03	2.17E-03	1.56E-03	8.69E-04	9.09E-04
B. rapa(n=3)	9.62E-03	3.29E-04	1.93E-03	4.49E-03	1.68E-03	1.68E-03	1.05E-02	7.69E-03	1.63E-03	5.16E-04	1.49E-03	1.49E-03	2.04E-03	8.56E-04	1.38E-03	8.00E-04
S. alba (n=3)	7.78E-03	2.02E-04	6.59E-04	2.12E-04	9.73E-03	2.05E-03	1.61E-02	7.62E-03	8.40E-03	4.21E-03	5.87E-03	8.12E-04	4.79E-03	3.53E-03	1.47E-03	1.48E-03
C. sativum(n=4)	8.36E-04	2.01E-04	1.67E-03	9.69E-04	1.38E-02	2.12E-03	1.10E-02	8.34E-03	7.26E-03	3.07E-03	4.87E-04	4.87E-04	3.47E-03	6.38E-05	2.40E-03	1.09E-03
D. carota(n=3)	7.36E-03	2.27E-04	3.95E-04	3.90E-03	2.74E-03	1.15E-03	1.32E-02	7.55E-03	2.00E-03	9.60E-05	6.31E-05	6.27E-05	2.03E-03	ND	1.09E-03	ND
P. sativum(n=4)	7.50E-03	1.85E-04	3.01E-04	4.93E-03	1.96E-03	1.96E-03	7.51E-03	6.36E-03	1.99E-03	2.00E-03	3.54E-03	1.90E-04	4.47E-03	1.05E-03	5.23E-04	4.67E-04
R. sativus(n=4)	9.01E-03	2.34E-04	5.39E-04	5.35E-03	8.89E-03	3.41E-03	8.32E-03	1.78E-02	2.75E-03	5.68E-03	6.68E-03	2.85E-04	4.94E-03	3.80E-03	8.36E-03	1.63E-03

Children

B. oleracea C.(n=3)	1.19E-02	3.09E-04	2.13E-03	6.34E-03	2.28E-03	2.28E-03	1.18E-02	1.55E-02	3.05E-03	3.05E-03	3.53E-03	2.32E-03	6.29E-03	1.83E-03	4.83E-04	1.31E-03
B. oleracca B. (n=3)	8.34E-03	2.48E-04	4.17E-03	1.93E-04	3.73E-03	2.38E-03	1.52E-02	9.91E-03	6.17E-03	6.19E-03	3.34E-03	2.73E-03	3.12E-03	2.24E-03	1.25E-03	1.31E-03
B. rapa(n=3)	1.38E-02	4.73E-04	2.78E-03	6.46E-03	2.42E-03	2.42E-03	1.51E-02	1.11E-02	2.35E-03	7.43E-04	2.15E-03	2.15E-03	2.93E-03	1.23E-03	1.99E-03	1.15E-03
S. alba (n=3)	1.12E-02	2.91E-04	9.49E-04	3.05E-04	1.40E-02	2.95E-03	2.32E-02	1.10E-02	1.21E-02	1.21E-02	8.46E-03	1.17E-03	6.89E-03	5.09E-03	2.12E-03	2.14E-03
C. sativum(n=4)	1.20E-03	2.89E-04	2.40E-03	1.39E-03	1.99E-02	3.05E-03	1.58E-02	1.20E-02	1.04E-02	4.41E-03	7.01E-04	7.01E-04	5.00E-03	9.18E-05	3.46E-03	1.57E-03
D. carota(n=3)	1.06E-02	3.27E-04	5.69E-04	5.62E-03	3.94E-03	1.65E-03	1.90E-02	1.09E-02	2.89E-03	1.38E-04	9.08E-05	9.03E-05	2.92E-03	ND	1.57E-03	ND
P. sativum(n=4)	1.08E-02	2.66E-04	4.34E-04	7.10E-03	2.82E-03	2.82E-03	1.08E-02	9.16E-03	2.87E-03	2.87E-03	5.09E-03	2.73E-04	6.44E-03	1.52E-03	7.53E-04	6.72E-04
R. sativus(n=4)	1.30E-02	3.37E-04	7.76E-04	7.70E-03	1.28E-02	4.91E-03	1.30E-02	2.57E-02	3.96E-03	8.17E-03	9.52E-03	4.11E-04	1.25E-02	5.47E-03	1.20E-02	2.34E-03

Adults

Karak

B. oleracca B. (n=3)	6.69E-03	1.99E-04	3.34E-03	1.55E-04	3.03E-03	1.90E-03	1.22E-02	7.94E-03	4.94E-03	2.87E-03	3.00E-03	9.01E-04	2.34E-03	1.80E-03	9.70E-04	1.05E-03
B. oleracea C. (n=3)	9.52E-03	2.48E-04	1.70E-03	5.09E-03	1.83E-03	1.83E-03	9.42E-03	1.24E-02	2.44E-03	2.45E-03	2.83E-03	1.15E-03	3.17E-03	1.47E-03	3.87E-04	1.05E-03
S. alba (n=3)	8.66E-03	2.13E-04	3.48E-04	5.69E-03	2.26E-03	2.26E-03	8.66E-03	7.34E-03	2.30E-03	2.30E-03	4.08E-03	2.19E-04	1.45E-03	1.22E-03	6.04E-04	5.39E-04

B. rapa(n=3)	1.11E-02	3.80E-04	2.23E-03	5.18E-03	1.94E-03	1.94E-03	1.21E-02	8.87E-03	1.88E-03	5.95E-04	1.72E-03	1.72E-03	1.67E-03	9.87E-04	1.59E-03	9.23E-04
C.sativum(n=5)	9.64E-04	2.32E-04	1.93E-03	1.12E-03	4.06E-03	2.45E-03	3.38E-03	9.62E-03	2.51E-04	8.32E-04	5.62E-04	5.62E-04	1.52E-03	7.36E-05	2.58E-04	1.26E-03
D. carota(n=4)	8.50E-03	2.62E-04	4.56E-04	4.50E-03	3.06E-03	1.32E-03	6.85E-03	8.72E-03	2.31E-03	1.11E-04	7.28E-05	7.23E-05	8.86E-04	ND	2.48E-03	ND
P. sativum(n=3)	8.97E-03	2.34E-04	7.61E-04	2.44E-04	2.53E-03	2.37E-03	2.96E-03	6.54E-03	8.94E-04	1.31E-03	2.59E-03	9.37E-04	3.43E-03	7.26E-04	1.67E-03	8.76E-04
R.sativus(n=4)	1.04E-02	2.70E-04	6.22E-04	6.17E-03	4.88E-03	1.00E-03	5.05E-03	1.48E-02	1.75E-03	1.75E-03	3.52E-03	3.29E-04	4.16E-03	1.04E-03	1.27E-03	1.04E-03
Children																
B. oleracca B. *(n=3)*	9.63E-03	2.86E-04	4.81E-03	2.23E-04	4.35E-03	2.74E-03	1.75E-02	1.14E-02	7.12E-03	7.14E-03	4.31E-03	1.30E-03	3.37E-03	2.59E-03	1.40E-03	1.51E-03
B. oleracea C. *(n=3)*	1.37E-02	3.57E-04	2.45E-03	7.32E-03	2.63E-03	2.64E-03	1.36E-02	1.78E-02	3.52E-03	3.52E-03	4.07E-03	1.66E-03	4.56E-03	2.11E-03	5.57E-04	1.51E-03
S. alba (n=3)	1.25E-02	3.07E-04	5.00E-04	8.20E-03	3.25E-03	3.25E-03	1.25E-02	1.06E-02	3.31E-03	3.32E-03	5.88E-03	3.16E-04	2.09E-03	1.75E-03	8.69E-04	7.76E-04
B. rapa(n=3)	1.60E-02	5.46E-04	3.21E-03	7.45E-03	2.79E-03	2.79E-03	1.74E-02	1.28E-02	2.71E-03	8.57E-04	2.48E-03	2.48E-03	2.41E-03	1.42E-03	2.29E-03	1.33E-03
C.sativum(n=5)	1.39E-03	3.34E-04	2.77E-03	1.61E-03	5.85E-03	3.52E-03	4.87E-03	1.39E-02	3.61E-04	1.20E-03	8.09E-04	8.09E-04	2.19E-03	1.06E-04	3.71E-04	1.81E-03
D. carota(n=4)	1.22E-02	3.78E-04	6.56E-04	6.48E-03	4.40E-03	1.90E-03	9.86E-03	1.25E-02	3.33E-03	1.59E-04	1.05E-04	1.04E-04	1.28E-03	ND	3.58E-03	ND
P. sativum(n=3)	1.29E-02	3.36E-04	1.09E-03	3.52E-04	3.64E-03	3.41E-03	4.27E-03	9.42E-03	1.29E-03	1.89E-03	3.73E-03	1.35E-03	4.94E-03	1.04E-03	2.40E-03	1.26E-03
R.sativus(n=4)	1.50E-02	3.89E-04	8.96E-04	8.88E-03	7.02E-03	1.44E-03	7.27E-03	2.13E-02	2.52E-03	2.51E-03	5.06E-03	4.74E-04	5.98E-03	1.49E-03	1.82E-03	1.50E-03
Adults																
Lakki Marwat																
B. oleracca B. *(n=3)*	4.64E-03	1.38E-04	2.31E-03	1.07E-04	9.02E-04	1.32E-03	8.45E-03	3.51E-03	3.43E-03	3.44E-03	7.65E-04	3.46E-04	1.82E-03	1.23E-03	7.79E-04	7.27E-04
B. oleracea C. *(n=3)*	6.60E-03	1.72E-04	1.18E-03	3.53E-03	1.27E-03	1.27E-03	6.53E-03	8.60E-03	1.69E-03	1.70E-03	1.54E-03	1.96E-03	1.99E-04	1.02E-03	2.68E-04	7.27E-04
S. alba (n=3)	6.00E-03	1.48E-04	2.41E-04	3.95E-03	1.56E-03	1.56E-03	6.01E-03	5.09E-03	1.60E-03	1.60E-03	2.83E-03	1.52E-04	1.06E-03	8.43E-04	4.19E-04	3.74E-04
B. rapa(n=3)	7.70E-03	2.63E-04	1.55E-03	3.59E-03	1.34E-03	1.34E-03	8.36E-03	6.15E-03	1.31E-03	4.13E-04	1.19E-03	1.19E-03	1.46E-03	6.85E-04	1.10E-03	6.40E-04
C. sativum(n=3)	6.68E-04	1.61E-04	1.34E-03	7.75E-04	2.66E-03	1.70E-03	2.60E-03	3.15E-03	3.29E-03	7.78E-04	3.90E-04	3.90E-04	1.10E-03	5.10E-05	1.50E-03	8.73E-04
D. carota(n=4)	5.89E-03	1.82E-04	3.16E-04	3.12E-03	2.44E-03	9.16E-04	3.43E-03	6.04E-03	1.60E-03	7.68E-05	5.05E-05	5.02E-05	3.63E-04	ND	1.29E-03	ND
P. sativum(n=4)	6.22E-03	1.62E-04	5.27E-04	1.69E-04	2.76E-03	1.64E-03	2.44E-03	3.59E-03	4.35E-04	4.35E-04	5.10E-04	6.49E-04	7.31E-04	3.13E-04	6.76E-04	3.50E-04
R.sativus(n=4)	7.21E-03	1.87E-04	4.31E-04	4.28E-03	3.43E-03	1.47E-03	3.97E-03	1.09E-02	9.41E-04	9.38E-04	1.15E-03	2.28E-04	6.86E-04	5.28E-04	8.23E-04	4.63E-04
Children																
B. oleracca B. *(n=3)*	6.67E-03	1.91E-04	3.33E-03	1.54E-04	1.30E-03	1.90E-03	1.22E-02	7.93E-03	4.93E-03	4.95E-03	1.10E-03	4.98E-04	2.62E-03	1.79E-03	1.12E-03	1.05E-03
B. oleracea C. *(n=3)*	9.50E-03	2.47E-04	1.70E-03	5.08E-03	1.82E-03	1.83E-03	9.40E-03	1.24E-02	2.44E-03	2.44E-03	2.22E-03	2.82E-03	2.86E-04	1.46E-03	3.86E-04	1.05E-03
S. alba (n=3)	8.64E-03	2.13E-04	3.47E-04	5.68E-03	2.25E-03	2.25E-03	8.65E-03	7.33E-03	2.30E-03	2.30E-03	4.08E-03	2.19E-04	1.53E-03	1.21E-03	6.03E-04	5.38E-04
B. rapa(n=3)	1.11E-02	3.79E-04	2.32E-03	5.17E-03	1.93E-03	1.94E-03	1.20E-02	8.85E-03	1.88E-03	5.94E-04	1.72E-03	1.72E-03	2.10E-03	9.86E-04	1.59E-03	9.21E-04
C. sativum(n=3)	9.62E-04	2.32E-04	1.92E-03	1.12E-03	3.83E-03	2.44E-03	3.74E-03	4.54E-03	4.74E-03	1.12E-03	5.61E-04	5.61E-04	1.59E-03	7.34E-05	2.77E-03	1.26E-03
D. carota(n=4)	8.48E-03	2.62E-04	4.55E-04	4.49E-03	3.51E-03	1.32E-03	4.94E-03	8.70E-03	2.31E-03	1.11E-04	7.26E-05	7.22E-05	5.23E-04	ND	1.86E-03	ND
P. sativum(n=4)	8.96E-03	2.33E-04	7.59E-04	2.44E-04	3.97E-03	2.36E-03	3.51E-03	5.16E-03	6.26E-04	6.26E-04	7.34E-04	9.33E-04	1.05E-03	4.51E-04	9.73E-04	5.04E-04

R.sativus(n=4)	1.04E-02	2.70E-04	6.21E-04	6.16E-03	4.93E-03	2.12E-03	5.72E-03	1.57E-02	1.36E-03	1.35E-03	1.66E-03	3.29E-04	9.88E-04	7.61E-04	1.18E-03	6.67E-04
Adults																
Bannu																
B. oleracca B. *(n=3)*	8.90E-03	2.65E-04	3.61E-03	2.06E-04	4.25E-03	2.54E-03	1.62E-02	1.06E-02	6.58E-03	2.42E-03	2.58E-03	4.81E-04	1.58E-03	2.39E-03	1.41E-03	1.40E-03
B. oleracea C. *(n=3)*	1.27E-02	3.30E-04	2.27E-03	6.77E-03	2.43E-03	2.44E-03	1.25E-02	1.65E-02	3.25E-03	3.26E-03	3.77E-03	2.09E-03	2.47E-03	1.53E-03	5.16E-04	1.40E-03
S. alba (n=3)	1.15E-02	2.84E-04	4.63E-04	7.58E-03	3.00E-03	3.00E-03	1.15E-02	9.77E-03	3.06E-03	3.07E-03	1.25E-03	2.92E-04	1.78E-03	1.62E-03	8.04E-04	7.18E-04
B. rapa(n=3)	1.48E-02	5.05E-04	2.97E-03	6.89E-03	2.58E-03	2.58E-03	7.68E-03	1.18E-02	2.51E-03	7.93E-04	1.46E-03	1.04E-03	1.72E-03	1.31E-03	8.62E-04	1.23E-03
C. sativum(n=6)	1.28E-03	3.09E-04	2.56E-03	1.49E-03	3.10E-03	3.26E-03	7.51E-03	1.70E-02	2.77E-03	2.77E-03	7.49E-04	7.48E-04	1.45E-03	9.80E-05	1.60E-03	1.68E-03
D.carota(n=4)	1.13E-02	3.49E-04	6.07E-04	5.99E-03	4.02E-03	1.76E-03	7.70E-03	1.16E-02	3.08E-03	1.47E-04	9.69E-05	9.63E-05	7.49E-04	ND	2.99E-03	ND
P.sativum(n=3)	1.19E-02	3.11E-04	1.01E-03	3.25E-04	5.39E-03	3.15E-03	6.40E-03	1.01E-02	1.59E-03	1.59E-03	1.06E-03	1.25E-03	5.43E-04	1.24E-03	6.78E-04	1.44E-03
R. sativus(n=4)	1.38E-02	3.60E-04	8.29E-04	8.21E-03	5.85E-03	1.89E-03	4.96E-03	1.12E-02	6.35E-03	6.76E-03	1.04E-03	4.39E-04	1.94E-03	8.14E-04	1.53E-03	2.50E-03
children																
B. oleracca B. *(n=3)*	1.28E-02	3.81E-04	5.19E-03	2.97E-04	6.12E-03	3.65E-03	2.33E-02	1.52E-02	9.47E-03	3.48E-03	3.71E-03	6.93E-04	2.27E-03	3.44E-03	2.03E-03	2.01E-03
B. oleracea C. *(n=3)*	1.82E-02	4.75E-04	3.27E-03	9.75E-03	3.50E-03	3.51E-03	1.81E-02	2.38E-02	4.68E-03	4.69E-03	5.42E-03	3.01E-03	3.55E-03	2.21E-03	7.42E-04	2.01E-03
S. alba (n=3)	1.66E-02	4.09E-04	6.66E-04	1.09E-02	4.32E-03	4.33E-03	1.66E-02	1.41E-02	4.41E-03	4.42E-03	1.80E-03	4.20E-04	2.56E-03	2.33E-03	1.16E-03	1.03E-03
B. rapa(n=3)	2.13E-02	7.27E-04	4.27E-03	9.92E-03	3.72E-03	3.72E-03	1.11E-02	1.70E-02	3.61E-03	1.14E-03	2.10E-03	1.49E-03	2.48E-03	1.89E-03	1.24E-03	1.77E-03
C. sativum(n=6)	1.85E-03	4.45E-04	3.69E-03	2.14E-03	4.47E-03	4.69E-03	1.08E-02	2.44E-02	3.98E-03	3.98E-03	1.08E-03	1.08E-03	2.08E-03	1.41E-04	2.30E-03	2.41E-03
D.carota(n=4)	1.63E-02	5.03E-04	8.74E-04	8.63E-03	5.78E-03	2.53E-03	1.11E-02	1.67E-02	4.43E-03	2.12E-04	1.39E-04	1.39E-04	1.08E-03	ND	4.30E-03	ND
P.sativum(n=3)	1.72E-02	4.48E-04	1.46E-03	4.68E-04	7.76E-03	4.34E-03	9.21E-03	1.45E-02	2.29E-03	2.29E-03	1.53E-03	1.79E-03	7.81E-04	1.78E-03	9.76E-04	2.08E-03
R. sativus(n=4)	1.99E-02	5.18E-04	1.19E-03	1.18E-02	8.42E-03	2.72E-03	7.13E-03	1.62E-02	9.14E-03	9.74E-03	1.50E-03	6.31E-04	2.79E-03	1.17E-03	2.20E-03	3.60E-03

3.7 Conclusion

Long term application of wastewater has led to enrichment of soil and plants with PAHs. In high urbanized districts, long term application of wastewater is the main cause of building up PAHs in the soil as compared to less urbanized districts. PAH concentrations in the soil and vegetable were exceeded the MPLs. The transfer factor was in order of *D. carota*> *L.satuva* L> *S. alba*> *S. oleracea*> *R. sativus* >*B. oleracea* C> *C. sativum* However, the highest intake of total PAHs was calculated through consumption of *C. sativum*, *B. oleracea* B., *S. oleracea*, while lowest intake calculated in both adults and children through the consumption *S. alba*, *A. cepa*. DI-PAH was used to evaluate the human health risk and calculate the carcinogenicity of the 16 PAH linked with consumption of PAH-contaminated vegetable. In high urbanized districts the highest intake of PAHs was recorded in both adults and children though the consumption of *C. sativum*, *B. oleracea* B.and *S. oleracea*, while the lowest intake was estimated through consumption of *S. alba*, *A. cepa*.In less urbanize districts, the highest intake was calculated through consumption of *R. sativus* and *C. sativum*, while the lowest intake was recorded through ingestion of *D. carota* and *B. rapa* for both adults and children.

Chapter-4

Characteristics of biochars prepared and used for control environment experiments

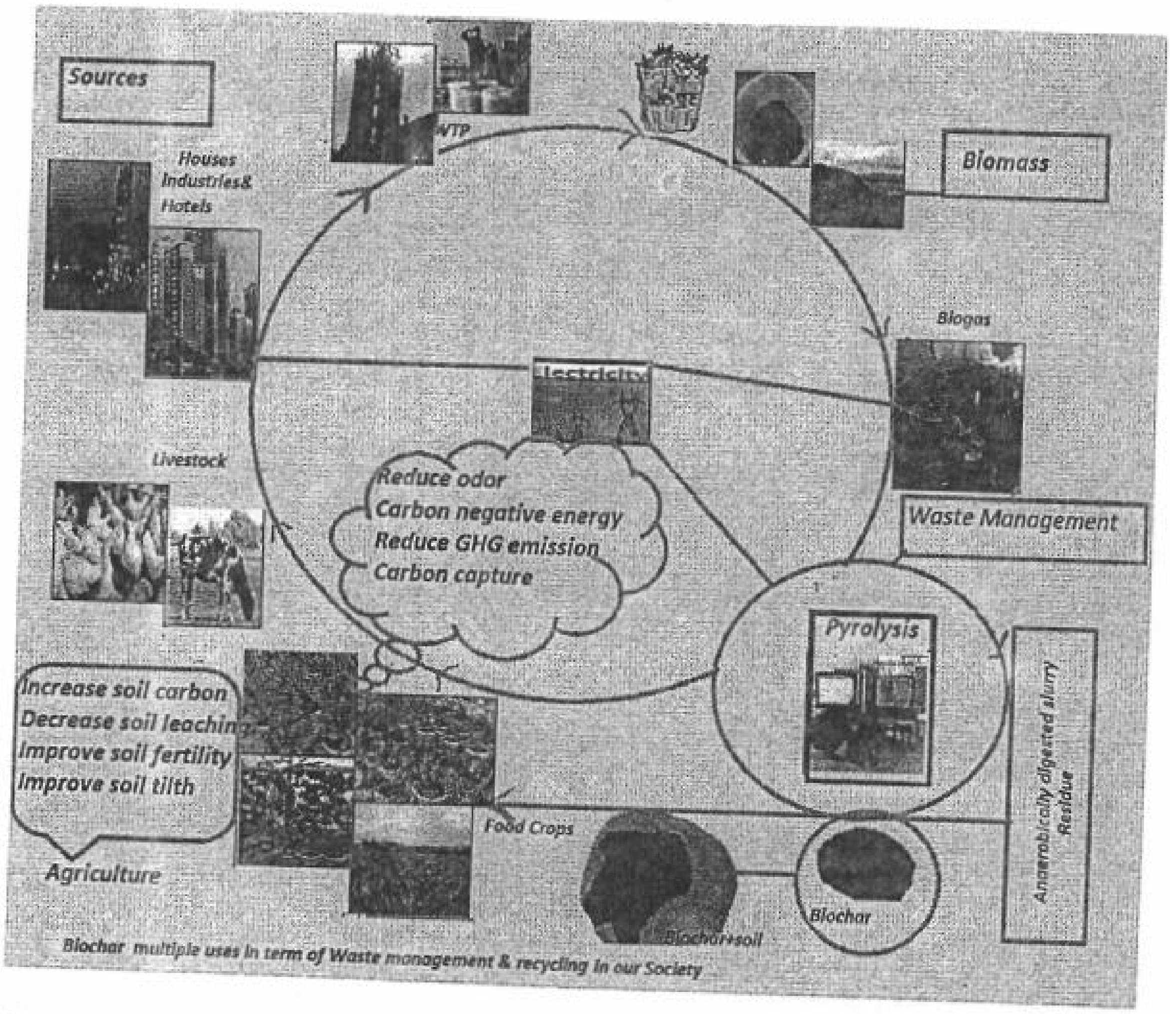


Fig.4.1Biochar multiple uses in term of waste management and recycling in our society.

4.1 Introduction

Biochar is carbonaceous organic material which is formed in the absence or under low oxygen condition from organic biomass by pyrolysis or hydrothermal process (Shackley and Sohi 2010). It is also defined as a recalcitrant organic carbon (C) compound, from biomass burning between 300 °C and 1000 °C under low oxygen concentrations (Verheijen et al., 2010).

Biochar causes sequestration of carbon and cause reduction in the emission of Green House Gases. Biochar improve soil nutrients and water quality (Lehmann and Joseph, 2009). Sustainable biochar practices can produce oil and gas byproducts that can be used as fuel, providing clean, renewable energy (IBI 2010).

The charcoal and biochar can be differentiating by its utilitarian intention. Biochar is deliberately used to improve soil fertility and carbon sequestration while charcoal is used for other purposes such as barbeque, heating (Marris 2006; Lehmann 2007; Verheijen et al., 2009).

Biochars provides a mechanism for mitigating anthropogenic climate change to sequester carbon and improves soil fertility through retaintion its nutrients and water (IBI 2010).

Research has established the benefits of biochars including:to improve ground water quality attributed to leaching of nitrogen into water :Reduction in the emission of Green House Gases such as nitrous oxide (N_2O), improve soil by increasing cation exchange capacity:to moderatesoil acidity:to increase soil beneficial microbes (Lehmann et al., 2011) :to increase water retention. (Lehmann et al., 2006).

Biochars can be prepared from different types of organic biomass such as crop residues, poultry waste, sewage waste and animal wastes (Luo et al., 2011) which shows the advantages of biochars due to cost effectiveness.In addition biochar provide an environmental tool to remediate contaminated soil.

4.2 Biochar physiochemical characteristics and preparation

Sewage sludge (SS) has been collected from Bingbei waste treatment Plant.The SS was air dried and then by pyrolysis.The SSBC has been prepared from dried SS under continuous flow of nitrogen at 500 °C for 8 hours(Khan et al., 2013).Similarly SBBC , RSBC and PNBC has prepared in the Institute of Urban Environment(IUE) Xiamen China. All biochars were analyzed for basic physiochemical properties such as pH, EC, BET surface area, pore volume, TN, TS, TC. The NO_3-N concentration decreased in the soil amended with SSBC where as theTOC value was only decreased 11.50 % and 2.36% in the soil by adding 2% and 5% PNBC(Table 4.1) .The pH value is also decreased by 2.31% and 1.50 % by adding 2% and 5% biochar whereas the pH increased in soil amended with SSBC,SBBC, RSBC(Table 4.1) .The EC was also increased in all amended soil by adding different biochars.The NH_4-N concentration was significantly increased by adding biochars to the soil. N% and C% was increased in the soil amended with different biochar (Table 4.1).

Table 4.1Changes (%) in soil amended with different Biochars.

Parameters	C-SSBC2	C-SSBC5	C-SBBC2	C-SBBC5	C-RSBC2	C-RSBC5	C-PNBC2	C-PNBC5
pH	0.14	0.54	5.17	6.12	3.95	17.82	-2.31	-1.50
ECmScm^{-1}	22.41	104.80	43.60	43.60	130.70	260.17	20.06	27.12
NH_4-N (mgkg^{-1})	112.18	367.55	97.90	335.99	84.50	417.05	106.87	204.21
NO_3-N (mgkg^{-1})	-16.61	-40.40	25.18	50.01	66.22	33.47	56.15	29.48
TOC(mgkg^{-1})	17.20	54.46	8.00	26.79	18.94	41.36	-11.50	-2.36
N (%)	21.43	185.71	28.57	64.29	21.43	35.71	28.57	50.00
C (%)	2.81	77.11	20.88	59.44	34.94	36.14	44.98	64.66
S (%)	100.00	500.00	40.00	40.00	40.00	40.00	-20.00	0.00

\- Represents decrease in % whereas + values represent increase in %

Biochar characteristics greatly alter due to burning temperature and parent materials from which biochar are prepared. The increase of burning temperature wide the surface area of biochars enhances sorption ability for organic contaminants such as PAHs. Charcoal prepared at 500-700 °C from wheat straw shows high sorption capability, had a surface area (>300 m^{2}g^{-1}), whereas the charcoal produced at 300-400°C, had low surface area (<200 m^{2}g^{-1})(Chun et al.,2004).Biochar characteristic alters due to parent materials. The properties of charcoal fly ash (black carbon), rice straw & soot studied and noted that the surface are (234.9 m^{2}g^{-1}), porosity (0.4392 mlg^{-1}) and the amount of functional groups (2.995 mmolg^{-1}) all were the prevalent in rice straw charcoal. (Luo et al., 2011).

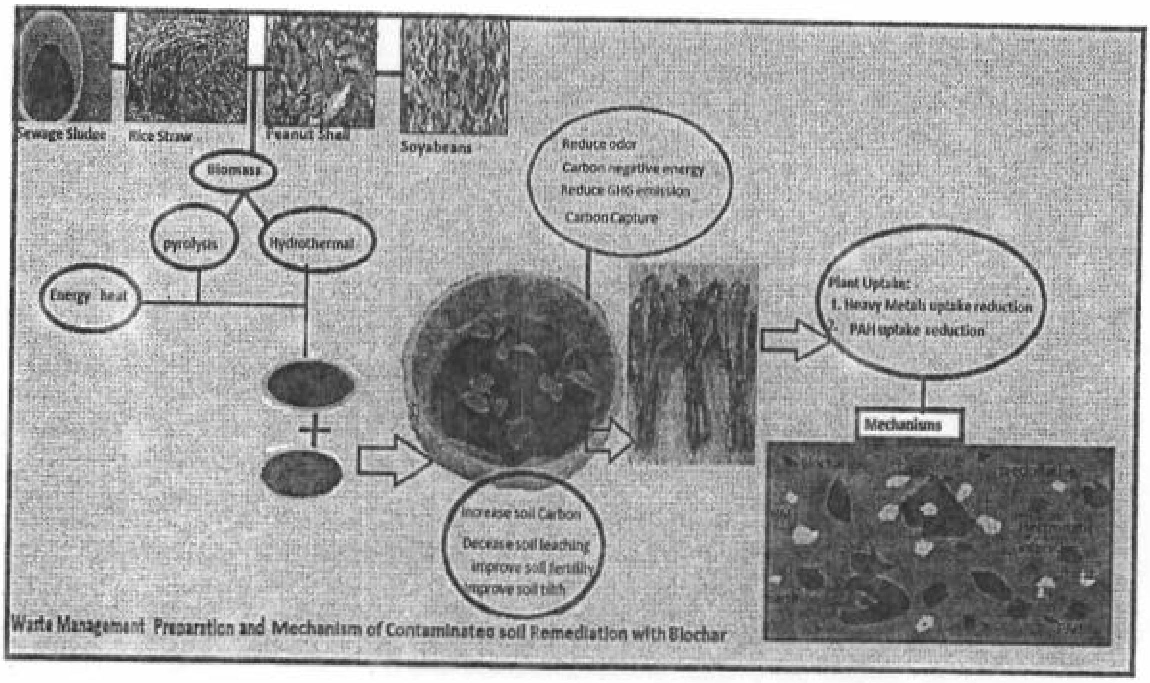


Fig.4.2Waste management and mechanism of contaminated soil remediation with Biochar.

Our present study shows great change in physiochemical properties by making biochars from different biomass at 500 °C (See table 4.3). The pH value was in order of SBBC >RSBC > PNBC >SSBC where as the EC was RSBC > SSBC >PNBC >SBBC. The BET surface area was highest for SSBC (4.07 m^2g^{-1}) following by PNBC (3.45 m^2g^{-1}). The pore size was of SSBC, RSBC SBBC, and PNBC was 10.31, 6.95, 6.81 and 3.82nm respectively.The NH_4-N, NO_3-N was highest in the RSBC whereas the lowest for SSBC. The TOC value was high in SBBC following by RSBC, the order was SBBC> RSBC >SSBC > PNBC. The N concentration was 2.83 % in SSBC following by 1.72% in SBBC.The carbon percentage was in order of SBBC > PNBC >RSBC >SSBC. The sulfur percentage was in order of SSBC >PNBC >RSBC >SBBC.

Table 4.2 Physiochemical properties of biochars at 500°C.

Parameters	SSBC*	SBBC**	RSBC***	PNBC****
pH	7.43±0.03	10.06±0.04	10.05±0.03	9.68±0.02
ECmScm^{-1}	6.69±0.24	5.12±0.45	10.37±0.39	6.57±0.11
BET Surface area(m^{2}g^{-1})	4.07134	2.6267	2.2071	3.4548
Pore volume (cm^{3}g^{-1})	0.02	0.004470	0.003833	0.003300
Pore size (nm)	10.31	6.806775	6.94602	3.82040
Moisture content (%)	1.7	1.5	1.3	1.9
NH_4-N (mgkg^{-1})	8.89±0.37	10.56±0.37	11.55±0.32	9.34±0.34
NO_3-N (mgKg^{-1})	3.39±0.51	3.39±0.51	6.65±0.54	3.63±0.56
TOC(mgkg^{-1})	283.05±16.59	2079±26.54	797.75±13.86	230.60±28.61
N (%)	2.83±0.03	1.72±0.02	1.65±0.07	1.58±0.01
C (%)	20.64±0.29	65.59±0.56	47.43±0.82	52.54±0.94
S (%)	2.25±0.09	0.13±0.02	0.40±0.044	0.43±0.01

*=sewage sludge biochar **= soya bean strawbiochar ***=rice straw biochar ****= peanut shell biochar

Different treatment methods can be used to improve the biochars adsorption capability.Non activated carbon are less effective in nutrients holding and uptake by plants in comparison with activated carbon (Borchard et al.,2012).Biochars prepared by hydrothermal process had double positive effects in all instance in comparison with pyrolysis biochars.

Biochar added to soil help in sequestration of carbon, besides these properties biochar amended soil pH, EC and soil buffering increased in comparison with control soil (Liang et al., 2006).The study has been conducted to investigate the bioavailablity of P in soil with the interation b/w P and F(ferrihydrit) in the presence of biochars ,the results shows greater enhancement in the bioavailability of P(Cui et al.,2011) this is due to the changes of soil environment for microorganisms(Atkinson et al.,2010). The further research work is needed to investigate the mechanism of soil fertility.

The biochars added to soil can increase crop yield in sense of nutrients uptake with increase biochars application rateas previousely mentioned research 15 and 20 tha^{-1} biochar added to maize crop and increased the productivity 98% and 150 % respectively (Uzoma et al., 2011).

The biochars also immobilize the HMs and release with less rate to the environment and exert less impacts on the environment. The study has been conducted on the immobilization of HMs in multi elements contaminated soil, the results show biochar can retain As, Cd and Zn and reduced Cd and Zn by 300- and 45- fold respectively but leaching concentration was not reduced (Beesley and Marmiroli, 2011).Many studies have been conducted on the green waste compost, biochars and SS, that effect the solubility and bioavailability of PTEs.The concentration of Cu and Pb was less in Reygrass shoots by adding green waste compost and biochars to soil (Karami et al., 2011). Similarly, in case of the application of SS and SSBC on soil characteristics has been studied, the result shows that SSBC less plant availability of Cd Ni, Pb and, Zn (Mendez et al.,2012).

According to current research the mechanisms removal of HMs with biochar addition might be accredited to precipitation, electrostatic interactions, and other reactions (Dong et al., 2011).Biochar addition to soil increase the negative charge on soil surface due to decreasing zeta potential and increasing cation exchange capacity(Peng etal.,2011).The addition of biochar to the soil increase soil pH and enhance the electrostatic interaction between the HMs and soil which cause precipitation of HMs on biochar surface and reduce the mobilization of HMs.The mechanism of contaminated soil remediation with biochars is shown in Fig. 4.2.The conversions of SS to SSBC decrease the risk of HMs leaching and also occur reduction in the bioavailability of HMs to plants (Mendez et al., 2012).Converting of agriculture waste into biochar is a best way of waste management (Fig.4.1; 4.2).

Composting is one of method for recycling of agriculture waste but direct use of this waste to plant can cause toxicity (Bulter et al.,2001).this process can promote by converting this waste into biochar which is source for carbon and energy source for micro organisms (Adhikari etal.,2009). Composting of poultry manure reduce the smell and the loss of nitrogen with a balance nutrient composition (Dias et al, 2010). Biochars provide alternative methods for disposing of agriculture wastes.

However biochar is also a matter of great concern for research because of its toxicity, because biochar consist of PAH. If the level of pollutants in material of biochar surpass the standard, it will cause threaten to environment once they are added to soil or water system but the amount of this PAH concentration depends pyrolysis temperature, the sum of 16 PAH significantly decreased in SSBC in our study as compare to SS, Various research shows that PAHs inside biochar is unavailable to plants, however biochar adsorb the organic contaminants on the surface of biochar and cause partition of organic contaminats molecule in the micro pore of biochar (Kwon et al., 2005; Chen et al., 2011).

Total PAH concentrations varied in different biomas biochar depend biochar parent materials , time , temperature and the method of burning .Biochars synthesized at high temperature shows less PAH concentration and had the great immobilization effect of PAH in soil which is recommended to use in contaminated soil (Hale et al ., 2012).It is also recommended to make precaution criteria in regarding of selection of biomass stock and preparation temperature to mitigate concentrations of PTE, in resultant the biochars. More research work are required on the biochar effectiveness, advantages to ensure the ecosystems benefits.

The rule of biochars is explained with the diagram in our society in terms of waste management and recycling in Fig. 4.1.

4.3 Conclusions

Most of the literature published about biochars which suggest improved in crop yield, absorption of organic pollutants such as PAH.The benefits of biochars are vast, the use of biochars in remediation can increase crop yield, If the biochar is used in proper dose to soil, the results will be more fruitful and the bioaccumulation rate of PAH and HMs will be reduced, the health risks associated with the PAHs and HMs will be minimized in food crops. The results of our research summarize that PNBC are more effective in the reduction of accumulation of PAHs in food crops, but it should be apply in a proper quantity. The PAHuptake rate was in order of PNBC >SBBC>RSBC>SSBC. Similarly HMs uptake can be reduced by increasing the application rate of different biochars. The uptake rate of HMs varied because of the vegetables varieties, but all the biochars shows great effectiveness in removal of HMs, the results depends on the application. With the increase of application rate more HMs will be trapped in the soil and less will be available for the bioaccumulation in plants.

Chapter-5

The effects of SS and SSBC on PAHs and potentially toxic element bioaccumulation in *Cucumis sativa* L.

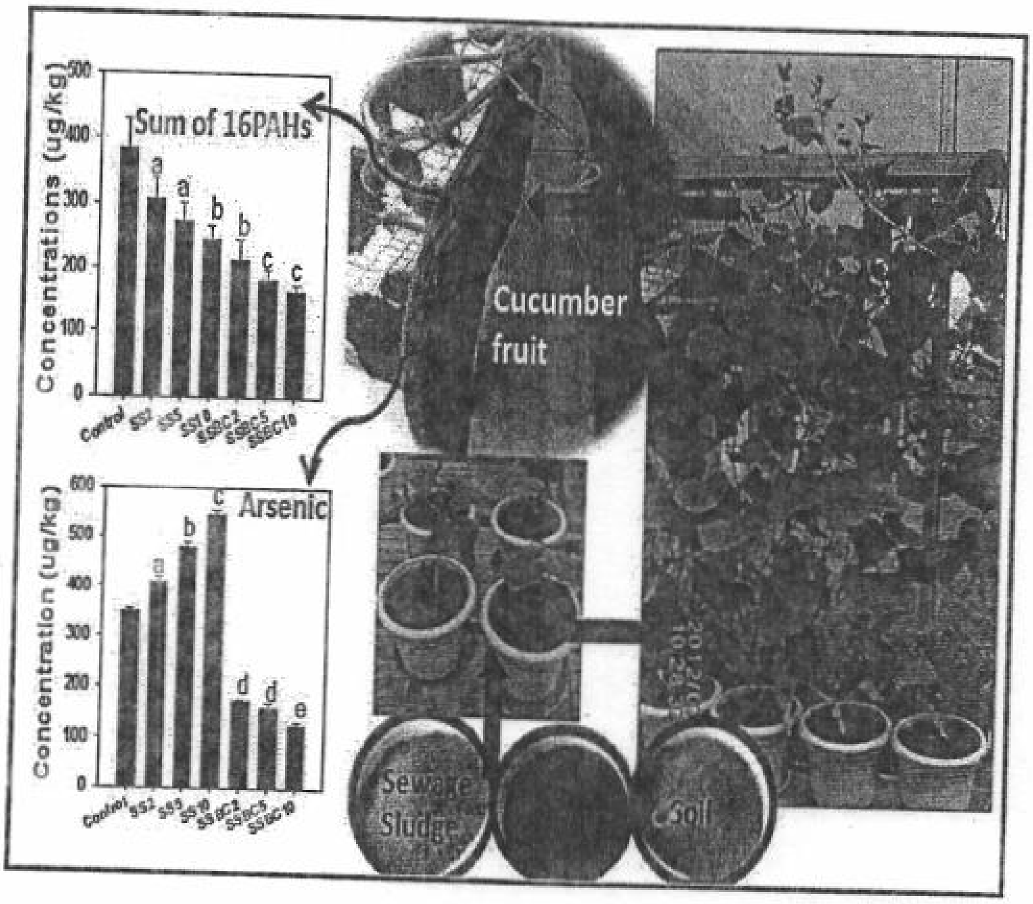


Fig.5.1 Graphical abstract of cucumber *(Cucumis sativa L).*

5.1 Introduction

Sewage sludge is produced in massive quantities as a by-product in wastewater treatment plants (WTPs). In China, WTPs produced approximately 30 milliontonsof SS per year (Yu, 2011). This quantity is expected to increase in keeping with China's rapid urbanization and industrial development.WTPs in the United States produced about 6.2 million metric tons dry weight of SS per year (Fytili and Zabaniotou, 2008). SS material contains high concentrations of micro- and macro-nutrients and the agronomic benefits of SS application to land are well recognized (USEPA, 1995; Roca-Pérez et al., 2009). However, the presence of toxic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and potentially toxic elements (PTEs) such as As, Cd, Cu, Pb and Zn restrict the application of SS to farmland(USEPA, 1995; SEPA, 1995; CEC, 2000).In contrast, PAH and PTE levels in a range of biochars have recently been reported (Freddo et al., 2012; Hale et al., 2012; Uchimiya et al., 2011a). Encouragingly, levels of both PAHs and PTEs in biochars (but not SSBC specifically) have been found to be in keeping with concentrations found in background soils and below levels used in the regulation of composts (Freddo et al., 2012).

Through the conversion of SS to SSBC it may be possible to destroy PAHs anddecrease PTE availability. Thus, the conversion of SSinto SSBC represents an interesting opportunity to potentially mitigate PAH/PTE issues relating to SS (while at the same time retaining the soil improvement properties associated with SS) and in so doing to produce a safer soil amendment for application to agricultural land. In addition to the possibility of mitigating toxicity associated with SS through its conversion to biochar wider environmental benefits could be realized (particularly given the ready availability of SS); most significant would be the opportunity to divert SS waste to its use in heat and power generation.

The directed application of SSBC to contaminated soils may hold opportunities to mitigate PAH/PTE risks. Specifically, biochar, on account of its sorptive capacity, could be an effective soil amendment to drive the re-partitioning of PAHs and PTEs and thereby reduce their potential for bioaccumulation into food and on into the food chain (Sneath et al., 2013). In support of this biochar has been shown to reduce the bioavailability of contaminants such as PAHs and PTEs (Cao et al., 2011; Hilber et al., 2012;Keiluweit et al., 2012; Khan et al., 2013). Regarding PAHs biochar application to contaminated soil and SS has been shown to cause significant decreases in freely dissolved PAH concentrations (Oleszczuk et al., 2012; Khan et al., 2013). The mass transfer of PAHs to biocharsorptive sites has been proposed as the mechanism through which this is achieved (Uchimiya et al., 2011b).With respect to PTEs the oxygen-functional-groups of biochar have been reported to form complexes with PTEs which reduce theirmobility and their bioaccumulation in plants grown in biochar amended soil (Uchimiya et al., 2011b;Jiang and Xu, 2013).

While bioaccumulation of PAH/PTE into plant tissue following biochar application to soil has recently been reported (Zheng et al., 2012; Méndez et al., 2012); the specific influence of SSBC has not. Furthermore, the direct comparison of how SS and SSBC perform with respect to each other has not been reported on either. Cucumber (*C. sativa* L) was chosen as a representative plant belongs to cucurbitaceae family. In China cucumber is an economically important plant (with4.74 million tonnes being produced each year; this making China the largest producer in the world) (FAO, 2011). Cucumber grown in field under different agricultural systems can be contaminated with organic compounds and PTEs and caused health problems (Mansour et al., 2009).

It was the aim of this research to assess the extent to which conversion of SS to SSBC destroyed PAHs and decreased the availability of PTEs. Thereafter, it was the aim of this research to establish the relative effectiveness of SS and SSBC,once added to contaminated soil, to: i) sustain plant growth, ii) alter the partition of PAHs and PTEs, and, iii)influence bioaccumulation of PAHs and PTEs into a commonly cultivated salad crop (*C. sativa* L).

5.2 Materials and methods

5.2.1 Soil sampling and characterization

Soil samples (0-20 cm) contaminated withPAHs and PTEswere collected from farmland nearan iron refinery plant in Fujian Province, China. Soil samples were passed through 2 mm mesh tohomogenize a composite sample. Sub-samples were freeze-dried (-50°Cat20 ±2 Pa) and stored at -20°Cin paper envelopes. Basic properties of the soil, including:pH, electrical conductivity (EC), porosity, surface area,carbon (C), nitrogen (N) sulfur (S) and PTEs are given in Table 5.1, while PAH concentrations in control and amended soils are given in Figure 5.2. The procedural detailsfor these measurements are given below.

5.2.2 Chemicals

All solvents were HPLC/spectro grade purchased from Tedia Company Inc, USA. Surrogate standards (PAH-Mix 24 deuterated, LA20950024HE) and reference materials (PAH-Mix 9, XA20950009CY) were purchased from the laboratory of Dr. Ehrenstorfer, Augsburg, Germany.Silica gel, alumina and sodium sulfate were purchased from Sinopharm Chemical Reagent Co Ltd., China.

5.2.3 pH and Electrical conductivity(EC)

pH and EC (n=3) weremeasured in deionized water suspensions of soil (1:2.5 w/v), SS and SSBC (1:5 w/v)using Accumet XL 60 meter equippedwithboth pH and EC electrodes.

5.2.3 Surface area and porosity

Surface area and porosityof soil, SSBC and SS samples (0.3 g, n=3) were measured using Surface Area and Porosity Analyzer (Micromeritics, ASAP 2020). All these samples were first out-gassed at 105°C for 16 h and used for N_2 sorption. The cold free space of48.3 cm^3, the bath temperature 77.3 K and the equilibrium interval 10 S. The Brunauer-Emmett-Teller method of multiple points was employed for surface area calculation, while four data points, with relative pressures of 0.05 to 0.3, were used to construct the monolayer adsorptioncapacity.

5.2.4 *Total C,N and S in soil,SS and SSBC*

These parameterswere measured (n=3) using amacroelementor(VarioMax CNS, Germany). For this dry combustion process, soil (400 mg) and SS and SSBC (300 mg) samples were processed using combustion temperature (H1) 1140°C, post combustion (H2) 800°C and reduction (H3) 850°C, while He flow was 680 ml/min as mentioned in the operational manual.

5.2.5 *Soil particle size*

Soil particle size was measured using the Mastersizer 2000 (Malvern Instruments Ltd, UK). Soil particles size distribution was: sand (70.8%), silt (27.3%) and clay (0.9%).

5.2.6 *Available NO_3-N and NH_4-N*

Available NO_3-N and NH_4-Nwere extracted (n=3) with KCl solution and analyzed using QuikChem QC 5800 (Lachat Instruments, USA. Briefly, a2 M KCl solution (40 ml) was added to soil and centrifuged after shaking for 1 h at 200 rpm. The supernatant was filtered and analyzed for NO_3-N and NH_4-N using QuikChem QC 5800 (Lachat Instruments, USA) according to the QuikChem methods (10-107-04-1-A and 10-107-06-1-B, respectively).

5.2.7 *Dissolved organic carbon (DOC)*

The value of DOC was extracted (n=3) with 40 ml of K_2SO_4 solution (0.5 M) from 4.0 g moist sample by shaking for 1 h at 200 rpm (Feng et al., 2012). After centrifugation, the supernatant was filtered and analyzed for DOC using Total Carbon Analyzer (TOC-V_{CPH} Shimadzu, Japan).

5.2.8 *Available P (Colwell P)*

Available P was measured using the method of Rayment and Higginson (1992). Briefly, soil was extracted (n=3) with 0.5 M $NaHCO_3$ solution (pH 8.5) and shaked for 16 h at 25°C. Centrifuged, filtered and analyzed for available P.

5.2.9 Total PTE in soil SS and SSBC

In brief, samples (0.5 g) were placed in digestion tubes and 10 mL mixture of HCl and HNO_3 (3:1) was added. The digestion tubes were capped with small funnels with coiled tubes to reduce the loss of acids and kept overnight in fume hood. Thereafter, the digestion tubes were placed in an AIM programmable digestion system (AIM600, AIM Lab, Milwaukee, WI, USA) and a progressively heating program was set: 50°C for 1 h, 100°C for 3 h and 160°C for 24 h. After cooling, the suspensions were filteredand diluted to 50 mL with Milli-Q water.

5.2.10 PAH purification

Total PAH concentrations in soil, SS, SSBC, the SS/SSBC treatments and cucumbers were extracted with DCM and acetone (1:1 ratio) using accelerated solvent extraction system (ASE, Dionex-350). The solvent extract was then cleanedusing silica adsorption chromatography prepared with silica gel,alumina and capped with anhydrous sodium sulfate. Silica gel, alumina and sodium sulfate wereactivatedbefore using at 180, 250, and 450°C for at least 12 h. For columns elution, a 60 ml mixture of hexane and DCM (7:3) was used and the eluted fractions were again evaporated up to 1 ml, transferred to a Kuderna-Danish concentrator and rinsed with 10 ml of n-hexane. Thereafter, the eluted fraction was again reduced to 1 ml under nitrogen and transferred to a 2 mlvial capped with a Teflon-lined septum ready for analysis of PAHs.

5.2.11 GC-MS column conditions

The GC-MS was equipped with an inert XL MSD with a triple axis detector and used under the selected ion monitoring mode. A HP-5 silica fused capillary column (60 m × 0.25 mm inner diameter × 0.25 μm film thickness) was used with helium as the carrier gas at a constant flow rate of 1 mL min^{-1}. The GC oven temperature was programmed to ramp from 50°C to 200°C at 10°C min^{-1}, then to 300°C at 10°C min^{-1} and to then hold for 8 min at this temperature. The injector and detector temperatures were 280°C and 300°C, respectively. Mass spectra were acquired at the electron ionization mode, while selected ion monitoring (SIM) mode was carried out using the molecular ions selective for individual PAHs.

5.2.12 Quality control

The efficiency of ASE extraction and silica column purification for PAH recovery from soil, SS/SSBC treatments andcucumber samples was checked using surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10). The results showed satisfactory recovery, with the average recovery ranging from 83.7±8.2% to 94.6±5.4%. Reagent blanks were also included to check the contamination of PAHs during extraction and purification processes. For PTEs, sample blanks and standard reference materials (GBW07603-GSV-2 and GBW07406-GSS-6 for plant and soil, both obtained fromthe National Research Center for Standards in China) wereincluded to verify the accuracy and precision of the digestionand subsequent analysis procedure. The recovery rates were satisfactory and ranging from 92.7±9.5% to106.4±7.1%.

5.2.13 Data analysis

The data were statistically analyzed using the statistical package SPSS 11.5. The measurements were expressed in terms of means, while the figures presented the mean values and standard deviation of four replicates. Treatment differences were tested using analysis of variance (ANOVA), while for mean significance Tukey'stestwas usedwith a level of $P<0.05$.

5.3 Biochar preparation and characterization

Wet SS was obtained from Bingbei WTP, Xiamen, China. Following itstransportation the SS was air dried. SSBC was prepared from the dried SS through pyrolysis (550°Cfor 8 h) under a continuous flow of nitrogen (Khan et al., 2013).Like soil, SS and SSBC characteristics such as pH, EC, C, N, S, porosity and surface area were determined (Table 5.1).

5.4 Greenhouse experiment

The SS or SSBC(<2 mm) was mixed with the contaminated soil at concentrations of 2, 5 and 10% on dry weight basis (defined as SS2, SS5, SS10 and SSBC2, SSBC5, SSBC10,respectively). A control treatment of un-amended soil was also included. These treatments were prepared in 4 replicates and each treatment had a total mass of 4 kg. After filling, the pots were irrigated with deionized water and kept for one week before cultivation. Cucumber (*Cucumis sativa* L) seedlings were prepared according to the method described in our previous work (Khan et al., 2008) and finally the transplanted seedlings was thinned to one per pot.The treatments were incubated in a greenhouse and irrigated with deionized water to maintain the moisture content (at 60% field capacity). During thegrowthperiod (15 weeks), the plants were fertilized twice with NH_4NO_3 solution (on each occasion 1 g $NH_4NO_3$was dissolved in 100 ml of deionized water and the solution added to each pot).The ripe cucumber fruits were harvested and fresh weights recorded. Samples were freeze-dried and powdered prior to further analyses.

5.5 Results and discussion

5.5.1*Changes in PAH/PTE concentrations following conversion of SS to SSBC*

Table 5.1 shows the basic characteristics of SSBC and SS. Pyrolysis increased the pH, surface area and pore volume of SSBC as compared to SS material.SSBC had pH (7.43), EC (6.69 $mScm^{-1}$), surface area (4.07 m^2g^{-1}) and pore volume (0.02 cm^3g^{-1}). The pore size of SSBC (10.31 nm) decreased as compared to SS (15.28 nm). Total N, C and S contents were 3.78, 28.2 and 2.53%, respectively which were decreased to 2.83, 20.6 and 2.25%, respectively in SSBC. Furthermore, the pyrolysis decreased NH_4-N (10.22 $mgkg^{-1}$) as compared to SS (38.5 $mgkg^{-1}$), while NO_3-N increased (3.93 $mgkg^{-1}$) as compared to SS (2.88 $mgkg^{-1}$).

Basic properties of SSBC and SS materials used in this study are presented in Table 5.1. The addition of SSBC and SS caused various changes in soil properties such as pH, EC, DOC, NH_4-N, NO_3-N, P, surface area and pore size (Table 5.2). Such changes have been well reported in the literature (Keithet al., 2011; Quilliamet al., 2012).The addition of SSBC increased soil pH by 0.01-0.06 pH units, while SS decreased pH by 0.07-0.34 pH unit. As compared to the control soil, the SS effect on EC change (0.28-1.59 $mScm^{-1}$) was higher than SSBC (0.1-1.25 $mScm^{-1}$). Total N concentrations significantly increased ($P<0.05$) in SSBC amended soil and this increase ranged from 1.40-3.02 fold.Total N concentrations also increased, but to a lesser extent, in the SS amended soil (1.54-3.70fold). The influence of SSBC on total C concentrations (1.08-1.41 fold)was slightly lower than SS (1.11-1.57 fold) upon amendments in the soil.Further, SSBC amendments increased S and available P by 1.58-4.58 fold and 1.2-2.5 fold, respectively, while SS amendments increased by 1.68-2.94 fold and 1.49-3.19 fold, respectively as compared to the control soil. SSBC and SS amendments increased available NH_4-N by 1.9-4.5 fold and 3.7-5.7 folds, respectively. NO_3-N concentrations were also significantly ($P<0.01$) increased in the 2% SSBC and SS amended soils but decreased with increasing of application rates. DOC concentrations increased dramatically and significantly ($P<0.01$) following SS application (5.5-16 fold) while SSBC had only a modest effect (increasing DOC 1.1-1.8 fold) that was significant ($P<0.01$) for

SSBC5 and SSBC10 only.

Solvent extractable Σ16PAHwas 5.78 mg kg^{-1} in SS; this value being below the maximumallowable limits (MAL) (Σ9PAHs, 6 mg kg^{-1}) set by the Council of the European Community (CEC, 2000). The Σ16PAHsconcentration in SSBC (1.70 mg kg^{-1})complied with the International Biochar Initiative recommended standards (6-20 $mgkg^{-1}$) (IBI, 2012). These results indicate that the conversion of SS to SSBC resulted in a significant ($P \leq 0.01$) reduction in PAH concentration. In the case of this SS, and its specific pyrolysis conditions, the Σ16PAH concentration was reduced by a factor of approximately four.It is stressed that dissimilar biochars have been reported to have different PAH levels; these being dependent upon type of feedstock used and the conditions under which they are pyrolised (Freddo et al., 2012; Hale et al., 2012). Higher temperatures and long pyrolysis time have been shown to reduce PAH concentrations in biochar (Freddo et al., 2012; Hilber et al., 2012).PAH concentrations in SS have been noted to depend upon on the nature of the wastewater treated by WTPs (Dai et al., 2007). SS has been reported to contain variable levels of PAH, ranging ranged from 2 to 36 $mgkg^{-1}$(Baran et al., 2003) (given this range the SS used in this research has a comparatively low PAH loading (5.78 $mgkg^{-1}$)). It follows that pyrolysis of SS with different PAH loadings (and compound profiles) are likely to give rise to SSBCs also with contrasting PAH loadings. With concentrations of PAHs in SS designated for application to agricultural land regulated (USEPA, 1995; SEPA, 1995; CEC, 2000) this result indicates opportunities to "treat" SS that contain PAH concentrations that exceed guidance values using pyrolysis to produce SSBC. Further research is, however, required to establish how contrasting pyrolysis conditions and the use of dissimilar SS feedstock influence PAH levels in the resultant SSBCs.

The distribution of solvent extractable individual PAHs in SSBC and SS varied greatly (Fig. 5.6). In SSBC: naphthalene, phenanthrene, fluoranthene and pyrene predominated.

Phenanthrene was most abundant individual PAH in SS following by Benzo(b)fluoranthene, while benzo(a)pyrene was not detected in either SS nor SSBC (Fig. 5.6).

Total PTE concentrations in SSBC were significantly ($P<0.01$) higher than those in SS (Table 5.1). Such increases in PTE concentrations upon pyrolysis are to be expected because PTEs are conserved during pyrolysis, while much of the SS mass is converted to gaseous and liquid fuels (Fagbemi et al., 2001). There is an overall concentrating effect. PTE levels were enhanced by the following factors: As (x4.8) >Pb (x1.9) >Zn (x1.7) >Cd (x1.6) >Cu (x1.3).

For all total PTE concentrations were increased when SS was converted to SSBC their availabilities were greatly reduced (Table 5.1). Available PTE concentrations in SSBC were significantly ($P<0.01$) lower than those in SS; PTE bioavailability reduced by the following factors: As (x28)>Cu (x11)>Cd (x6)=Zn (x6)>Pb (x3). These results highlight the opportunity conversion of SS to SSBC has to produce a soil amendment that represents a lower risk in terms of its available PTE concentrations. If follows that lower PTE availability in the amendment matrix should translate into lower PTE level available for plant uptake.

5.5.2 Effects of amendment on soil PAHs

Basic properties of SSBC and SS materials used in this study are presented in Table 5.1. The addition of SSBC or SS caused various changes in soil properties such as pH, EC, DOC, NH_4-N, NO_3-N, P, surface area and pore size (Table 5.2).

The smallest decrease in PAH bioaccumulation was noted for naphthalene; bioaccumulation decreases in the SSBC and SS treatments varied depending on application rate of amendment, respectively, by 27.8-38.5% and 11.3-29.2% (Fig. 5.3A). Among 3-ringed PAH compounds, phenanthrene was the predominant PAH in cucumber grown in control and SSBC/SS amended treatments. The highest decrease in bioaccumulation (55.3-59.5%) was observed for acenaphthylene, while the lowest decrease (27.9-39.5%) for anthracene in SSBC amendments (Fig.5.3A). Like SSBC, the effect of SS addition on these 3-ringed PAHs was also varied and again acenaphthylene exhibited thehighest decreasein bioaccumulation (26.8-38.6%), followed by acenapthlene (12.7-36.2%) (Table 5.4).

The addition of SSBC significantly ($P<0.001$) reduced the bioaccumulation of 4-ringed PAHs such as fluoranthene (43.7-56.3%), pyrene (44.4-61.1%), benzo(b)anthracene (61.2-70.9%) and chrysene (41.4-68.3%), as compared to the control soil (Fig. 5.3B). Similarly, SS addition reduced the bioaccumulation of fluoranthene (15.5-24.7%), pyrene (19.2-42.8%), benzo(b)anthracene (26.9-45.8%) and chrysene (21.1-26.0%) (Table 5.5). Among the 5-ringed PAHs, benzo(b)fluoranthene and benzo(k)fluoranthene were the most prevalent PAHs in cucumber grown in all treatments (Fig. 5.3B). The decrease in bioaccumulation of benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene were 71.3-86.8, 70.9-84.7 and 53.1-75.8%, respectively in SSBC amended soil, while 36.7-57.6, 38.9-57.8 and 23.6-60.8%, respectively in SS amended soil (Table 5.6). In cucumber fruit, the bioaccumulation of 6-ringed PAHs was the lowest compared to other groups of PAHs (Fig. 5.3B). SSBC addition significantly ($P<0.001$) reduced bioaccumulation of indeno(1,2,3-c,d)pyrene (51.8-81.0%), dibenzo(a,h)anthracene (53.2-80.3%) and benzo(g,h,i)perylene (60.0-84.2%), while the effects of SS addition was lower than this (35.3-51.9, 29.2-59.8 and 31.1-65.9%, respectively) (Table 5.7).

The soil used in this study had a Σ16PAH concentration of 9.90 mg kg^{-1}. Concentrations of mutagenic Σ7PAHs (5.49 mg kg^{-1}) exceeded the limit (4.23 mg kg^{-1}) set by the Ministry of Environmental Protection, China (MEP, 2007). Owing to the lower Σ16PAH concentrations in SS and SSBC their amendment to soil served to dilute Σ16PAH concentrations. This dilution made a significant difference ($P<0.05$) in the SS10 and all SSBC treatments.

SSBC significantly reduced the available Σ16PAH concentrations, as compared to the control soil (Fig. 5.2A). The available Σ16PAH concentration in the SSBC2 treatment was reduced by 30.8% as compared to the control soil. This decrease in Σ16PAH concentrations was even greater in the SSBC5 (38.5%) and SSBC10 (43.4%) treatments. AvailableΣ16PAH concentrations were not significantly reduced in the SS2 and SS5 treatments, while the highest application rate (10%) of SS resulted in a significant decrease in available Σ16PAH concentrations(27.5%) (Fig.5.2A).

It is stressed that the decreases in PAH availability in the SS and SSBC amended treatments represent changes that are far greater than those that can be attributed to dilution alone (based upon PAH concentration in the soil, SS and SSBC (section 3.1) the treatments with 10% amendment had dilutions of only 3% (SS) and 8% (SSBC). The magnitude of changes reported above reflect compound re-partitioning not simply a dilution effect.

The addition of SSBC significantly ($P \leq 0.05$) reduced different PAH compounds (defined by number of benzene rings) and this reduction increased with increasing application rates (Fig. 5.2B). The results indicate that the reduction in available fractions of low molecular weight (LMW) PAHs (2-3 ringed) was low, but increased for high molecular weight (HMW) PAHs (4-6 ringed). This is reasonable on account of LMW-PAHshavinggreatermobility than HMW-PAHs (Arp et al., 2011; Xing and Pignatello, 1996).Regarding SS the reduction in available PAH was not significant for SS2 (for both LMW and HMW PAHs) and SS5 (for LMW-PAHs) amendments, while only SS10 amendment showed a significant decrease (for both LMW and HMW PAHs). However, with increasing SS application different groups of PAHs were influenced to contrasting extents (Fig. 5.2B).

Recently Oleszczuk et al. (2012) reported the influence ofbiochar on available concentrations of PAHs following its addition to SS. The results reported in this research are in keeping with Oleszczuk et al. (2012) in so much as both investigations have shown biochar to reduce PAH availability and that this reduction is most considerable for HMW-PAHs.

It is of note to mention that 7 days were allowed for the amended soils to 'interact/equilibrate'. While this amount of time is unlikely to have been sufficient for all of the PAHs to fully re-equilibrate with the amendments the results clearly indicate that this was sufficient time to markedly reduce PAH availability in the SSBC amended soils. Significantly the results indicate clear differences between the influence of SS and those of SSBC in this regard. Oleszczuk et al. (2012) noted that the influence of different biochars on free dissolved PAHs added to sewage sludge was also different. In our study, greater mass transfer from soiltoSSBC and SS was observed for 5-and 6-ringed PAH compounds. It is stressed that affinity of PAHs for biochar will depend upon the properties of the biochar; these being governed by feedstock type and pyrolysis conditions such as temperature and time (Chen et al., 2008; Hale et al., 2012). Biochar characteristics such as pH, elemental composition, surface area and structure could be changed with changes in these conditions, and as a consequencealter the sorptionbehavior of biochar (Cao et al., 2011). In this study the SSBC and SS materials had different surface areas and pore volumes (Table 5.1). In both cases the values for SSBC were double those observed for SS; this may, in part, be the reasons for different extents of PAH sorption observed.

After desorption from a contaminated matrix, the sorption and sequestration of PAHsinto SS/SSBC occurs through different means such as hydrophobic effect, π-electroninteractions and pore-filling mechanisms (Chen et al., 2008).These mechanisms would contribute differently in the SSBC and SS materials used in this study. Furthermore, the presence of surfactants in SS (Smith et al., 2009) may have increase desorption/solubility of PAHs from contaminated matrices (Zhu et al., 2010) thereby promoting their availability.

5.5.3 Effects of amendments on PTEs

The concentrations of PTEs in the contaminated soil were determined (Table 5.1). Of these PTEs Zn and Cd exceeded the MALs (250 $mgkg^{-1}$ and 0.3 $mgkg^{-1}$, respectively) set by the State Environmental ProtectionAdministration, China (SEPA, 1995), while the As concentration was lower than its MAL limit (30 $mgkg^{-1}$).

The addition of SSBC decreased available concentrations of As (52.4-66.7%), Pb (20.5-49.5%), Cu (7.1-48.0%), and Zn (2.1-8.4%), while Cd concentration increased slightly (2.2-13.9%) as compared to the control soil (Table 5.2). There was no significant decrease in available concentrations of As, Cu, Pb and Zn at low SSBC application rate (2%), while significantly ($P \leq 0.05$) decreased concentrations (except Zn) were observed at higher SSBC application rates (5 and 10%).(Table 5.2).

In contrast to SSBC, available concentrations of the PTEs As, Cd, Cu and Zn significantly ($P < 0.001$) increased in SS amended soil as compared to the control soil(Table 5.2). The increase in available concentrations of PTEs in SS amended soils increased with increasing application rates (Table 5.2). Of the PTEs, the highest increase in available concentration was observed for As (57.1-229%). After As, increases in available concentrations weremost marked for Cd (69.4-80.6%). The increase in available concentrations for Cu and Zn were 30.2-142% and 21.6-149%, respectively; while Pb showed the lowest increase (2.0-7.1%) in available concentrations.

Collectively these results highlight the propensity of SS to increase available concentrations of PTEs following its amendment to soil. In contrast, once converted to SSBC, PTE availability in the amended soil was decreased markedly. This outcome is consistent with several other works that have shown biochar to be effective in reduced the available concentrations of PTEs in contaminated soils (Beesley et al., 2011; Lehmann, et al., 2011; Ahmad et al., 2012). Reduced PTE availability in soil amended with biochar has been linked to changes in soil pH, cation exchange and DOC values (Keith et al., 2011; Zheng et al., 2012). In addition, biochar also contains oxygen-functional-groups which are expected to form complexes with PTEs (Kookanaet al., 2011) and led to PTE immobilization in soil and then reduced bioaccumulation in plants grown on biochar amended soil. Previous studies have been shown that these oxygen-functional-groups are more effective than increasing alkalinityeffects in suppression of PTEs such as Cu (Jiang and Xu, 2013).

5.5.4 Plant biomass

Fruit biomass was significantly higher ($P \leq 0.01$) in the SSBC amended soil than in the control soil (Fig. 5.7). These SSBC amendments appreciably improved fruit yields when compared to the control, showing increases of: 31.7, 57.1 and 63.0% in SSBC2, SSBC5 and SSBC10 amendments, respectively. Similarly, fruit biomass in the SS amendments was also significantly ($P \leq 0.05$) increased with respect to the control soil (Table 5.3). It was noted that the highest application of SS had no appreciable benefit in terms of fruit biomass, as compared to SS2. Besides other nutrients, the increase in NH_4-N and NO_3-N concentrations by SSBC and SS (Table 5.2) could be the reason for higher fruit yield (Clough and Condron, 2010). As noted earlier, SS and SS amended soil had higher concentrations of available PTEs than SSBC and SSBC amended soils (Table 5.1 and 5.2), therefore, the higher application rate of SS (10%) may have exerted a negative effect on plant biomass production that off-set potential gains due to increases in plant nutrient levels. Theresults indicate that SSBC has greater potential over SS withrespect topromoting improved cucumber fruitbiomass.

5.5.5 PAH bioaccumulation

All 16 PAHs were detected in cucumber fruit grown in control soil and treatments amended with SSBC and SS (Fig. 5.3). The addition of SSBC significantly ($P<0.01$) reduced the concentrations of Σ16PAH by 44.3-57.1% in cucumber fruits. SS addition was less effective but stillsignificantly ($P \leq 0.05$) reduced Σ16PAH bioaccumulation by 19.9-

35.6% (Fig. 5.8). Results indicated that the extent of decrease in Σ16PAHbioaccumulation increased with increasing application rates of SSBC and SS (Fig. 5.8). In the case of SS maximum reduction in Σ16PAHbioaccumulation was observed in the SS10 treatment (35.6%) this reduction was comparable to the reduction observed in the SSBC2 treatment (44.3%) with no significant ($P>0.05$) difference being observed between these treatments. Further significant ($P<0.05$) reductions in bioaccumulation were observed in the SSBC5 and SSBC10 treatments (Fig. 5.8).

Figure 5.3 summarizes the overall influence of SSBC and SS amendments on PAH bioaccumulation in cucumber fruit. At all application rates the effectiveness of SSBC was greater than SS at reducing PAH bioaccumulation in cucumber fruit (i.e. data points fell in the upper left portion of the frame (Fig.5.4)).In SSBC2 amendments, the decrease in bioaccumulation of individual PAH was more than 40% (except for naphthalene, fluorene and anthracene). In SS2 amendments, this decrease was lower than 30% (except for benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene, and benzo(g,h,i)perylene (Fig. 5.4A)). SSBC5 amendments reduced PAH bioaccumulation by more than 50% in cucumber except for naphthalene, fluorene and anthracene. In SS5 amended treatments, benzo(a)pyrene, 5-and 6-ringed PAHsonly justexceeded the 40% decrease rate, while other PAH compounds (except pyrene) were lower than 30% (Fig. 5.4B). At 10% application rate, again SSBC was more effective in decreasing PAH bioaccumulation than SS (Fig. 5.4C). The decrease in PAH bioaccumulation was higher for HMW-PAHs than LMW-PAH (i.e. data points for HMW-PAH fell in the upper left of the frame (Fig. 5.4)).These HMW-PAH compounds would be expected to be of lower availability and their bioaccumulation greatly reduced by SSBC amendments as compared to LMW-PAH compounds (Papadopoulos et al., 2007). The trends reported regarding bioaccumulation into fruits reflect the trends in available PAH concentrations in the soil and the amended soils.

The reduced bioaccumulation of PAHs in to cucumber is in keeping with results indicating reduced PAH bioaccumulation into lettuce when grown in the presence of SS and SSBC (Khan et al., 2013). This lower accumulation of PAHs in cucumber fruits grown in SSBC amended soil than SS could be attributed to the reduced concentration of PAHs in the soil solution. Our resultsdemonstrated that SSBC addition to contaminated soil reduces the risk associated with the transfer of PAHs in to food and thereafter into the food chain.

These results confirmed thatSSBC has higher sorptive capacity than SSfor PAHs. The structural composition and elemental characteristics of SSBC are different to those of SS (Table 5.1).These play an important role in determining the sorptive capacity for these materials. The results reported here are in agreement with the findings of other researchers who have reported the application of other (not SSBC) biocharsto PAH contaminated soil to decrease their availability (Chen and Yuan, 2011). Specifically wood biocharhas also been observed to decrease the bioavailability of PAHs (Beesley et al., 2010) but its effectiveness in this regard has been shown to be dependent uponPAH ageing or contact time (Cao et al., 2011). It has been suggested that biochar not only reduces the available PAHs in the soil liquid phase but also minimizes the transfer of PAHs from soil to air to plant (this pathway being particularly relevant for more volatile PAHs) (Kipopoulou et al., 1999; Howsam et al., 2001). The impediment to both transfer mechanisms would contribute to reduce soil to plant transfer of PAHs.

5.5.6 PTE bioaccumulation

The SSBC addition significantly ($P \leq 0.01$) reduced bioaccumulation of As, Cu and Pb in cucumber fruit as compared to the control, while the decrease for Zn bioaccumulation was negligible (Fig 5.5). The concentration of Cd in the cucumber fruit significantly ($P \leq 0.05$)increased in the presence of SSBC. The average Cd concentration increased by 40.5-72.6% in cucumber grown in SSBC amendments (Table 5.8).

The addition of SS significantly ($P \leq 0.05$)increased bioaccumulation of selected PTEs as compared to the control. Changes in metal concentrations observed in cucumber fruits grown on SS amended soil are given in Table 5.8.

The concentration of As was significantly ($P \leq 0.05$) elevated in cucumber grown on SS amended soil, reaching 0.41-0.55 $mgkg^{-1}$ as compared to control (0.35 $mgkg^{-1}$). These levels of As exceeded the maximum permissible limits (0.05 $mgkg^{-1}$ dw) set for food by SEPA (2005).The addition of SSBC decreased As concentration (0.13 $mgkg^{-1}$ in SSBC10 treatments) in cucumber but this concentration exceededth limit set by SEPA (0.05 $mgkg^{-1}$).

The concentrations of Cd in cucumber significantly increased in both SSBC and SS amended soils, and exceeded the permissible limits (0.2 $mgkg^{-1}$ dw) set by SEPA (2005) for food. It should be noted that the soil used in this research had total Cd concentration that exceeded the MAL set by SEPA (1995)for agricultural practice (Table 5.1).

The concentration of Zn was also significantly ($P \leq 0.05$) elevated in cucumber grownin SS amended soil and exceeded the MAL (100 $mgkg^{-1}$ dw) set for food by SEPA (2005). Again, as was the case for Cd total Zn concentration in the soil exceeded the MAL (300 $mgkg^{-1}$) set by SEPA (1995) for agricultural practice (Table 5.1). Thus soil contaminated with such high levels of As, Cd and Zn may still pose a health risk through soil to food transfer of PTEs even after SS or SSBC amendments.

The concentrations of DOC in SSBC treatments may have played an important role in reducing bioavailability and bioaccumulation of PTEs. However, in the case of SS treatments, PTE bioaccumulationincreased inspite of increased DOC. It is suggested that PTEsmay be bound more weakly to SS-DOC than to SSBC-DOC (see Table 5.1 for available metals) and are thereforemore easily released to soil solution for plant uptake even where DOC levels are elevated. The higher surface area, higher pore volume and smaller pore size of SSBCwould also contribute to reducing PTE bioavailability. The SSBC amendments increased P and S contents in soil (Table 5.2) which interact strongly with Asduring plant uptake, which affect its bioaccumulation. Arsenateis aphosphate analog and taken up into plants through P transporters (Meharg and Macnair, 1992), therefore, P associated with SSBC might be responsible for the reduced*in planta* As bioaccumulation. In the case of SS amendments, elevated concentrations of P and S were also present (Table 5.2). These did not, however, reduce As and other metal bioavailabilities through the above mentioned mechanisms. It seems that the different physical and chemical structure of SS increase the availability of PTEs and facilitate their subsequent bioaccumulation in cucumber. In contrast, the addition of SSBC decreased As, Cu, Pb and Zn availability and subsequent uptake through combined effects attributed to (Uchimiya et al., 2011a): increasing soil pH; higher P and S availability; oxygen-functional-groups present on biochar, and; metal-complexation (Lehmann and Joseph, 2009). It is also likely thatchanges in microbial activities (Quilliamet al., 2012; Steinbeiss et al., 2009) would also have influenced PTE bioavailability.

Conclusions

Results indicated that theconversion of SS to SSBC was effective in destroying PAHs and in reducing the availability of PTEs. The addition of SSBC to contaminated soil was far more effective in reducing the availability of PAHs and PTEs than SSand as a consequence SSBC was more effective in reducing pollutant transfer from soil to water to plant. In addition to reduced pollutant transfer from soil to plant it is noteworthy that pathogens associated with SS are killed during SSBC production. Thus, SSBC offers additional benefits in terms of mitigating pathogen transfer to food.It should be emphasized that how well these results can be extrapolated for other SSBC, other biochars and other soil types is uncertain, and further investigation in this regard is required. Nonetheless it can be concluded, from several perspectives, that SSBC represents a promising alternative to SS as a soil amendment particularly where PAH and PTE concentrations are elevated in soil.

Table5.1

Physical and chemical properties of soil, SS and SSBC and concentrations to PTEs. Mean values are shown±1 standard deviation (n =3).

[a]Permissible limits set for sewage sludge by the United States Environmental Protection Agency(USEPA) [3]

[b]Permissible limits set for soil pH>6.5 by the State Environmental Protection Administration(SEPA) [5]

[c]Soil background values taken from Chen et al. (24) for Fujian province, China

Parameters	SS	SSBC	Soil	Permissible limits		Background[c] soil
				USEPA[a] (sludge)	SEPA[b] (soil)	
pH(H_2O)	6.69±0.06	7.43±0.14	7.35±0.12			
EC(mS/cm)	10.8±0.52	6.69±0.31	0.43±0.01			
N (%)	3.78±0.21	2.83±0.43	0.14±0.02			
C (%)	28.3±2.38	20.6±1.07	4.98±0.12			
S (%)	2.53±0.12	2.25±0.10	0.05±0.01			
NH_4-N (mg kg^{-1})	38.6±4.02	10.2±0.59	9.05±0.05			
NO_3-N (mg kg^{-1})	2.88±0.45	3.93±0.36	1.41±0.13			
TOC(mg kg^{-1})	13073±231	283±27.1	342±31.2			
BET Surface area(m^2 g^{-1})	2.34±0.03	4.07±0.02	8.83±0.05			
Pore volume (cm^3 g^{-1})	0.01±0.00	0.02±0.00	0.02±0.00			
Pore size (nm)	15.3±0.05	10.3±0.10	9.48±0.08			
	Total PTE concentrations (mg kg^{-1} dw)					
As	2.56±0.13	12.4±0.71	5.80±0.48	75	30	5.88
Cd	1.65±0.06	2.72±0.03	1.00±0.01	85	0.3	0.05
Cu	160±7.94	213±5.83	44.9±1.25	4300	100	19.8
Pb	43.9±1.25	81.9±2.11	146±4.32	840	300	35.6
Zn	1215±81.7	2079±114	325±17.6	7500	250	79.5
	Available PTE concentrations (mg kg^{-1} dw)					
As	1.12±0.05	0.04±0.00	0.21±0.01			
Cd	1.07±0.08	0.18±0.01	0.36±0.02			
Cu	36.6±1.21	3.35±0.08	4.21±0.52			
Pb	8.22±0.25	2.50±0.05	20.8±2.13			
Zn	371±31.4	65.7±5.30	29.7±3.21			

Table 5.2

Physical and chemical properties of soil and soil amended with SS and SSBC. Mean values are shown ±1 standard deviation (n =4). The values with lower case letters shown alongside indicate significant difference between the control and amended soils;values with no letters indicate no significant difference.The values shown in bold indicate significant difference between values for SSBC amendments with respect to SS amendments at like application rates.

Parameters	Control	SS2	SS5	SS10	SSBC2	SSBC5	SSBC10
				Basic characteristics			
pH	7.35±0.10	7.28±0.06	7.13±0.05	7.01±0.04	7.36±0.02	7.39±0.03	7.41±0.02
EC ($mScm^{-1}$)	0.42±0.02	0.70^{a}±0.11	1.38^{b}±0.10	1.59^{b}±0.05	0.52±0.01	0.87^{a}±0.04	1.25^{a}±0.03
N (%)	0.15±0.01	0.25^{a}±0.01	0.38^{b}±0.02	0.61^{c}±0.04	0.22±0.01	0.34^{a}±0.02	0.56^{a}±0.01
C (%)	4.95±0.13	5.75^{a}±0.10	6.95^{b}±0.21	8.71^{c}±0.11	5.59±0.17	6.31^{a}±0.15	7.85^{b}±0.23
S (%)	0.06±0.00	0.12^{a}±0.01	0.20^{b}±0.01	0.39^{b}±0.02	0.10±0.01	0.18^{a}±0.01	0.30^{b}±0.02
P	14.4±1.21	21.4^{a}±2.84	32.1^{b}±3.15	45.9^{c}±3.46	17.2^{a}±1.03	28.2^{b}±2.40	35.8^{c}±2.76
NH_4-N (mg kg^{-1})	9.03±0.15	33.0^{a}±2.28	44.1^{b}±1.94	51.3^{b}±3.76	17.2^{a}±1.02	**32.2^{b}±2.51**	**40.8^{c}±3.19**
NO_3-N (mg kg^{-1})	1.36±0.07	54.3^{a}±4.15	22.8^{b}±2.30	15.0^{c}±1.06	**96.4^{a}±5.47**	40.2^{b}±3.12	34.1^{b}±3.61
DOC (mg kg^{-1})	342±7.23	1879^{a}±23.6	4008^{b}±41.4	5641^{c}±59.3	**368±6.94**	**495^{a}±14.4**	**618^{a}±17.5**
BET Surface area(m^2 g^{-1})	8.83±0.05	7.60±0.03	7.25±0.06	6.45±0.02	7.83±0.05	7.60±0.07	6.78±0.03
Pore volume (cm^3 g^{-1})	0.020±0.00	0.0203±0.00	0.0211±0.00	0.0190±0.00	0.0211±0.00	0.0210±0.00	0.0195±0.00
Pore size (nm)	9.48±0.08	10.7±0.42	11.7±0.37	11.8±0.29	10.8±0.16	11.1±0.11	12.0±0.24
				Available PTE concentrations (mg kg^{-1} dw)			
As	0.21±0.01	0.33^{a}±0.01	0.5^{b}±0.02	0.69^{c}±0.03	**0.09^{a}±0.01**	**0.08^{a}±0.01**	**0.07^{a}±0.01**
Cd	0.36±0.02	0.61^{a}±0.03	0.62^{a}±0.04	0.65^{a}±0.02	**0.37±0.01**	**0.38±0.02**	**0.41±0.04**
Cu	4.21±0.52	5.48±0.81	7.26^{a}±0.72	10.2^{b}±1.25	**3.91±0.91**	**3.64±0.80**	**2.19^{a}±0.13**
Pb	20.8±2.13	21.2±1.46	21.9±2.33	22.3±3.04	**16.5^{a}±1.02**	**13.8^{b}±0.95**	**10.5^{c}±1.17**
Zn	29.7±3.21	36.1^{a}±5.47	57.6^{b}±4.86	74.2^{c}±3.98	29.1±3.06	**27.5±4.55**	**27.2±4.12**

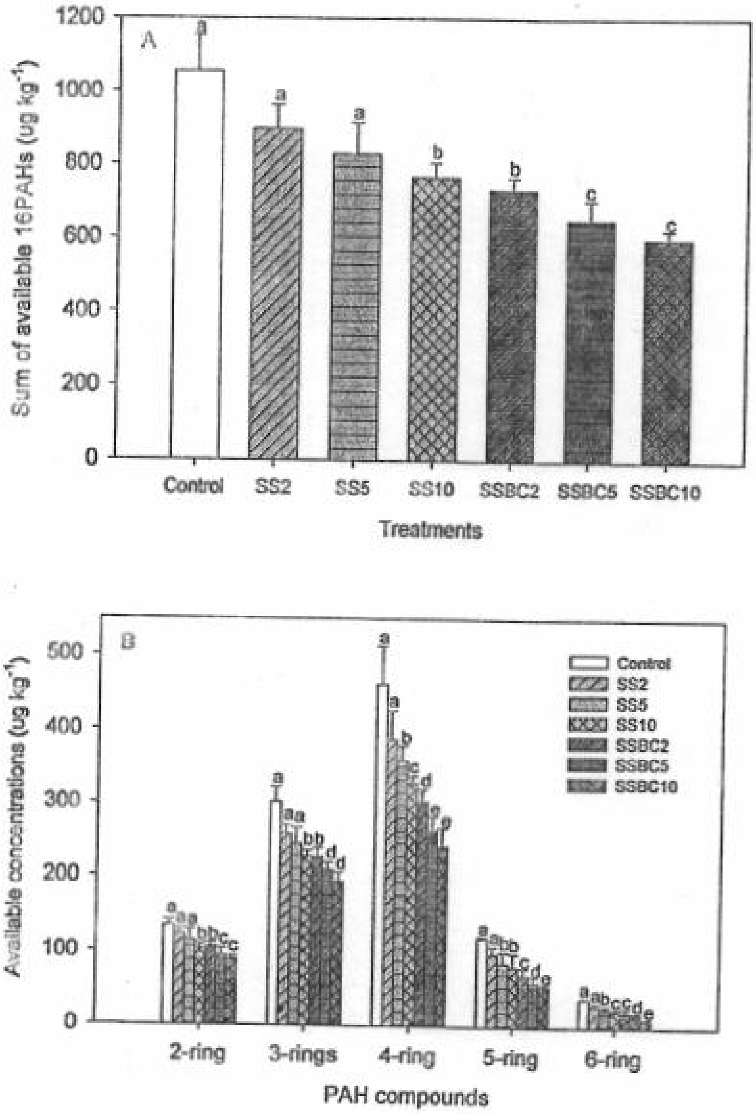


Fig. 5.2. PAHs in the control soil and soil amended with SSBC and SS: A) supercritical water extracted Σ16PAHs B) supercritical water extractedPAHs grouped by number of benzene rings (2-6). Error bars represent standard deviations (n=4). Different letters on the bars indicate significant difference (P≤0.05), while similar letters indicate no significant difference between the treatments.

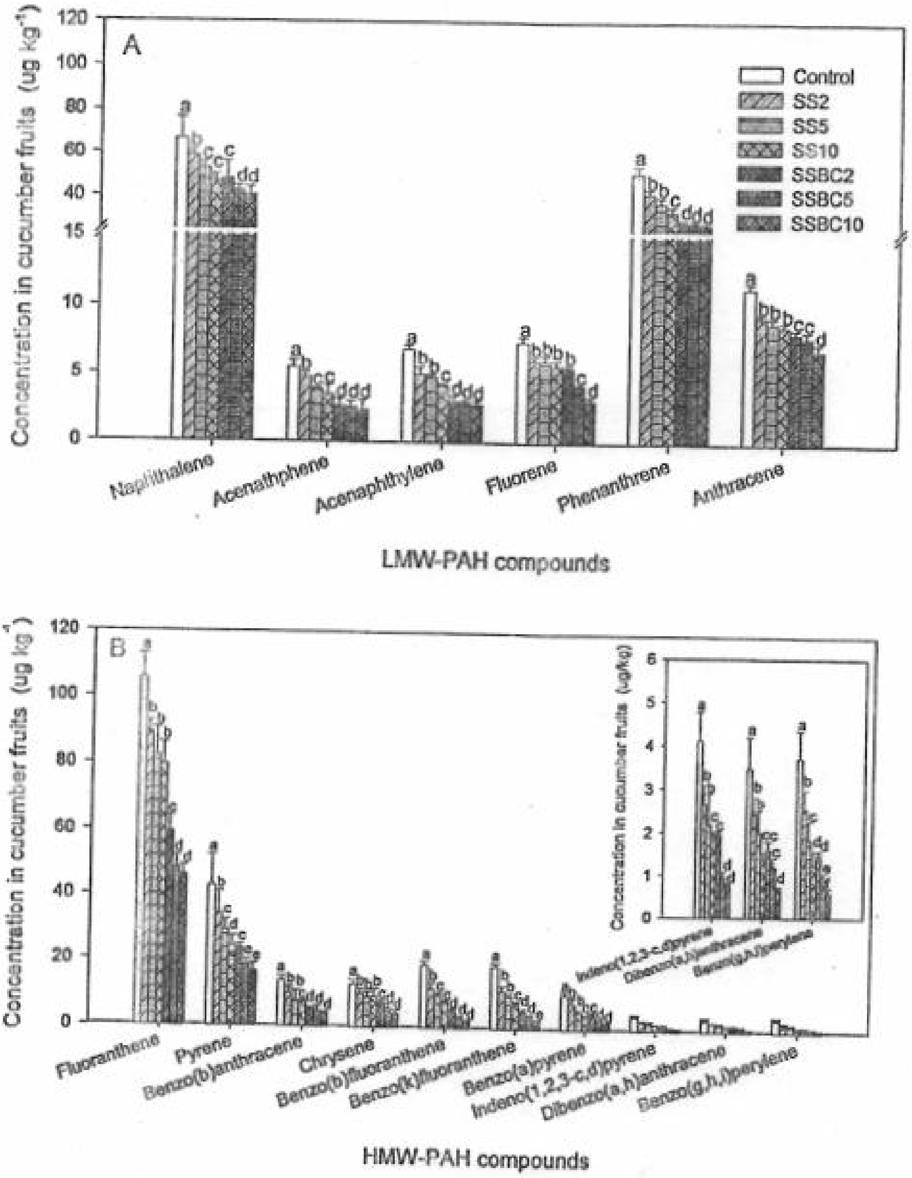


Fig.5.3. Bioaccumulation of PAH compounds in cucumber fruit grownin soil and soil amended with SSBC and SS. A) LMW-PAH compounds, B) HMW-PAH compounds.Error bars represent standard deviations (n=4). Different letters indicate significant difference ($P \leq 0.05$), while same letters indicate no significant difference between the treatments.

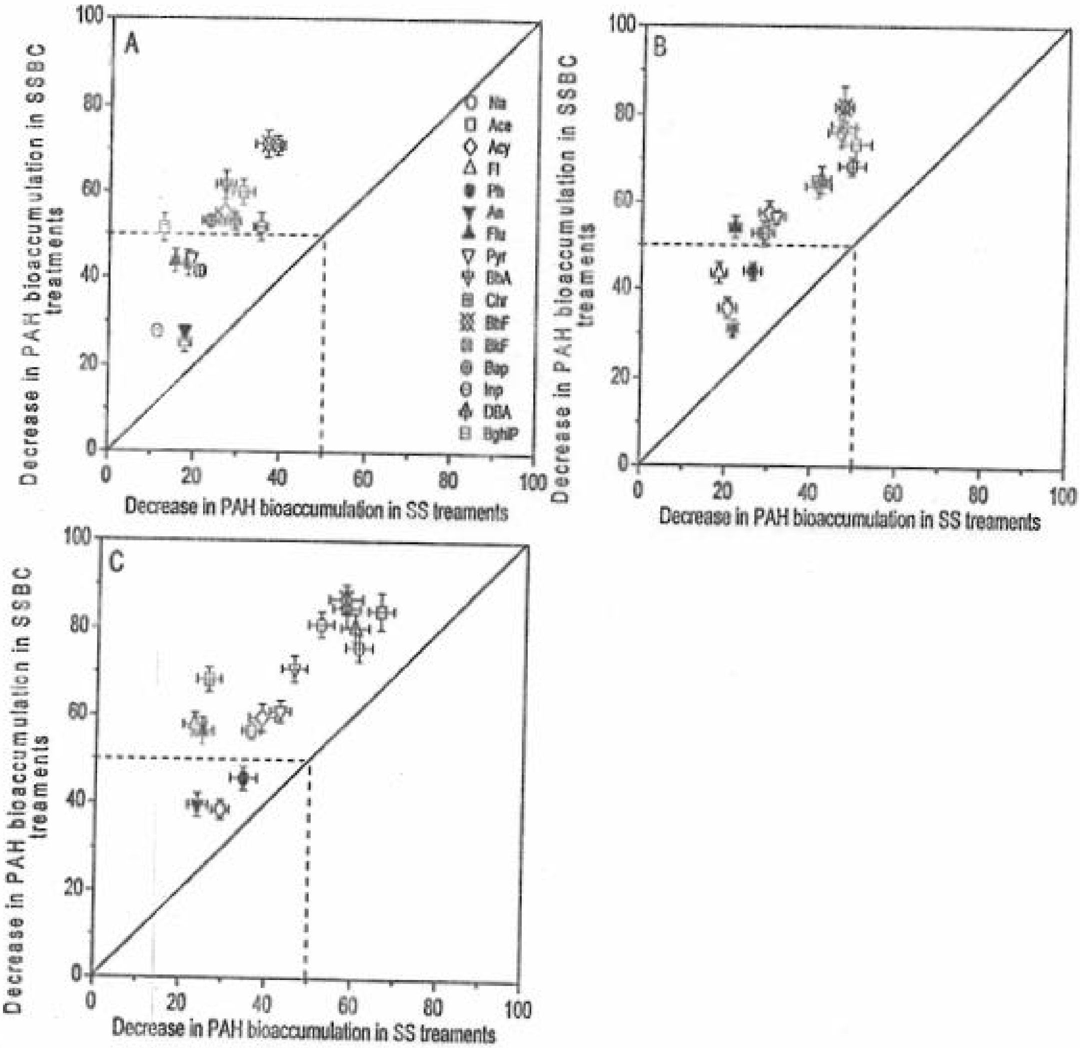


Fig.5.4. Comparison of reduction (%) in bioaccumulation of PAHs in cucumber fruits grown in the presence of SSBC and SS at application rates of A) 2%,B) 5% and C) 10%. The solid lines are lines of 1:1 relationship, while the dashed lines indicate the 50% reduction in bioaccumulation of PAHs. The legend abbreviations are: Na (naphthalene), Ace (acenathphene), Acy (acenaphthylene), Fl (fluorine), Ph (phenanthrene), An (anthracene), Flu (fluoranthene), Pyr (pyrene), BaA (benzo(b)anthracene), Chr (chrysene), BbF (benzo(b)fluoranthene), BkF (benzo(k)fluoranthene), Bap (benzo(a)pyrene), Inp (indeno(1,2,3-c,d)pyrene), DBA, (dibenzo(a,h)anthracene) and BghiP (benzo(g,h,i)perylene).

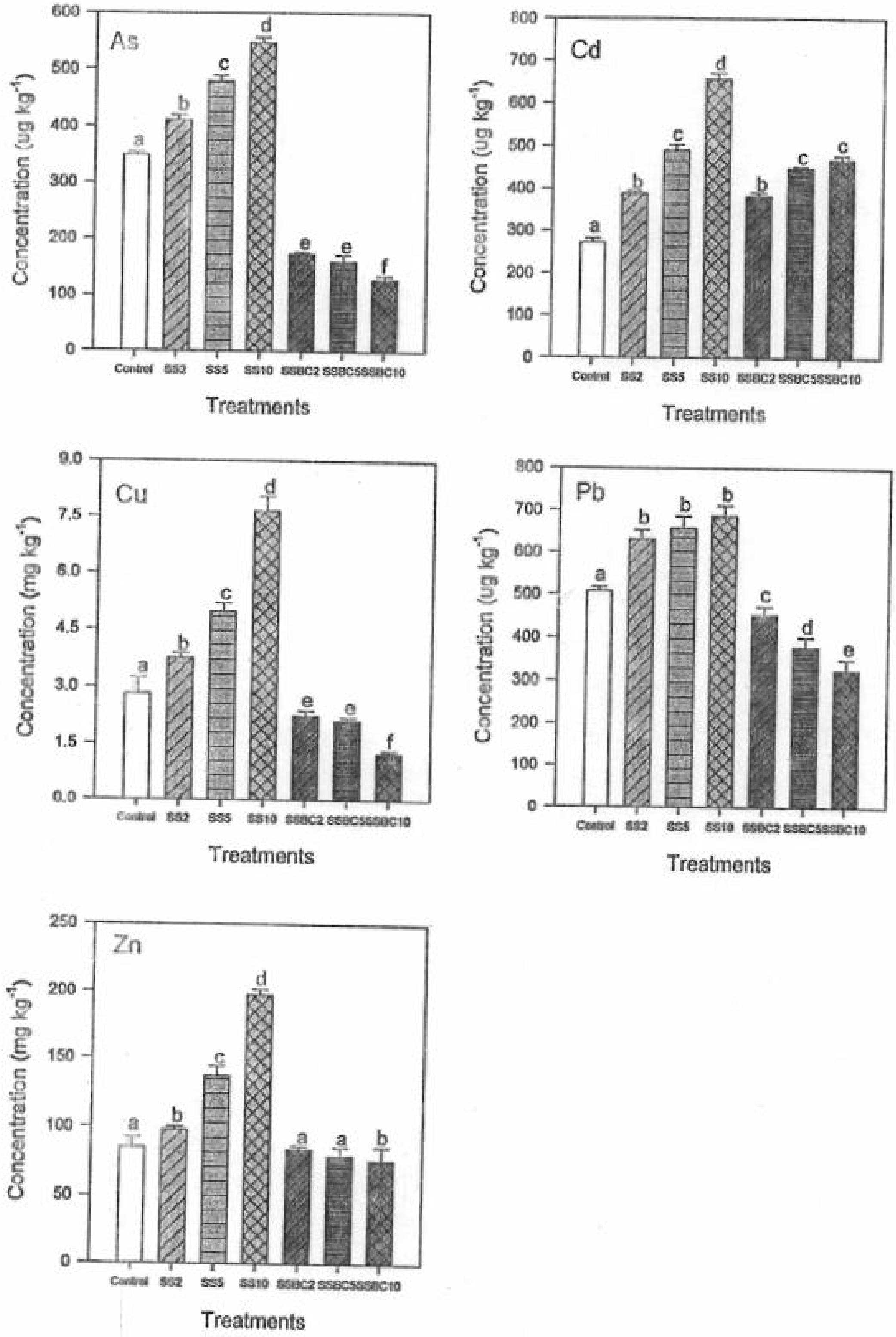


Fig.5.5. Bioaccumulation of PTEs in cucumber fruit grown in soil and soil amended with SS and SSBC. Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤0.05), while same letters indicate no significant difference.

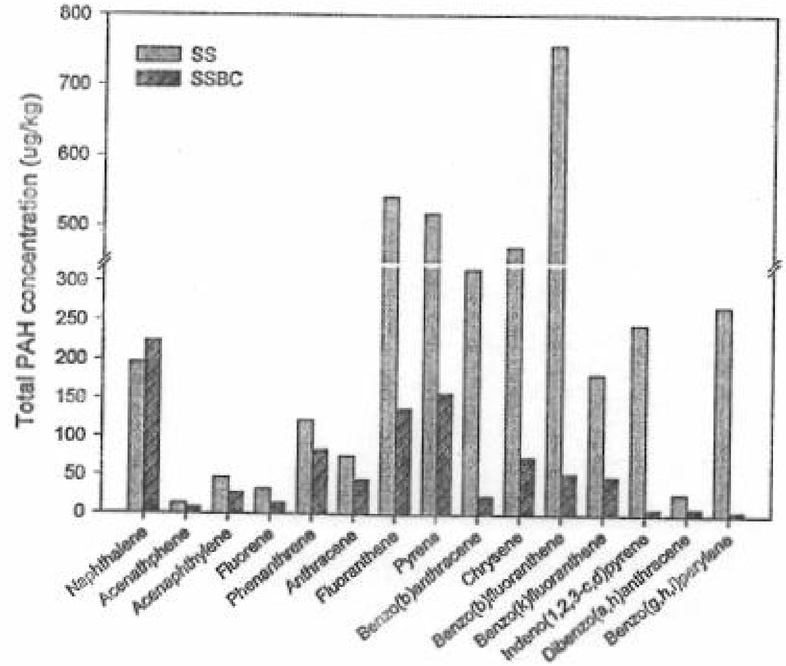


Fig.5.6.Total concentrations of PAHs in SS (grey) and SSBC (dark grey hatched) materials used in this study.

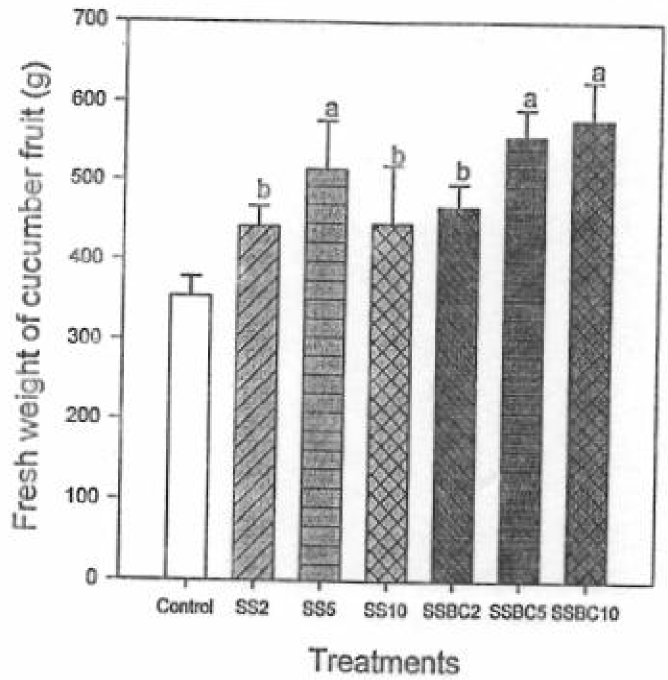


Fig. 5.7. Fresh weight (g/pot) of cucumber fruit grown incontrol soil (white)and soil amended with SS2 (grey hatched), SS5 (grey horizontal), SS10 (grey cross-hatched),SSBC2 (dark grey hatched), SSBC5 (dark grey horizontal), SSBC10 (dark greycross-hatched).Error bars represent standard deviations (n=4). Letters indicate significant difference ($P \leq 0.05$) from the control, while similar letters indicate no significant difference between the treatments.

Table 5.3
Mean fresh weight (g/pot) of cucumber yield (n=4) grown incontrol, SSBC and SS amended soil. Increase changes, due to SSBC and SS addition, are given in percentage.

Treatments	Cucumber biomass	
	Fresh weight (g/pot)	Changes (%)
Control	355	
SS2	442	24.6
SS5	515	45.2
SS10	446	25.7
SSBC2	467	31.7
SSBC5	557	57.1
SSBC10	578	63.1

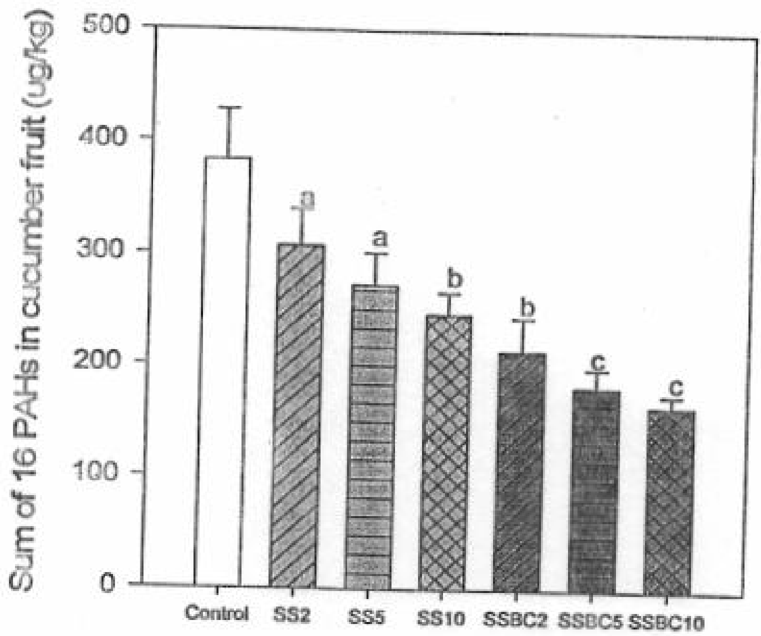


Fig. 5.8. Sum of 16PAHs in cucumber fruit grownincontrol soil (white)and soil amended with SS2 (grey hatched), SS5 (grey horizontal), SS10 (grey cross-hatched),SSBC2 (dark grey hatched), SSBC5 (dark grey horizontal), SSBC10 (dark greycross-hatched).Error bars represent standard deviations (n=4). Letters indicate significant difference ($P \leq 0.01$) from the control, while similar letters indicate no significant difference between the treatments.

Table 5.4

Mean concentrations of 2-3 rings PAH compounds (LMW-PAH) in cucumber fruit (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	Naphthalene		Acenathphene		Acenaphthylene		Fluorene		Phenanthrene		Anthracene	
	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	66.5		5.487		6.85		7.38		51.3		11.3	
SS2	58.9	-11.3	4.7892	-12.7	5.01	-26.8	6.07	-17.8	41.9	-18.4	9.30	-17.8
SS5	52.9	-20.4	3.892	-29.1	4.80	-29.9	6.03	-18.3	37.9	-26.2	8.86	-21.7
SS10	47.0	-29.2	3.502	-36.2	4.21	-38.6	5.69	-22.9	33.6	-34.5	8.62	-23.8
SSBC2	48.0	-27.8	2.6601	-51.5	3.07	-55.3	5.52	-25.3	29.1	-43.2	8.16	-27.9
SSBC5	42.7	-35.7	2.5845	-52.9	2.92	-57.4	4.17	-43.5	28.8	-43.9	7.79	-31.1
SSBC10	40.8	-38.5	2.3836	-56.6	2.77	-59.5	3.11	-57.8	27.9	-45.7	6.84	-39.5

Table 5.5

Mean concentrations of 4 ring PAH compounds in cucumber fruit (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	Fluoranthene		Pyrene		Benzo(b)anthracene		Chrysene	
	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	106		42.7		13.9		12.9	
SS2	89.3	-15.5	34.5	-19.2	10.2	-26.9	10.2	-21.1
SS5	82.4	-21.1	29.2	-31.6	8.16	-41.3	9.25	-28.6
SS10	79.6	-24.7	24.5	-42.8	7.53	-45.9	9.58	-26.0
SSBC2	59.5	-43.7	23.8	-44.4	5.34	-61.6	7.59	-41.4
SSBC5	48.5	-54.1	18.6	-56.5	5.07	-63.6	6.12	-52.7
SSBC10	46.1	-56.3	16.6	-61.1	4.05	-70.9	4.12	-68.3

Table 5.6

Mean concentrations of 5 ring PAH compounds in cucumber fruit (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	Benzo(b)fluoranthene		Benzo(k)fluoranthene		Benzo(b)pyrene	
	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	19.0		18.7		10.4	
SS2	12.0	-36.7	11.4	-38.92	7.96	-23.6
SS5	10.0	-47.2	9.89	-47.1	5.29	-49.2
SS10	8.04	-57.6	7.90	-57.8	4.08	-60.8
SSBC2	5.44	-71.3	5.43	-71.0	4.89	-53.1
SSBC5	3.46	-81.8	4.21	-77.5	3.30	-68.4
SSBC10	2.50	-86.8	2.86	-84.7	2.52	-75.8

Table 5.7

Mean concentrations of 6 ring PAH compounds in cucumber fruit (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	Indeno(1,2,3-c,d)pyrene		Dibenzo(a,h)anthracene		Benzo(g,h,i)perylene	
	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	4.11		3.48		3.71	
SS2	2.66	-35.3	2.46	-29.2	2.56	-31.1
SS5	2.20	-46.6	2.02	-42.0	1.86	-50.0
SS10	1.98	-51.9	1.40	-59.8	1.27	-65.9
SSBC2	1.98	-51.8	1.63	-53.2	1.49	-60.0
SSBC5	0.98	-76.1	1.21	-65.2	0.99	-73.3
SSBC10	0.78	-81.0	0.68	-80.3	0.59	-84.2

Table 5.8

Concentrations of toxic metals in cucumber fruit on dry weight basis. Increase or decrease changes due to SSBC and SS amendments are given in percentage.

Treatments	As		Cd		Cu		Pb		Zn	
	Conc. (µg/kg)	Changes (%)	Conc. ((µg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)
Control	349		271		2.82		0.52		85.1	
SS2	411	18.0	387	42.6	3.75	33.0	0.63	24.3	98.0	15.2
SS5	480	37.6	490	80.5	4.98	76.6	0.66	29.4	137	61.3
SS10	546	56.5	656	141.6	7.66	171.6	0.69	35.1	197	132
SSBC2	174	-50.2	381	40.5	2.26	-19.9	0.45	-10.7	84.5	-0.76
SSBC5	160	-54.3	448	65.1	2.13	-24.5	0.38	-25.6	79.7	-6.33
SSBC10	127	-63.6	469	72.6	1.25	-55.7	0.33	-36.0	76.6	-10.0
SEPA limits	50		200		20		9		100	

Chapter -6

Quantification of bioavailability and uptake of PAHs and toxic heavy metals in *Solanum lycopersicum* using sewage sludge biochar

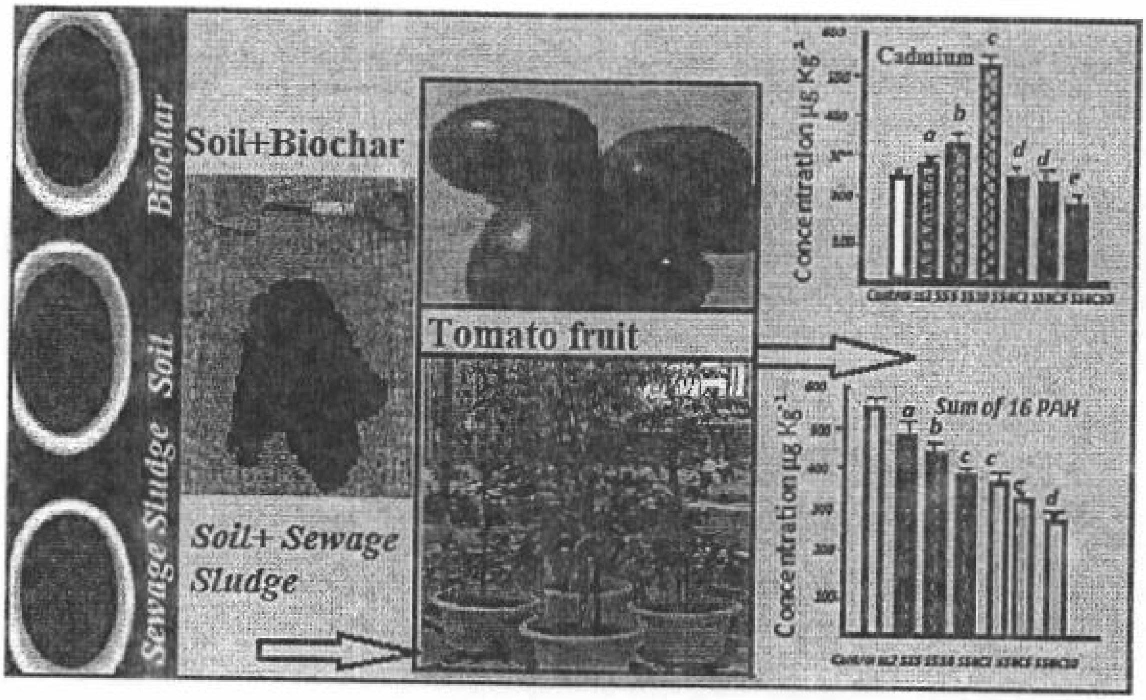


Fig.6.1 Graphical abstract of Tomato *(S. lycopersicum).*

6.1 Introduction

Unsustainable development creates numerous environmental problems including deterioration of air, water and soil quality along with depletion of natural resources. Sewage sludge (SS) production is tremendously increased with rapid urbanization and industrialization throughout the world. SS is mostly used for landfill, combustion, industrial utilization, composting or direct application into farmlands (Sán- chez Monedero and Mondini, 2004). Farmland application is the most sustainable use of SS was it improves soil fertility and productivity through increasing nutrients and organic matter concentrations (Roca-Pérez et al., 2009; Romanyà and Rovira, 2011).

Characteristically, SS contains high concentrations of toxic heavy metal(oid)s (THMs), organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Khan et al., 2013) and pathogens. The presence of these pollutants restricts the application of SS into agricultural lands. However, SS can be used as a biomass for pyrolysis to produce biochar (SSBC). Like other biochars, SSBC also enhances soil fertility due to presence of both macro and micro nutrients. However, SSBC sorptive capacity can reduce mobility and uptake of THMs and PAHs (Wang et al., 2011; Khan et al., 2013).

It is recommended that the relevance of biochar to soils that have elevated burdens of metals and HOCs may decrease problems linked with their toxicity and their transfer into the human food chain. This situation is substantial in Pakistan where rapid urbanization has lead to contamination of urban soil with high levels of metals and HOCs particularly, PAHs which is extensively used for agricultural purpose.

This research provides a direct comparison of sewage sludge (SS) and sewage sludge biochar (SSBC) effectiveness upon crop yields and to immobilize metals and reduce the bioavailability of PAH into tomato plants grown in contaminated soil.

6.2 Materials and methods

6.2.1 Soil characterization

The soil was collected from surface layer of THM and PAH contaminated site present in Fujian, China. Soil was sieved (2 mm mesh) and thoroughly homogenized. A 50 g sub-sample was dried at -50±3°Cat22 ±3 Pa and then stored in the freezer at -20°C. Soil properties such as electrical conductivity (EC), pH, surface area, porosity nitrogen (N), carbon (C),sulfur (S) and THMs were measured (Table 6.1), while initial concentrations of PAHs in controland SS/SSBC amended soils are shown in Fig. 6.2.

6.2.2 Preparation and characterization of biochar

Moisture containing SS samples were obtained from wastewater treatment plant (called Bingbei)present in Xiamen, China. The wet SS was dried in air. SSBC was prepared according to the method mentioned in our previous work (Waqas et al., 2013). Briefly, the dried SS was pyrolysed using 550°C temperature for 8 h in the presence of nitrogen. The SSBC was cooled inside the furnace to room temperature and then removed to pass through 2 mm mesh. Sub samples of SS and SSBC were used to determine the basic characteristics including pH, EC, N, C, S, surface area and porosity(Table 6.1).

6.2.3 Pot experiment in greenhouse

The contaminated soil was amended with prepared (<2 mm) SS or SSBC materials at different application rates (2, 5 and 10% on dry weight basis). The SS amended treatments were defined as SS2, SS5 and SS10, respectively, while SSBC treatments were defined as SSBC2, SSBC5 and SSBC10, respectively. The soil without SS and SSBC was also included as a control treatment. The plastic pots were filled with these treatments and replicated 4 times. These pots were irrigated with deionized water and cultivated with the seedlings (n=2) of tomatoes (S. lycopersicum) after 7 d. After one week, these seedlings were reduced to one in each pot. The pots were kept in the control environment of a greenhouse and irrigation was done with deionized water. The ammonium nitrate solution (1 g per 100 ml) was used twice to fertilize the plants. The mature fruits of tomatoes were collected from each treatment and their fresh weightswere recorded. Fresh samples were dried using vacuum freez drier. The dried samples were powdered and stored in paper sacks at -20°C for further analyses.

6.2.4 Chemical analyses

The soil, SSBC, SS and the SSBC/SS amended samples were assessed for total THM concentrations using HNO_3 and HCl mix acid extraction procedure (Khan et al., 2008). Powdered sample (0.5 g) of tomato fruits were extracted with 3 ml of HNO_3 and H_2O_2(1:1 v/v) using microwave accelerated reaction system (MARS) (CEM-Mars,V. 194A05). The finally extracted suspensionswere filtered through membrane filter and the filtrate was diluted (50 mL) with Milli-Q water. The EDTA was used to extract available fraction of THMs in the soil, SS, SSBC and the SSBC/SS amended treatments (Zeng et al., 2011). The concentrations of THMswere determined using ICP-OES (Perkin Elmer Optima 7000 DV, USA) and ICP-MS (Agilent Technologies, 7500 CX, USA).

Total concentrations of 16 US-EPA PAH (Σ16PAH) in soil, SS, SSBC, SSBC/SS and the fruits of tomato were extracted with DCM and acetone (1:1 ratio) as a mix solvents using accelerated solvent extraction system (ASE, Dionex-350). The extracted solvents were reduced to 1 mL using rotary evaporation system and then used the silica chromatography columns to purify these samples according to method mentioned by Khan et al. (2008). The final refined sample (1 mL) was stored in a vial (2 mL) and used for PAH analysis.

A hot water (Milli Q) extraction was done using ASE to assess available PAH concentrations in both control and mended soils (Latawiec and Reid, 2009). Briefly, quartz sand (25-50 mesh, Sinoparma) was added to samples to ensure properdispersion of sample matrices. After filling the ASE cells, the program was set as 1) 200°Cextractiontemperature 2) static time 10 min and 3) flush volume 20%. The cooled extracts weretransferred to 200 mL separating funnels and further extracted with DCM (Latawiec and Reid, 2009). All extracts were quantified for PAHs using GC-MS (Agilent Technologies 5975C).Furthermore, available NO_3-N, NH_4-N, P (Colwell P) and dissolved organic carbon (DOC) were also analyzed in the samples.

6.2.5 Quality control and data analysis

In order to check the efficiency of ASE extraction and silica column purification for PAH recovery, surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10) were used.Reagent blanks, plant and soil standard reference materials were included to check PTE recovery. The obtained data were statistically analyzed using the statistical package SPSS 11.5.

6.3 Results and discussion

6.3.1Quantification of PAH/PTE changes in SS and SSBC materials

The pyrolysis changed the concentrations of PAHs in the SSBC as compared to SS. The total concentration of Σ16PAHs (5.78 mg kg^{-1}) in SS was below the maximumpermissible limits (MPL) (Σ9PAHs, 6 mg kg^{-1}) set by the Council of the European Community (CEC, 2000). Similarly, Σ16PAHconcentration (1.70 mg kg^{-1}) in SSBC was observed within the MPL (6-20 $mgkg^{-1}$) set by International Biochar Initiative (IBI, 2012). These findings show that the pyrolysis significantly ($P \leq 0.01$) reduced PAH concentrations in SSBC. In this study, the sum of 16PAHs was reduced approximately four times under selected pyrolysis conditions. This decrease in PAH concentration also depends on the type of SS.Previously, the biochars prepared from different feedstock materials have shown different levels of PAHs dependent upon the types of feedstocks and pyrolsis conditions (Freddo et al., 2012). Long pyrolysis times and higher temperatures have been observed to reduce PAH concentrations in biochar (Freddo et al., 2012; Hale et al., 2012). The concentrations of PAHs in SS depend upon on the type of wastewater treated (Dai et al., 2007). Previous studies have been reported that SS contain variable concentrations of PAHs (ranged from 2-36 $mgkg^{-1}$) (Baran et al.,

2003) but the SS used in the present research has a relatively lower concentration of PAH (5.78 $mgkg^{-1}$). The PAH loadings and their compound profile in different SSBCs are depended on the PAH loadings in SS materials used in pyrolysis. The results indicate that SS exceeding permissible limits for these pollutants can be used to produce SSBC. However, further research work is needed to investigate the suitable pyrolysis conditions and the PAH concentrations in SSBC prepared from different SS feedstock.

The total concentrations of individual PAHs were greatly varied in SS and SSBC materials used (Fig. 6.5). In SS materials phenanthrene was the dominant compound followed by Benzo(b)fluoranthene, while SSBC materials naphthalene, phenanthrene, fluoranthene and pyrene were dominant compounds (Fig. 6.5).

Table 6.1 summarizes the total THM concentrations in SSBC and SS which were significantly ($P<0.01$) higher in SSBC as compared to SS. The results indicate that THM concentrations were increased during pyrolysis which was also observed in different studies (Khan et al., 2013). This increase is to be expected because THMs are concentrated in SSBC during pyrolysis and large amount of SS mass is converted to liquid and gaseous fuels (Fagbemi et al., 2001).

However, available concentrations of THMs were greatly reduced in SSBC (Table 6.1). These results confirm that the pyrolysis is a good opportunity to convert high risks SS in to lower risk SSBC which has lesser concentrations of available THMs. These lower available THMs in the SSBC amended soil can reduce uptake rate in plants.

6.3.2 Quantification of changes in soil PAHs

Table 1 summarizes the basic characteristic of SS and SSBC used in this research,. SS and SSBC amendments changed the basic soil characteristics including pH, DOC, EC, NH_4-N, P, NO_3-N, pore size and surface area (Table 6.2).

The soil of this research was highly contaminated with PAHs (Σ16PAH = 1050.85μg kg^{-}

^{1}) and the concentration of seven mutagenic PAHs (Σ7PAHs = 5.49 mg kg^{-1}) exceeded the MPL(4.23 mg kg^{-1}) set by the Ministry of Environmental Protection, China (MEP, 2007). The soil amendments with SS and SSBC also diluted the concentration of Σ16PAH because of lower concentration of PAHs present in these materials. The results indicate that this dilution significantly ($P<0.05$) changed the concentration of PAHs in the SS10 and all SSBC amended soils.

Figure 6.2 shows that the sum of available PAH (Σ16PAH) concentrations were significantly reduced in SSBC treatments as compared to control. The available Σ16PAH concentrations in the SSBC2, SSBC5 and SSBC10treatments were reduced by 29.98, 40.17 and 47.27%, respectively as compared to the control treatment. The available concentrations of PAHs (Σ16PAH)wereonly significantly($P<0.05$) reduced (29.32%)in the SS10 treatment but not in SS2 and SS5 treatments (Fig. 6.2A).

The concentrations of PAHs in SS and SSBC were lesser than those observed in the soil used in this study, therefore, the SS10 amendment had caused dilution 3%, while SSBC10 amendment diluted by 8% (calculated on the basis of PAH concentrations in SS, SSBC and soil samples). The above-mentioned results indicate that the reduction in available PAH concentrations in the SSBC and SS amended soils was greater than the reduction attributed to SSBC and SS dilution alone. It means that the changes in PAH concentrations as reported above indicate PAH compounds re-partitioning not simply a dilution effect.

Figure 6.2B shows the concentrations of different PAH compounds present in SS and SSBC amended soils. SSBC amendment significantly ($P\leq0.05$) decreased the concentrations of different PAHs. Highest decrease was found for SSBC10 amendment. The data show that theeffects of SSBC on available fractions LMWPAHs (2-3 rings) was lower than those observed for HMW-PAHs (4-6 rings). This lower reduction in availability of LMWPAHs could be attributed with their greatermobility as compared to HMWPAHs (Xing and

Pignatello, 1996).Furthermore, the decrease in available PAHs in SS amendments (2 and 5%) was not significant (LMWPAHs and HMWPAHs in SS2 and only LMWPAHs in SS5). However, SS10 amendment significantly decreased both LMW and HMWPAHs in the amended soil (Fig. 6.2B).

There have only been a few studies evaluating the effects of biochar on bioavailable concentrations of PAHs (Oleszczuk et al., 2012; Khan et al., 2013). The results reported in this research are in in-keeping with Oleszczuk et al. (2012) in so much as both investigations have shown biochar to reduce PAH bioavailability and that this reduction is most considerable for HMW-PAHs. A clear difference was observed between the effects of SSBC and SS on available PAHs in soils. Mass transfer from soil to SSBC and SS materials depended on concentrations of compounds and their nature. Among individual PAHs, higher mass transfer was observed for HMWPAH, particularly, 5- and 6-rings containing PAH compounds. PAH affinity forC containing materials (biochars) depends on their properties such as pH, surface area, elemental composition and structure. These properties are governed by pyrolysis conditions (temperature and time) and types of feedstocks (Hale et al., 2012; Keiluweit et al., 2012) which in turn control biochar sorptionbehavior for PAH compounds. The SSBC and SS materials used in this study had different EC, pH, pore volume, surface area and porosity (Table 6.1) which may be responsible for different sorption rates of PAHs by these materials.

The sorption ofPAHs and their further sequestration into SSBC and SSmaterials occur through several means including π-electroninteractions,hydrophobic effect and porefilling mechanisms (Chen et al., 2008).These mechanisms could be contributed differently in the SS and SSBC materials because of their different nature and characteristics.

6.3.3 *Quantification of changes in soil PTEs*

Table 1 summarizes the PTE concentrations in the soil used in this research. Zn and Cd concentrations were exceeded the MPLs (250 $mgkg^{-1}$ and 0.3 $mgkg^{-1}$, respectively) set by the State Environmental ProtectionAdministration, China (SEPA, 1995), while As, Cu and Pb concentrations were lower than their resptive MPL limits(Table 6.1).

Table 6.2 summarizes the effects of SSBC and SS materials on availability of the selected PTEs. Among PTEs the available concentrations of As (56.52-65.2%), Pb (14.22-54.13%), Cu (2.5-45.83%) and Zn (6.92-17.21%) were decreased in SSBC amended soil, while concentration of Cdwas slightly (2.5-12.82%)increased than the control soil. This decrease in available PTEs (except Zn)was significant($P \leq 0.05$) at higher application rates (SSBC5 and SSBC10%).

The addition of SS significantly ($P<0.001$) increased the available concentrations of PTEs (As, Cd, Cu and Zn) than the control soil(Table 6.2). The available concentration of As was shown highest increase (55.17-204.53%) followed by Cu (47.45-140.28%). The available concentrations of Cd and Znwereranged from 64.10-74.36% and 7.48-132.70%, respectively;while lowest increase (4.98-8.32%) was shown by Pb. In SS amended soil, increase in available PTE concentrations was increased with increasing dose of SS (Table 6.2).

Characteristically SS has the tendency to increase PTE concentrations in the amended soil. The results of this research indicate that SS addition to soil increased the available concentrations of the selected PTEs. However, once SSBC produced from SS then the PTE availability was decreased. These findings are agree with several research studies that have reported a decrease in available concentrations of metals due to addition of biochars in to contaminated soils (Lehmann, et al., 2011; Ahmad et al., 2012). The decrease in available PTE concentrations in biochar amended soilscould be linked with the changes in pH of soil,DOC, cation exchange values and oxygen-functional-groups(Kookanaet al., 2011; Zheng et al., 2012). In the past, numerous research works have been confirmed that these oxygen-functional-groupsform complexes with PTEs to reduce their mobility and bioaccumulation and have shown more effectiveness in suppression of metals (Jiang and Xu, 2013).

6.3.4 Quantification of plant biomass

The addition of SSBC significantly ($P \leq 0.01$) increased the fruit yields as compared to control. The increases in yields were counted for 30.5, 58.42 and 51.6% in SSBC2, SSBC5 and SSBC10 amended soils, respectively (Fig. 6.6). In SS amendments, fruit biomass was also significantly ($P \leq 0.05$) increased as compared to the control soil (Table6.3) but no significant difference was observed between the highestapplicationrate (10%) of SS and SS2. The high nutrient contents particularly NH_4-N and NO_3-N in SSBC and SS amended soil(Table 6.2) could be given stimulus for plant growth and high fruit yield (Clough and Condron, 2010). As mentioned earlier, the SS material and SS amended soil hadcontained higher available PTE concentrations as compared to SSBC material and SSBC amended soils (Table 6.1-2), thus, the SS10 (the highest application rate)may have caused a negative effect on biomass yield that overcome the gains bacuse of increases the nutrient concentrations in plants. Thedata show that SSBC material has higher influence over SS for enhancing tomato fruityeild.

6.3.5 Quantification of PAH bioaccumulation

Figure 6.3 shows the concentrations of 16 PAHs observed in tomato fruitsgrown in SSBC and SS amended and control soils. SSBC addition reduced Σ16PAH (47.97-62.37%)in tomato fruits and this decrease was significantly ($P<0.01$) lower as compared to control. The addition of SS less effectively but stillsignificantly ($P\leq0.05$) reduced the accumulation of Σ16PAH (21.47-39.32%). The decrease in bioaccumulation of Σ16PAHwas increased with increasing doses of SS and SSBC materials (Fig. 6.7). Thus the maximum reduction in PAHaccumulation was detected in the SSBC10 and SS10 treatments (Fig. 6.7).

The addition of SSBC was shown greater influence than SS addition in term of PAH accumulation in tomato (Fig. 6.3). The SSBC effectiveness was greater than SS at all selected application rates (2, 5 and 10%)at reducing the accumulation of PAHs in tomomato fruits. This effect was clear with data points fitted with the upper left portion of the frame (Fig.6.3).The decrease of PAH bioaccumulation of selected individual PAHs was > 40% in SSBC2 treatments (except for naphthalene, fluorene and anthracene), while this decrease was < 30% in SS2 treatments (except for benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-c,d)pyrene, and benzo(g,h,i)perylene (Fig. 6.3A)). Except for naphthalene, fluorene and anthracene, SSBC5 addition reduced PAH accumulation by > 50% in tomato, while SS5 addition this decrease was <30% (except for benzo(a)pyrene, 5 and 6-ring PAHs reduced more than 40%) (Fig. 6.3B). Figure6.2 shows that SSBC10 influence on decreasing PAH accumulation was again higher than SS10. Farthermore, thisdecrease in accumulation for HMW-PAHs was greater than LMW-PAH (Fig. 6.3).Characteristically, these HMW-PAHshave lower availability; therefore, their accumulation was greatly decreased by addition of SSBC than LMW-PAHs (Papadopoulos et al., 2007).

This decrease in accumulation of PAH compounds in tomato fruits were agreed with the findings reported in our previous work (Waqas et al., 2013). In SSBC amended soil, the lower

PAH accumulation in tomato fruits as compared to SS amended soil could be associated with the reduced PAH concentrations in the soil water. These findings demonstrated that the addition of SSBCin to contaminated soil can reduce the health risk associated with PAHs uptake and transfer in to food chains.

The basic properties of SSBC (structure and element composition) are different than those observed for SS which play an essential role to specify the sorptive capacity of these selected materials. These results are agreed with the findings reported by our previous research (Waqas et al., 2013) and also with other authors (Beesley et al., 2010; Chen and Yuan, 2011). The effectiveness of Biochar on PAH availability depends on ageing/contact time (Cao et al., 2011).

6.3.6 Quantification of PTE bioaccumulation

Figure 4 shows the overall effects of SSBC and SS addition of accumulation of THMs in tomato fruits. The addition of SSBC significantly ($P \leq 0.01$)decreased the accumulation of Cu, As and Pb in tomato fruits than the control. This decrease was not significant for Zn accumulation in tomato fruits (Fig.6.4). Cd concentration in the tomato fruits wassignificantly ($P \leq 0.05$)increased by addition of SSBC as compared to control.

The bioaccumulation of THMs were significantly ($P \leq 0.05$)increasedin tomato grown in SS amended soils as compared to the control (Table 6.8). The concentrations of As were increased from 411.45-537.87$\mu g kg^{-1}$ which were significantly ($P \leq 0.05$) higher than the control (328 $\mu g kg^{-1}$). The concentrations of As were exceeded the MPLs (0.05 $mg kg^{-1}$ dw) set for food by SEPA (2005).SSBC addition markedly reduced the concentration of As (132.51 $\mu g kg^{-1}$ in the SSBC10 treatments) but could not to MPL (0.05 $mg kg^{-1}$) set by SEPA (2005). Furthermore, Cd concentrations were significantly increased in tomatoes grown on both SS and SSBC amended soils which exceeded the MPL (0.2 $mg kg^{-1}$ dw) set by SEPA (2005). In SS amended soils, Zn concentration was significantly ($P \leq 0.05$) higher in tomato grownin SS amended soil which exceeded the MPL (100 mgkg^{-1} dw) set by SEPA (2005). Cd and Zn concentrations in the soil used in this study were exceeded the MPL (0.3 and 300 mgkg^{-1}, respectively) set for agricultural sector by SEPA (1995) (Table 6.1). These findings show that the soil contaminated with high concentrations of THMs may still pose risk through soil-to-food transfer of toxic metals even after the addition of SSBC and SS.

In case of SSBC, the higher pore volume,higher surface area and smaller pore size of are contributed to reduce the availability of THMs.DOC concentrationsmay have an important role in decreasing the availability and uptake rate of THMs in plants. In SS amended soil DOC was also increased but the accumulationof THMs increased. This could be linked with the weak bondspresent between SS-DOC and metals than SSBC-DOC and metals. Therefore, these metals more easily released from soil to soil solution which further available for plant uptake even DOC are present at elevated level. The different basic properties of SSBC such as higher surface area, higher pore volume and smaller pore size would also be contributed to reduce metal availability. In the SSBC amended soils, the concentrations of P and S were higher than control (Table 6.2). These elements strongly interact and affect the bioaccumulation and uptake of Asby plants because of the same transporting mechanisms (Meharg and Macnair, 1992). SS addition also increased the concentrations of these elements in the amended soils but these were not reduced the availability and upatke of As. The different basic characteristics (both physical and chemical) of SS increase the availability and uptake of THMs in tomato. On the other hand, SSBC addition decreased the availability and accumulation of As, Cu, Pb and Zn (Uchimiya et al., 2011a) which could be linked with increasing pH of soil, higher availability of P and S, compleaxtion of metals and oxygen-functional-groups (Lehmann and Joseph, 2009).

6.4 Conclusions

On the basis of findings it is concluded that theconversion of SS to SSBC was effective in reducing the concentrations of PAHs and availability of THMs. SSBC amendment was far more effective in reducing the availability and accumulation of PAHs and THMs than SS.Pyrolysis for SSBC production killed the pathogens associated with SS, therefore, SSBC also provides additional benefits in terms of mitigating pathogen transfer to food. SSBC is a promising alternative of SS for amendment of PAH and THMs contaminated soil.

Table 6.1 Characteristics of SS and SSBC used for soil amendments in this study

[a]Permissible limits set for sewage sludge by the United States Environmental Protection Agency(USEPA, 1995)
[b]Permissible limits set for soil pH>6.5 by the State Environmental Protection Administration(SEPA, 1995)
[c]Soil background values taken from Lu et al. (2007) for Fujian province, China

Parameters	SS	SSBC	Soil	Permissible limits		Back ground[c] soil
				USEPA[a] (sludge)	SEPA[b] (soil)	
pH(H_2O)	6.64	7.40	7.25			
EC($mScm^{-1}$)	10.15	6.38	0.44			
N (%)	3.61	2.71	0.16			
C (%)	27.26	21.64	5.98			
S (%)	2.44	2.11	0.08			
NH_4-N (mg kg^{-1})	39.37	12.42	9.03			
NO_3-N (mg kg^{-1})	2.71	4.03	1.31			
TOC(mg kg^{-1})	11063	383.05	431.3			
BET Surface area(m^2g^{-1})	2.15	3.97	8.81			
Pore volume (cm^3g^{-1})	0.008	0.03	0.03			
Pore size (nm)	14.58	9.31	8.41			
Total metal concentrations (mgkg^{-1} dw)						
As	2.31	11.93	4.71	75	6.3	40
Cd	1.53	2.32	1.01	85	0.6	0.3
Cu	170.69	237.08	43.90	4300	100	50
Pb	53.79	71.85	142.34	840	350	250
Zn	1105.6	1878.9	421.33	7500	300	200
Bioavailable metal concentrations (mgkg^{-1}dw)						
As	1.07	0.05				
Cd	1.03	0.17				
Cu	35.31	4.35				
Pb	9.02	3.41				
Zn	387.13	56.71				

Table 6.2

Physical and chemical properties of soil and soil amended with SS and SSBC. Mean values are shown ±1 standard deviation (n =4).

Parameters	Control	SS_2	SS_5	SS_{10}	$SSBC_2$	$SSBC_5$	$SSBC_{10}$
			Basic characteristics				
pH	7.25	7.19	7.13	7.01	7.27	7.30	7.36
EC ($mScm^{-1}$)	0.39	0.81	1.32	1.71	0.49	0.79	1.26
N (%)	0.14	0.77	1.85	2.68	0.18	1.37	1.55
C (%)	5.08	8.35	13.56	20.03	4.92	12.81	19.32
S (%)	0.06	0.40	1.11	1.72	0.12	1.13	2.09
P	15.34	22.60	35.08	44.81	16.21	27.03	37.08
NH_4-N ($mgkg^{-1}$)	8.93	34.23	43.15	54.23	16.23	35.31	42.36
NO_3-N ($mgkg^{-1}$)	1.47	44.34	23.61	13.69	100.44	43.21	37.12
DOC ($mgkg^{-1}$)	361.21	1763.5	3989.0	5720.0	360.4	504.51	613.0
BET Surface area(m^2g^{-1})	8.71	7.55	7.31	6.41	7.71	7.58	6.61
Pore volume (cm^3g^{-1})	0.02	0.020291	0.020931	0.018166	0.0201135	0.021044	0.019426
Pore size (nm)	9.39	10.53	11.71	11.88	10.73	11.88	12.45
			Available PAHs (μgkg^{-1} dw)				
NA	141.43	127.35	119.53	105.13	110.71	85.12	79.87
CAN	30.32	24.73	22.31	20.44	19.28	18.72	17.73
AC	27.36	24.31	23.01	22.91	20.11	19.33	17.05
FI	32.36	27.01	25.04	24.01	21.66	19.86	18.56
PHE	150.32	145.01	138.34	127.01	130.31	119.98	104.51
AN	43.01	32.07	26.76	27.67	28.34	24.34	22.53
FLA	263.27	242.7	211.27	198.23	189.52	158.80	141.67
PY	123.28	102.38	96.05	73.12	76.84	59.01	50.04
B(b)A	37.21	30.84	28.82	26.21	22.01	20.01	18.07
CHR	36.21	29.43	26.32	24.98	23.01	20.65	16.87
B(b)F	48.98	40.21	36.23	34.74	25.32	23.31	20.61
B(k)F	48.91	40.5	34.45	21.03	30.63	24.56	19.12
B(a)P	28.35	23.22	18.98	17.75	17.09	16.63	11.93
I(1,2,3-c,d)P	14.31	11.97	8.03	6.32	7.01	5.89	5.32
D(a,h)A	13.01	10.01	9.33	8.01	7.41	6.32	5.31
B(g,h,i)P	12.52	9.06	7.59	5.21	6.52	6.20	4.98
2 rings PAH	141.43	127.35	119.53	105.13	110.71	85.12	79.87
3 rings PAH	283.37	253.13	235.46	222.04	219.70	202.23	180.38
4 rings PAH	459.97	405.35	362.46	322.54	311.38	258.47	226.65
5 rings PAH	126.24	103.93	89.66	73.52	73.04	64.5	51.66
	39.84	31.04	24.95	19.54	20.94	18.41	15.61
$\sum$ of 16 PAHs	1050.85	920.8	832.06	742.77	735.77	628.73	554.17
			Bioavailable heavy metals ($mgkg^{-1}$ dw)				
As	0.23	0.35	0.6	0.7	0.1	0.09	0.08
Cd	0.39	0.68	0.64	0.63	0.40	0.42	0.44
Cu	4.32	6.37	7.03	10.38	4.21	3.32	2.34
Pb	20.32	21.32	21.71	22.01	17.39	14.32	9.32
Zn	30.21	32.47	59.32	70.30	28.12	26.34	25.01

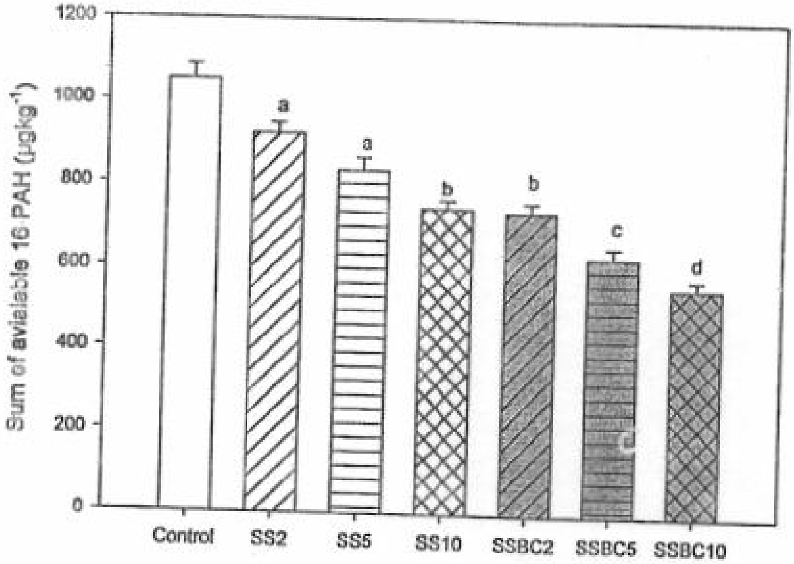


Fig.6.2. PAHs in the control soil and soil amended with SSBC and SS: A) supercritical water extracted Σ16PAHs. Error bars represent standard deviations (n=4). Different letters on the bars indicate significant difference (P≤0.05), while similar letters indicate no significant difference between the treatments.

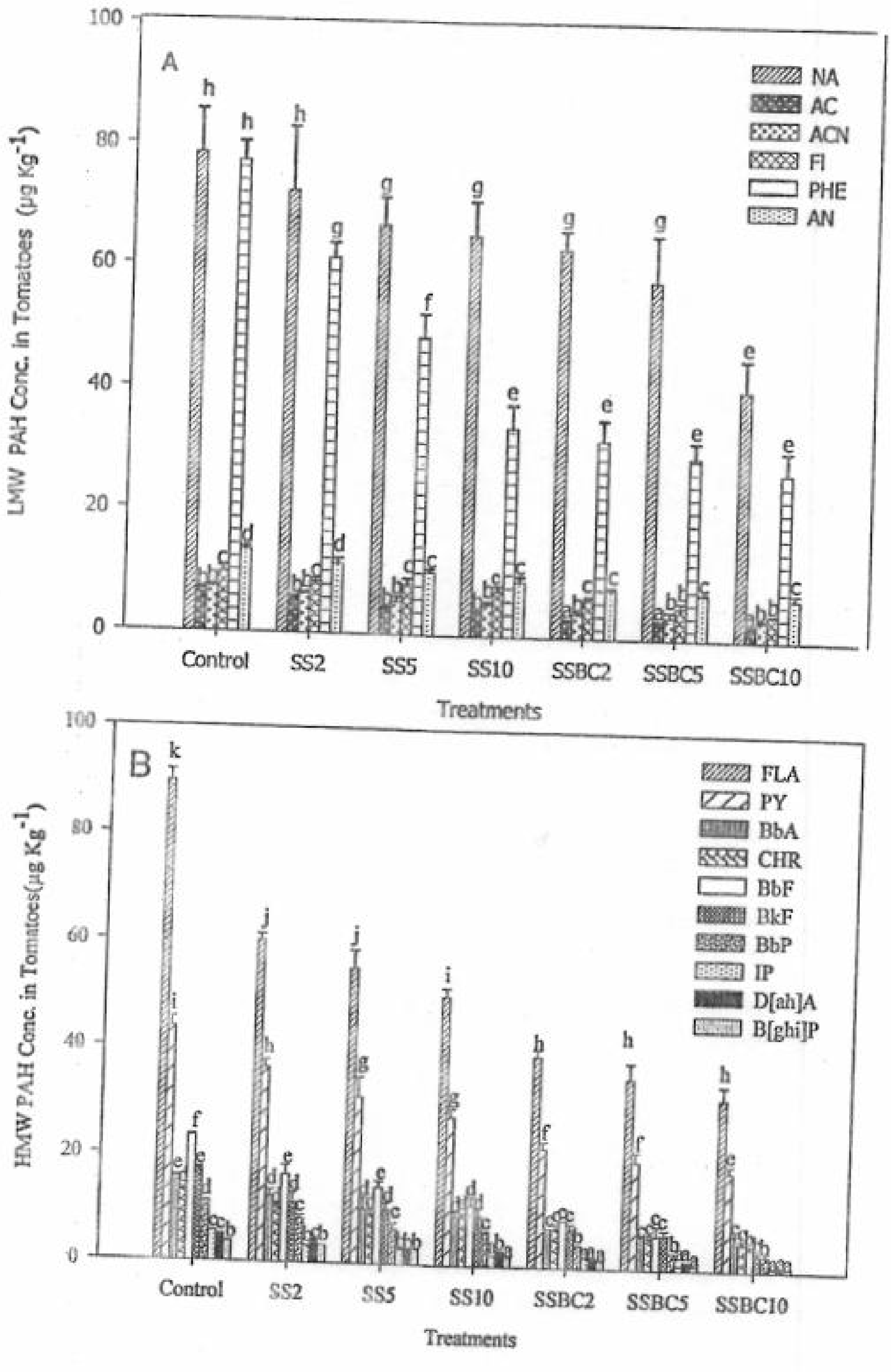


Fig.6.3Bioaccumulation of PAH compounds in Tomatoesgrownin soil and soil amended with SSBC and SS. A) LMW-PAH compounds, B) HMW-PAH compounds.Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤0.05), while same letters indicate no significant difference between the treatments.

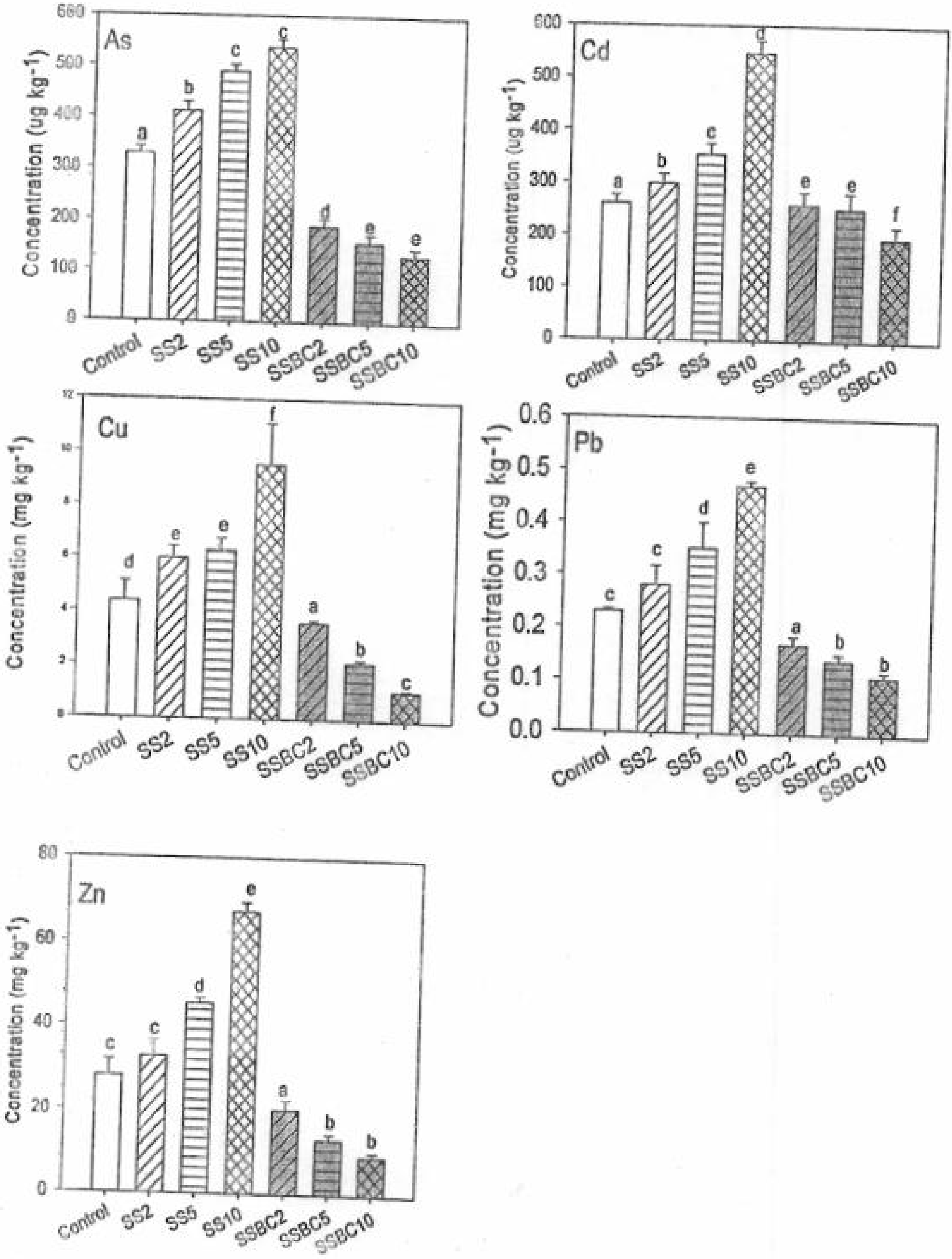


Fig.6.4 Bioaccumulation of PTEsin tomatoes grown in soil and soil amended with SS and SSBC. Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤0.05), while same letters indicate no significant difference.

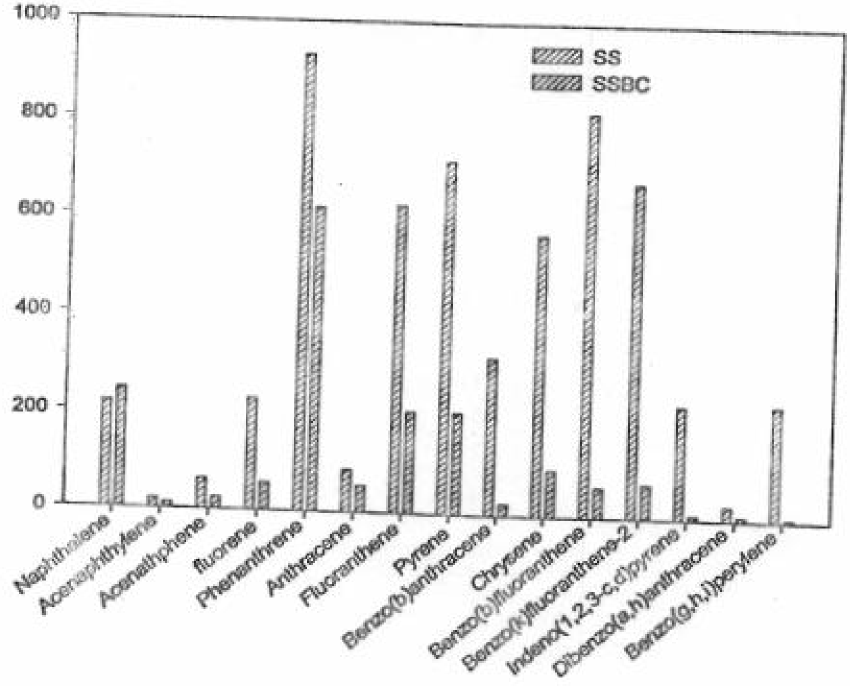


Fig.6.5 Total concentrations of PAHs in SSBC and SS materials used in this study.

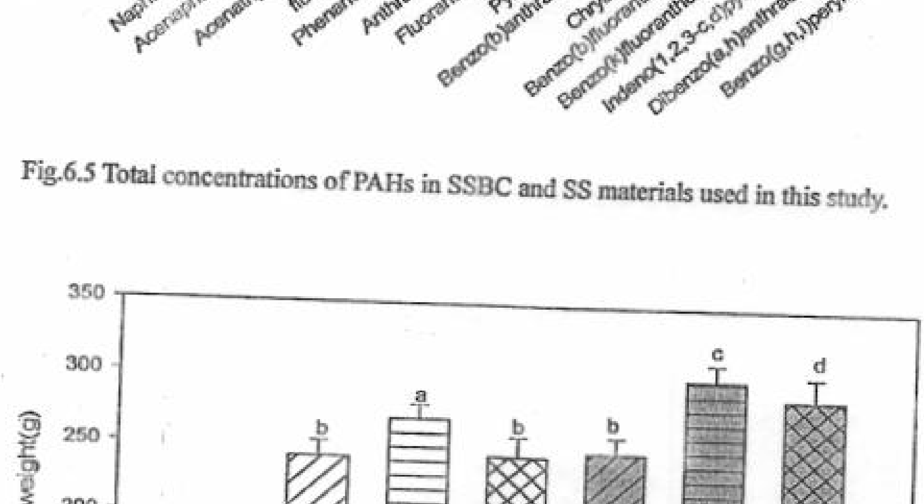


Fig.6.6Fresh weight (g/pot) of Tomatoes grown on control, SS and SSBC treatments. Error bars represent standard deviations (n=4). Different letters indicate significant difference ($P \leq 0.05$), while similar letters indicate no significant differenc

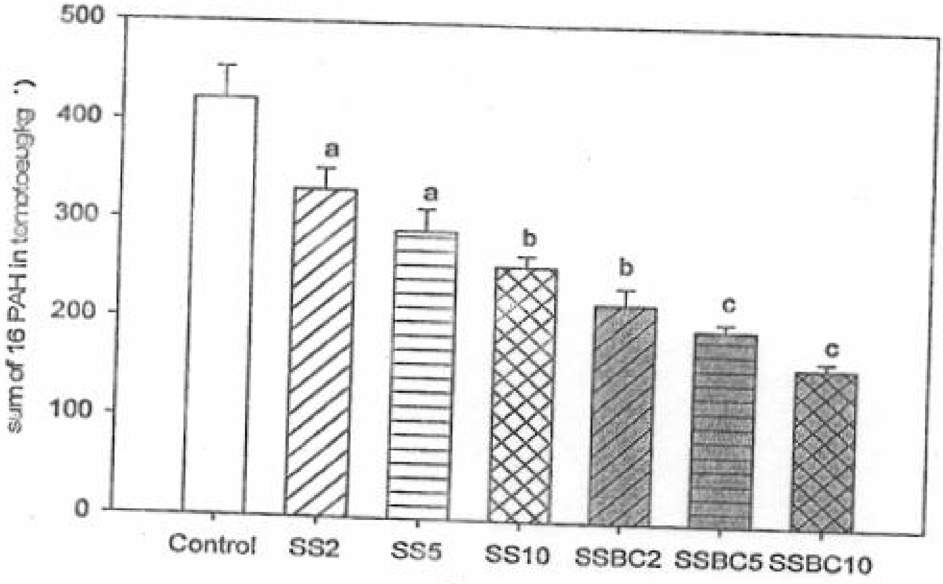


Fig. 6.7 Sum of 16PAHs in tomatoesgrown on control, SSBC and SS mended soil. Error bars represent standard deviations (n=4). Different letters indicate significant difference ($P \leq 0.01$), while similar letters indicate no significant difference.

Table 6.3

Mean fresh weight (g/pot) of tomatoes yield (n=4) grown on control, SSBC and SS amended soil. Increase changes due to SSBC and SS addition are given in percentage.

Treatments	Tomatoes biomass	
	Fresh weight (g/pot)	Changes (%)
Control	189.5	
SS2	241.31	27.34
SS5	269.14	42.03
SS10	243.51	28.50
SSBC2	247.37	30.54
SSBC5	300.21	58.42
SSBC10	287.31	51.61

Table 6.4

Mean concentrations of different 2-3 rings PAH compounds (LMW-PAH) in tomatoes (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	NA		ACN		AC		Fl		PHE		AN	
	Conc. (µgkg-1)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	78.45		6.87		7.23		9.7		77.23		13.4	
SS2	72.32	-9.11	5.73	-16.59	6.45	-10.79	8.32	-14.23	61.34	-20.57	11.32	-15.52
SS5	66.71	-16.21	4.32	-37.12	5.43	-24.90	8.32	-14.23	48.63	-37.03	10.23	-23.66
SS10	65.21	-22.71	4.21	-38.72	5.31	-26.56	7.45	-23.20	34.15	-55.78	8.98	-32.99
SSBC2	63.23	-24.45	2.98	-56.62	4.02	-44.40	6.56	-32.37	32.32	-58.15	7.98	-40.45
SSBC5	58.21	-26.74	2.56	-62.74	3.67	-49.24	5.32	-45.15	29.67	-61.58	7.45	-44.40
SSBC10	40.8207	-32.56	2.32	-66.23	3.27	-54.77	4.23	-56.39	27.34	-64.60	6.83	-49.03

Table 6.5

Mean concentrations of different 4 ring PAH compounds in tomatoes (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	FLA		PY		B(b)A		Chrysene	
	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)	Conc. (µg/kg)	Changes (%)
Control	105.6177		42.7343		13.9031		12.9464	
SS2	89.2542	-15.49	34.5236	-19.21	10.1584	-26.93	10.2097	-21.14
SS5	82.3822	-21.10	29.2358	-31.59	8.1584	-41.32	9.2497	-28.55
SS10	79.5516	-24.68	24.4542	-42.78	7.5289	-45.85	9.5774	-26.02
SSBC2	59.4537	-43.71	23.7782	-44.36	5.336	-61.62	7.592	-41.36
SSBC5	48.5191	-54.06	18.6129	-56.45	5.0683	-63.55	6.1214	-52.72
SSBC10	46.1233	-56.33	16.6339	-61.08	4.0515	-70.86	4.1076	-68.27

Table 6.6

Mean concentrations of different 5 ring PAH compounds in tomatoes (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	B(b)F		B(k)F		B(b)P	
	Conc. (μg/kg)	Changes (%)	Conc. (μg/kg)	Changes (%)	Conc. (μg/kg)	Changes (%)
Control	18.975		18.702		10.4143	
SS2	12.0107	-36.70	11.4233	-38.92	7.9581	-23.58
SS5	10.0107	-47.24	9.8923	-47.11	5.2906	-49.20
SS10	8.0454	-57.60	7.8992	-57.76	4.0846	-60.78
SSBC2	5.4373	-71.34	5.4321	-70.95	4.8857	-53.09
SSBC5	3.4620	-81.75	4.2056	-77.51	3.2962	-68.35
SSBC10	2.5024	-86.81	2.860	-84.71	2.5185	-75.82

Table 6.7

Mean concentrations of different 6 ring PAH compounds tomatoes (n=4) on dry weight basis. Changes due to SSBC and SS amendments are given in percentage.

Treatments	I(1,2,3-c,d)P		D(a,h)A		B(g,h,i)P	
	Conc. (μg/kg)	Changes (%)	Conc. (μg/kg)	Changes (%)	Conc. (μg/kg)	Changes (%)
Control	4.112		3.4780		3.7110	
SS2	2.6597	-35.33	2.4620	-29.21	2.5575	-31.08
SS5	2.1972	-46.58	2.0162	-42.03	1.8557	-49.99
SS10	1.9775	-51.92	1.3978	-59.81	1.2672	-65.85
SSBC2	1.9808	-51.84	1.6280	-53.19	1.4851	-59.98
SSBC5	0.9847	-76.06	1.2117	-65.16	0.9895	-73.34
SSBC10	0.7812	-81.01	0.6843	-80.32	0.5873	-84.17

Table 6.8

Concentrations of toxic metals in Tomatoes on dry weight basis. Increase or decrease changes due to SSBC and SS amendments are given in percentage.

Treatments	As		Cd		Cu		Pb		Zn	
	Conc. (μg/kg)	Changes (%)	Conc. ((μg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)	Conc. (mg/kg)	Changes (%)
Control	348.79		260.34		2.82		0.5179		85.11	
SS2	411.42	17.96	298.53	14.67	3.75	32.98	0.6313	24.30	98.03	15.18
SS5	479.93	37.60	354.31	36.10	4.98	76.60	0.6573	29.42	137.25	61.26
SS10	545.92	56.52	547.31	110.23	7.66	171.63	0.6864	35.14	197.38	131.91
SSBC2	173.71	-50.20	260.32	-0.01	2.26	-19.86	0.4535	-10.71	84.46	-0.76
SSBC5	159.56	-54.25	253.21	-2.74	2.13	-24.47	0.378	-25.58	79.72	-6.33
SSBC10	127.05	-63.57	197.21	-24.25	1.25	-55.67	0.3252	-35.97	76.59	-10.01
SEPA limits	50		200		20		9		100	

Chapter -7

The effects of different biochars on bioaccumulation of PAHs and PTEs in *B.rapa* L.

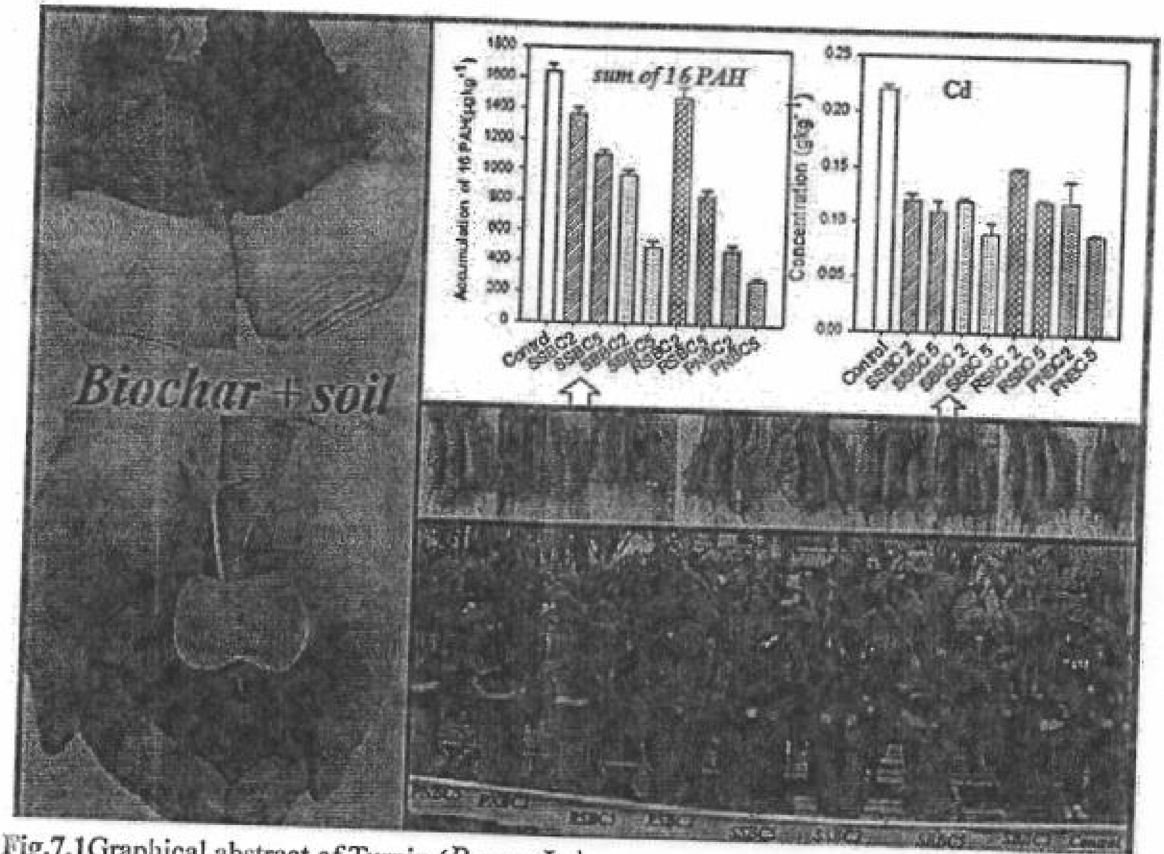


Fig.7.1Graphical abstract of Turnip (*B rapa*L.).

7.1 Introduction

SSis a byproduct of Wastewater treatment Plants (WTPs) which is used to agriculture land because of its agronomic benefits attributed to high concentration of nutrients vital for plant growth(Fytili and Zabaniotou, 2008). China is fast growing country in population which produce 30 million of SS every year (Yu,2011). The USA produce 6.2 million metric tons of dry sludge every year(Fytili and Zabaniotou, 2008) However, the existence of toxic contaminants such as PAHs and PTEs such as As, Cd, Cu, Pb ,Fe and Zn confine the application of SS to farmland(SEPA,1995; USEPA, 1995; CEC, 2000).The direct use of SS cause harms to agriculture land due to the presence of PAHs and PTEs ,many agroscientists have restricted SS uses in agriculture land(SEPA, 1995; USEPA, 1995; CEC, 2000)However to convert SS to SSBC(Waqas et al.,2013) and various agriculture biomass into biochars (Hale et al., 2012;Freddo et al., 2012; Uchimiya et al., 2011a).The level of PTEs, and PAHs were in the limits of with the concentration of background soil and below levels used in the regulation of composts (Freddo et al., 2012).

China produce 16Mtons of soybeans yearly, much of the soybeans discarded resulting in the loss of precious resources and environmental pollution (Zhu et al., 2008). In Asia it is estimated that total rice straw production was 623 million t and 125 million t rice husk is produced in 2008(FAOSTAT online data base).

Biomass is organic matter that is renewable including plant materials, animal manure and urban waste materials (Lal, 2005).Total crop residue produce in the USA 488×10^{6}Mg year $^{-1}$ where as in the world 3758×10^{6}Mg year $^{-1}$ (Lal, 2005). The crop residue production can be calculated by using formula.

Residue production = grain production ×straw /grain ratio

The straw /grain are calculated (see table 7.1).

Table 7.1 Straw/grain dry weight (DW) for various crops.

Crops	Lal(1995)	Stout,1990; Larson et al; 1982	Gupta etal.,1979	Residue production (10^6 Mg(2001)
Rice (Oryza sativa L.)	1.5	0.75-2.5	1.5	
Soya beans (Glycino max L.)	1	0.8-2.26	1.5	890
Peanut	-	-	-	177
Sugarcane (Saccharum officinarum)	0.25	0.2-0.25	-	-
Corn (Zee may L.)	1	0.55-1.50	1	314
Cotton (Gossypium hirsutum L.)	1.5	1.4-3.0	1	609
Wheat (Triticum aestivum)	1.5	1.10-2.57	1.3-1.7	24 875

The PAHs and PTEs are potentially toxic that is accumulating in the soil due to the continuous use of wastes biomass materials such as SS, by converting the different biomasses, SS into biochars that will destroy the PAHs and reduce the PTEs bioavailability to plants.It is also appreciable that during the biochars preparation heat and energy will be produced.

The risk to agriculture land due to the PAHs and PTEs can be reduced by addition of different biomass biochars , distinctively ,the sorption ability of different biochars ,could be more valuable in the re-partitioning of PAHs and PTEs ,and hence the reduction occurs in the accumulation of these contaminants in the food chain(Sneath et al., 2013).The SS biochars cause reduction in the bioavailability of PAHs and PTEs(Cao et al., 2011; Keiluweit et al., 2012; Hilber et al., 2012; Khan et al., 2013;Waqas et al.,2013).Freely dissolved PAHs concentration significantly decrease due to addition of biochars (Khan et al., 2013;Oleszczuk et al., 2012).The mass transfer of PAHs to biocharsorptive sites has been proposed as the mechanism through which this is achieved (Uchimiya et al., 2011b).The oxygen functional groups in the biochars forms complexes with PTEs which cause reduction in the mobility and bioaccumulation in plants grown in soil amended with biochars soil (Jiang and Xu, 2013;Uchimiya et al., 2011b).Biochar application to soil has recently reported that PAH/PTE accumulated in the plant tissue, not specifically due to the biochar application (Zheng et al., 2012; Méndez et al., 2012).

Turnip which is basically originated from Europe, which is nowadays cultivated throughout the world (McNaughton, 1976) including indo-pak subcontinent. In 2009 the turnip cultivated in Pakistan on 14873 ha, with total production 265,600 tones (Imran et al., 2011).

B. rapa L. were chosen as a representative plant belongs to brassicaceae family.Vegetable such as cucumber (Waqas et al.,2013)and tomatoes grown in field under different agricultural systems can be contaminated with organic compounds and PTEs and caused health problems (Mansour et al., 2009).

The aims under which this research was conducted to check the bioaccumulation of PAH and PTEs with the application of various biomass biochars, effectiveness of dissimilar biochars such as SSBC, SBBC , RSBC, and PNBC and sustainability of plant growth once added to contaminated soil into a universally cultivated *B. rapa L.*

7.2 Materials and methods

7.2.1 Soil sampling and characterization

Soil samples (0-20 cm) were collected from agriculture land near an iron refinery plant inFujian Province, China. The soil was contaminated with PAHs and PTEs. The soil sample were sieved 2nm mesh to make a homogenize sample. The sub-soil samples were freeze dried at

-50 °C at 22 Pa and enveloped at – 20°C.Basic properties of the soil including pH ,EC,BET surface area, pore volume,pore size, carbon(C),nitrogen(N) and Sulfhur(S)and PTE, are specified in Table 7.2. PAH concentrationhave been quantified in the control and amended soils are specified in Table 7.3The technical factsfor these measurements are set in thechapter

3. Soil particlesize distribution wasclay sand (71.2%),silts (27.6%) and clay (1.2%).

7.2.2 Biochar preparation and characterization

The SS has collected from the Bingbei WTP, Xiamen, and China.The wet sludge were dried in the air. Biomass of soya beans straw, rice straw, and peanut shell obtained from local villages near Xiamen. The biochars were prepared from these biomasses at a temperature of 500 °C for 6 hours under a continuous flow of N (Khan et al., 2013;Waqas et al.,2013).The basic properties such pH, EC, C, N, S, BET surface area, pore volume and pore size were determined (Table 7.2). The addition of SSBC RSBC and PNBC caused various changes in soil properties such as pH, EC, DOC, NH_4-N, NO_3-N, P, surface area and pore size (Table 7.3). Such changes have been well reported in the literature (Keithet al., 2011; Quilliamet al., 2012).The addition of SSBC increased soil pH by 0.01-0.04 pH units, The addition of SBBC increased soil pH by 0.38-0.45 whereas RSBC addition increase soil pH by 0.29-1.31 whilePNBC application decreased pH by 0.17-0.11 unit.

The EC of the SSBC amendmened soil with the application rate of 2 % and 5% was 0.52 and 0.87mScm^{-1}, whereas in SBBC application it was 0.61.The soil amended with RSBC with the application rate of 2% and 5% the EC becomes 0.98 and 1.53mScm^{-1} respectively and PNBC addition to soil changed the EC from 0.51 to 0.54 mScm^{-1} with the application rate of 2 and 5 % respectively. The total C concentration in the soil amended with SSBC increased 5.12 to 10.96% with the application rate of 2 and 5 % biochars .where the PNBC addition changes the C concentration from 7.22-8.20 respectively with the application rate of 2 and 5% .Total N and C concentrations increased significantly with the application of 2 and 5 % SSBC in comparison with the control soil. With the application of five percent dissimilar biochar such as SSBC, SBBC, RSBC and PNBC changes NH_4-N (mg kg^{-1}) concentration were in order of: $RSBC_5$ (6.69) >$SSBC_5$ (42.22)>$SBBC_5$ (39.37)>$PNBC_5$ (27.47) and with application of two percent dismilar biochar,the changes in NH_4-N (mg Kg^{-1}) were in order of

$SSBC_2$ (19.16) >$PNBC_2$ (18.68) >$SBBC_2$ (17.87)> $RSBC_2$ (16.66).

Whereas NO_3-N (mg kg^{-1}) concentrion with the application five percent were in order of $SBBC_5$ (93.54)> $RSBC_5$ (84.33)> $PNBC_5$ (82.11) >$SSBC_5$ (43.19) and two percent the order were $RSBC_2$ (102.57).>PNBC2 (96.96)> $SBBC_2$ (79.71)> $SSBC_2$ (56.44). Similarly for TOC (mg Kg^{-1}), the order were $SSBC_5$ (576)> $RSBC_5$ (474.35)>SBBC5 (425.45) > $PNBC_5$ (327.63) and $RSBC_2$ (399.1) $SBBC_2$ (393.25)> $SBBC_2$ (362.4)>$PNBC_2$ (296.95).

7.2.3 Greenhouse experiment

The SSBC, SBBC, RSBC, and PNBC,(<2 mm) was blended with polluted soil at concentrations of 2 and, 5 % on dry weight basis (defined as $SSBC_2$, $SSBC_5$, $SBBC_2$, SBBC $_5$, $RSBC_2$, $RSBC_5$ $PNBC_2$ and $PNBC_5$ respectively). A control soil treatment along with amended soil with various biomass biochars in three replicates has prepared with a total weight of 4kg.The pots were irrigated and kept it for 1 week before cultivation Turnips The seeds of the B. *rapa L.* germinated in petii dishes according to the method mentioned by (Khan et al., 2008; Waqas et al., 2013) and at last the germinated seeds were transplanted to pots prepared, in each pot three germinated seeds were placed. The experiment was fully developed in the greenhouse .The pots were irrigated with deionized water to uphold the 60% moisture contents according to the field capacity During the 8 months duration , fertilizer were taken100 mL of deionized water was dissolved 1 g of $NH_4NO_3$and the solution added to each pot). After 8 months the turnips were harvested and fresh weights recorded. Samples were freeze-dried and powdered prior to further analyses.

7.2.4 Chemical analyses

Available NO_3-N, NH_4-N, P (Colwell P) and dissolved organic carbon (DOC) were determined (see Table 7.2). Total PTE concentrations in soil, SSBC, SBBC, RSBC, PNBC and various biochars treatments were found using strong acid digestion (Waqas et al., 2013).PTE concentrations in powdered turnip samples were established following their digestionwith high purity concentrated HNO_3/H_2O_2(1/1 v/v) in a microwave accelerated reaction system (CEM-Mars,V. 194A05). The suspensionswere filtered and the filtrate was adjusted to 50 mL with Milli-Q water. To evaluate PTEs availability in soil SSBC, SBBC, RSBC, PNBC and various biochars treatments amixed reagentcontaining ethylene-diamine-tetra-acetic acid disodium(EDTA-Na_2; 0.05 M), $CaCl_2$ (0.01 M)and Tri-ethanolamine (TEA; 0.1 M) was used according to method adopted by Zeng et al. (2011). The quantification of PTEs concentrations were established by ICP: in the case of Cu, P and Zn using ICP-OES (Perkin Elmer Optima 7000 DV, USA); while As, Cd, and Pb were quantified using ICP-MS (Agilent Technologies, 7500 CX, USA).

Total 16 US-EPA PAH (Σ16PAH) concentrations in soil and SSBC, SBBC,RSBC,PNBC and the various biochar treatments and turnip were established using hot solvent extractions of PAHs with DCM and acetone (1:1 ratio) as solvents and an accelerated solvent extraction system (ASE, Dionex-350). After reducing these extracts to 1 mL through rotary evaporation, silica chromatography columns were used to purify the samples (Khan et al., 2008). The final fraction of 1 mLwas transferred to a 2 mL vial capped with a Teflon-lined septum ready for PAH analysis.

To assess PAH availability in soil and the SSBC, SBBC, RSBC and PNBC amended treatments a subcritical water (milli-Q) extraction method was used with the Dionex-350 ASE. Latawiec and Reid (2009) reported the use of this approach to release 'labile' PAH concentrationsin soil that reflected their bioaccessibility. The samples were mixed with quartz sand (25-50 mesh, Sinoparma) to provide properdispersion of matrices. An extractiontemperatureof 200°C, a 10 min static time and a 20% flush volume were selected. After cooling the extract was transferred to separating funnel (200 mL) and back extracted using DCM (Latawiec and Reid, 2009).PAH quantification in all extracts was achieved using GC-MS (Agilent Technologies 5975C).

7.2.5 Quality control and data analysis.

The efficiency of ASE extraction and silica column purification for PAH recovery from soil, SSBC,SBBC,RSBC and PNBC treatments andturnip samples was checked using surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10). The results showed satisfactory recovery, with the average recovery ranging from 83.7±8.2% to 94.6±5.4%. Reagent blanks were also included to check the contamination of PAHs during extraction and purification processes. For PTEs, sample blanks and standard reference materials (GBW07603-GSV-2 and GBW07406-GSS-6 for plant and soil, both obtained from the National Research Center for Standards in China) were included to verify the accuracy and precision of the digestion and subsequent analysis procedure. The recovery rates were satisfactory and ranging from 92.7±9.5% to106.4±7.1%.

The data were statistically analyzed using the statistical package SPSS 11.5. The measurements were expressed in terms of means, while the figures presented the mean values and standard deviation of threereplicates. Treatment differences were tested using analysis of variance (ANOVA), while for mean significance Tukey'stestwas usedwith a level of $P<0.05$.

7.3 Results and discussion

7.3.1*Changes in PAH/PTE concentrations using SSBC, SBBC, RSBC, and PNBC*

The sum of 16 PAHs concentration in the SSBC , SBBC, RSBC, and PNBC were 1671, 2548 ,5997 and 1499 μg kg^{-1} respectively .The Σ16PAHs in SSBC fulfilled with the International Biochar Initiative recommended standards (6-20 $mgkg^{-1}$) (IBI, 2012).The sum of 16 PAHs in the PNBC,SBBC significantly less in comparison with RSBC and SSBC.It is recommended that different biochars have been reported to have diverse PAH levels; these being dependent upon type of biomass used and the conditions under which they are burned (Hale et al., 2012;Freddo et al., 2012). PAH concentration reduced at high burning temperature and also depended upon duration of time under which it is pyrolized (Hilber et al., 2012; Freddo et al., 2012).The distribution of solvent extractable individual PAHs in SSBC, SBBC, RSBC and PNBC significantly varied (See table 7.5) The NA(Naphthalene) concentration was highest in the RSBC(2230.15μgkg^{-1}),SBBC(1199.23μgkg^{-1}),PNBC(256.96μgkg^{-1})andSSBC(223.52μgkg^{-1}).PH(Phenanthrene),was in RSBC(1420.25μg kg^{-1}),SSBC(832.45μg kg^{-1}),PNBC(758.57μg kg^{-1})and SBBC(737.66 μg kg^{-1})Similarly FLA(fluoranthene) and PY(Pyrene) concentration was highest in the RSBC following by SSBC. All the 16 PAH were detected in the various biomass biochars as shown in (Table 7.5).

Total PTEs concentrations in SSBC, SBBC, RSBC and PNBC were established. The PTEs concentrations varied in different biomass biochar as shown (Table 7.2). The PTEs concentration in the SSBC was in order of Zn (972.72) >Fe (21.05) >Cu (2.18) >Pb (2.03) >Cd (0.19) >As (0.15)

In SBBC was in order ofZn (566.98) >Fe (3.93) >Cu (1.13) >Pb (0.38) >Cd (0.04> As (0.04) In PNBC was in order of Zn (442.70) >Cu (1.34) > Fe (1.18) >Pb (0.19) > As (0.09) > Cd (0.03).and RSBC was in order of Zn(30.00) > Pb(1.57) >Cu(0.71) > Fe(0.44) > As(0.24) > Cd(0.09).When biochars were added to soil, it reduced greatly the availability of PTEs in the treatment soil in comparison with control soil, the application rate of 5 % significantly reduce the PTEs availability in comparison with 2% biochars application as shown in table 7.5.

7.3.2 Effects of amendment on soil PAHs

SSBC, SBBC, RSBC and PNBC basic characteristics are presented in Table 7.2. The addition of SSBC, RSBC, and PNBC causediverse changes in soil characteristics such as pH, EC, DOC, NH_4-N, NO_3-N, P, BET surface area and pore size (see Table 7.4).

The smallest decrease in PAHs bioaccumulation was noted for NA; bioaccumulation decreases with the application of SSBC, SBBC, RSBC and PNBC.The highest decrease occur in the PNBC treatment(70.77-82.64) , following by SBBC(40.46-69.43%) RSBC (23.61-48.50%) and in SSBC (16.90-32.50). NA bioaccumulation decreases in the turnip with the application of PNBC. The $PNBC_2$ and $PNBC_5$ treatment decreases the NA accumulation by (92.00-92.81%) following by SBBC treatments which decrease the accumulation upto (70.71-77.18%).The ACN bioaccumulation also decreases with the application of PNBC(72.41-75.95%) following by SBBC(45.35-54.03%) in turnip. The AC FLA, PHE, AN accumulation decreases with the application of PNBC (65-80.90%) following by RSBC.

The addition of PNBC significantly ($P<0.001$) reduced the bioaccumulation of 4-rings PAHs such as fluoranthene (52.45-72.26%), pyrene (50.39-68.77%), benzo(b)anthracene (83.19-92.99%) and chrysene (77.30-70.57%), as compared to the control soil (Fig. 26).
The highest bioaccumulation rate for 4-rings PAH was in order of PNBC > SBBC >RSBC >SSBC (see Table 7.9). The 5-rings PAHs, benzo(b)fluoranthene and benzo(k)fluoranthene, benzo(a)pyrene was in order of PNBC> SBBC >RSBC >SSBC (Table 7.10). In turnip, the bioaccumulation of 6-rings PAHs was the lowest compared to other groups of PAHs (Fig.7.5). PNBC addition significantly ($P<0.001$) reduced bioaccumulation of indeno(1,2,3-c,d)pyrene (80.80-87.80%), dibenzo(a,h)anthracene (86.85-93.83%) and benzo(g,h,i)perylene (60.75-76.11%), following by SBBC treatments compounds (See Table 7.11).The bioaccumulation for the 6 rings PAH compounds was in order of PNBC>SBBC >RSBC>SSBC.

The soil used in this study had a Σ16PAH concentration of 1050.85μg kg^{-1}. Concentrations of mutagenic Σ7PAHs (5.49 mg kg^{-1}) exceeded the limit (4.23 mg kg^{-1}) set by the Ministry of Environmental Protection, China (MEP, 2007).SSBC, SBBC , RSBC and PNBC application to soil significantlydecrease the available Σ16PAH concentrations, as compared to the control soil (Fig. 7.5). The available Σ16PAH concentration in the SSBC treatment was reduced by (25.38-44.40%) as compared to the control soil. The decrease in Σ16PAH concentrations was even greater in the PNBC (67.81-79.91 %).In the RSBC treatment the availableΣ16PAH concentrations was reduced by (14.17-44.71%)where as in the SBBC treatment available concentration was reduced by 40.82-67.50% in comparison with control soil(See Table 7.4).

It is stressed that the decreases in PAH availability in the SSBC, SBBC, RSBC and PNBC amended treatments represent changes that are far greater than those that can be attributed to dilution alone (based upon PAH concentration in the soil, SSBC, SBBC, RSBC and PNBC. The magnitude of changes reported above reflect compound re-partitioning not simply a dilution effect.

The addition of SSBC, SBBC, RSBC, and PNBC significantly ($P \leq 0.05$) reduced different PAHs compound(defined by number of benzene rings) and the PAHs concentration reduced significantly by adding 2 % biochars in comparison with 5% addition of biochars (See table 7.3). The results showed that the reduction in available fractions of low molecular weight (LMW) PAHs (2-3 rings) was low, but increased for high molecular weight (HMW) PAHs (4-6 rings). This is reasonable on account of LMW-PAHshavinggreatermobility than HMW-PAHs (Pignatello, 1996; Arp et al., 2011).

The results of Oleszczuk et al. (2012) and the results of our research indicate that biochar application reduce PAH availability, mostly in the HMW-PAHs.

It is of memo to declare that 7 days were acceptable for the amended soils to 'interact/equilibrate'. While this sum of time is unlikely to have been enough for all of the

PAHs to entirely re-equilibrate with the amendments the consequences obviously specify that this was enough time to noticeably decrease PAHs availability in the SSBC, SBBC, RSBC and PNBC amended soils. The results significantly indicate clear differences between the influence of PNBC SBBC, RSBC and PNBC Oleszczuk et al. (2012) noted that the influence of different biochars on free dissolved PAHs added to SS was also different. In our study, greater mass transfer from soilto SSBC.SBBC, RSBC and PNBC was observed for 5-and 6-rings PAHs compound. It is stressed that affinity of PAHs for biochar will depend upon the properties of the biochar; these being governed by feedstock type and pyrolysis conditions such as temperature and time (Chen et al., 2008; Hale et al., 2012). Biochar characteristics such as pH, elemental composition, surface area and structure could be changed with changes in these conditions, and as a consequencealter the sorptionbehavior of biochar (Cao et al., 2011). In this study the SSBC and SBBC, RSBC and PNBC materials had different surface areas and pore volume (See Table 7.2). The BET surface areas for above mention biochars were different; this may, in part, be the reasons for different extents of PAHs sorption observed.

After desorption from a contaminated matrix, the sorption and sequestration of PAHsinto SSBC, SBBC, RSBC and PNBC occurs through different means such as hydrophobic effect, π-electroninteractions and pore-filling mechanisms (Chen et al., 2008).These mechanisms would contribute differently in theSSBC, SBBC, RSBC and PNBC materials used in this study.

7.3.3 Effects of amendments on PTEs

The concentrations of PTEs in the contaminated soil were determined (Table 7.2). Of these PTEs Zn(131.51) and Cd(0.21) within the MALs (250 $mgkg^{-1}$ and 0.3 $mgkg^{-1}$, respectively) set by the State Environmental ProtectionAdministration, China (SEPA, 1995), while the As concentration was lower than its MAL limit (30 $mgkg^{-1}$).

The addition of SSBC, with the application rate of 2 % and 5 % to soil decreased available concentrations of As (56.76-59.46%), Pb (49.43-39.46%), Cu (35.79-53.81%), and Zn (63.10-77.07%),Fe(9.19-33.10) while Cd concentration decrease slightly (13.51-24.32%) as compared to the control soil The addition of SBBC to soil decrease the available concentration of As (83.78-29.73%),Cd (83.78-37.84 %), Cu(42.89-57.87), Fe(48.42-57.43), Pb(71.48-40.89), Zn(25.91-73.82) as compared to the control soil. The application rate of 5 % of SBBC decrease the PTEs availability as compared to 2 % application of SBBC except Fe and Zn reduction in the bioavialablity occurs with the increasing of biochar.In the rice straw biochar As (83.78-62.16), Cd (97.30-86.49), Cu (61.42-57.36%), Fe (77.63-81.83%), Pb (69.18-58.57), and Zn (29.45-26.27%) With the increase of biochar quantity the concentrations of availability of PTEs decease except in the caseof Fe and Pb. By adding PNBC to the soil with the application rate of 2% and 5 % reduced the availability of PTEs such as As (43.24-48.65%),Cd (16.22-43.24%), Cu (36.80-39.85%) Fe (27.93-37.38), Pb (17.52-21.78%) and Zn (16.61-33.34%) as compare to control soil .The Application rate of 2 % PNBC shows less significant as compared to the 5 % addition of PNBC to control soil, The PTEs values significantly ($P \leq 0.05$) decreasedat higherPNBC application rates(5%) The SBBC and RSBC show great reduction in the PTEs availability with the 2 % application rate as compared to SSBC and PNBC (See Table 7.3).

In biochars amended soil PTEs availability decrease noticeably. This result is reliable with numerous other mechanism that have revealed biochar to bevaluable in reduced the available concentrations of PTEs in contaminated soils (Lehmann, et al., 2011; Ahmad et al., 2012; Beesley et al., 2011). Reduced PTEs availability in soil amended with biochar has beenconnectedto changes in soil pH, cation exchange and DOC values (Zheng et al., 2012; Keith et al., 2011). In addition, biochar also contain oxygen-functional-groups which are expected to form complexes with PTEs (Kookanaet al., 2011) and led to PTEs immobilization in soil and then reduced bioaccumulation in plants grown on biochar amended soil. Previous studies have been shown that these oxygen-functional-groups are further effective than increasing alkalinityaffects in suppression of PTEs the same as Cu (Jiang and Xu, 2013).

7.3.4 Plant biomass

Fruit biomass was significantly higher ($P \leq 0.01$) in the SSBC, SBBC, RSBC and PNBC, amended soil than in the control soil (see table 7.6). These SSBC SBBC, RSBC, and PNBC amendments appreciably improved fruit yields when compared to the control, The 2 % application of SSBC SBBC, RSBC, and PNBC to soil significantly ($P \leq 0.05$) increase the turnip biomass, 48.55, 21.86, 43.86 and 28.79% where as with the application of 5 % of SSBC, RSBC and PNBC the biomass reduced by 20.15, 3.21and 16.36 % except SBBC 5 % increased the biomass by 11.44 (see Table 7.6). It was noted that the highest application of biochars had no significant advantage in terms of fruit biomass, Besides other nutrients, the increase in NH_4-N and NO_3-N concentrations by SSBC, SBBC, RSBC and PNBC (See Table 7.3) could be the reason for higher fruit yield (Clough and Condron, 2010). The result suggest that the SSBC and RSBC with the application of 2 % can significantly increase the turnip production (Fig.7.2).

7.3.5 PAH bioaccumulation

All 16 PAHs were detected in turnip grown in control soil and treatments amended with SSBC, SBBC, RSBC and PNBC (Fig. 7.7). The addition of different biomass biochars significantly ($P<0.01$) reduced the concentrations of Σ16PAH.the SSBC (16.90-32.54 %). SBBC (40.46-69.43%), RSBC (23.61-48.50%) and PNBC (70.78-82.57%) reduced the Σ16PAH with application rate of 2 % and 5%. in turnip .PNBC addition was more effective following by SBBC (Fig. 7.7). Results indicated that the extent of decrease in

Σ16PAHbioaccumulation increased with increasing application rates of SSBC, SBBC, RSBC and PNBC (Table 28). Maximum reduction in Σ16PAHbioaccumulation was observed in the $PNBC_5$ treatments (39.32%).Further significant ($P<0.05$) reductions in bioaccumulation were observed in the $SBBC_5$ and $RSBC_5$ treatments.

Figure 7.7summarizes the overall influence of SSBC, SBBC, RSBC, and PNBC amendments on PAH bioaccumulation in turnip. The effectives of different biomass biochars was in order of PNBC >SBBC >RSBC >SSBC in terms of reducing PAHsbioaccumulation in turnip. NAreduce in the SSBC amendments (22.32-37.67%),SBBC amendments(70.71-77.18%), RSBC(7.51-47.51%) and PNBC (92-92.81%)whereas ACN reduced dominantly in the PNBC(72.41-75.99%) application to the soil following by the SBBC(45.39-54.03%). AC bioaccumulation in the turnip decrease with the application of SSBC, SBBC, RSBC and PNBC except the 89.22 % increase occurred due to application of rice straw biochar with 5 % whereas 2 % application rate reduced AC accumulation 1.62%.

FL bioaccumulation rate in the turnip in order of$PNBC_2$> $PNBC_5$> $SBBC_5$> $RSBC_5$>$SSBC_5$> $SSBC_2$> $SBBC_2$> $RSBC_2$.

The PHE is reduced dominantly by adding PNBC in comparison with control soil (70.87-83.72%) whereas the AN accumulation in turnip plant reduced by SSBC (3.12-15.31%), SBBC (2.33-67.79%), RSBC (42.55-58.56%) PNBC (67.23-80.90%).

The 4 rings PAHs accumulation significantly reduced in the $PNBC_2$ and $PNBC_5$ treatments in comparison with other biochar application. The 5 rings PAH bioaccumulation reduced by addition of PNBC (67.98-75.01%) following by SBBC (49.39-80.00%) for Benzo(b)fluoranthene. In 6 rings PAH bioaccumulation in turnip significantly reduced by adding different biomass biochars, but the more effective is PNBC that has reduced the bioaccumulation in turnip by 60.75-87.80%. Thereduction in PAHs bioaccumulation was higher for HMW-PAHs than LMW-PAH. These HMW-PAH compounds would be expected to be of lower availability and their bioaccumulation reduced by SSBC amendments as compared to LMW-PAH compounds (Papadopoulos et al., 2007).

The reduced bioaccumulation of PAHs in turnip is in keeping with results indicating reduced PAH bioaccumulation into lettuce when grown in the presence of SS and SSBC (Khan et al., 2013). This lower accumulation of PAHs in turnip grown in SSBC, SBBC, RSBC and PNBC amended soil could be attributed to the reduced concentration of PAHs in the soil solution. Our resultsdemonstrated that SSBC, SBBC, RSBC and PNBC addition to polluted soil decreases thehazardconnected with the transfer of PAHs into food and subsequently into the food chain.

These results confirmed that PNBC has higher sorptive ability than SSBC, RSBC and PNBC for PAHs. The structural composition and elemental characteristics of PNBC are different to those ofSBBC, RSBC and SSBC (See Table 7.2).These play significant role in determining the sorptive ability for these materials. The consequences reported here are in accord with the conclusions of other researchers who have reported the application of other (not SSBC) biocharsto PAH pollutedsoil to reduce their availability (Chen and Yuan, 2011). particularly wood biocharhas also been observed to decrease the bioavailability of PAHs (Beesley et al., 2010) but its efficacy in this regard has been revealed to be dependent uponPAH ageing or contact time (Cao et al., 2011). It has been recommended that biochar not only reduces the available PAHs in the soil liquid phase but also minimizes the transfer of PAHs from soil to airand then to plant (this pathway being particularly important for more volatile PAHs(Kipopoulou et al., 1999; Howsam et al., 2001). The obstruction to both transfer mechanisms would contribute to reduce soil to plant transfer of PAHs.

7.3.6 PTE bioaccumulation

The SSBC, SBBC,RSBC and PNBC addition significantly ($P \leq 0.01$) reduced bioaccumulation of PTEs in the turnip the SSBC2,SBBC2,RSBC5,PNBC2 treatment cause 45.45% reduction in the As as compared to control soil, RSBC2 cause 31.82 % reduction in As, whereas PNBC5 and SBBC5 reduce it by 59.09% and SSBC5 cause 50 % reduction in the accumulation of As in turnip.

The Cd bioaccumulation reduced in SSBC (7.14-28.57%), SBBC (21.43-35.71%), RSBC (21.43-28.57%) and PNBC (7.14-42.86%).The Cu bioaccumulation in turnip reduced by adding different biomass biochar such as the SSBC(6.55-19.37%),SBBC(10.83-26.50%),RSBC(23.36-26.50%) and PNBC(27.64-31.62 %).Fe bioaccumulation decrease in SSBC (12.42-18.69%), SBBC (32.60-49.22%), RSBC (7.60-22.87%) and PNBC (15.08-41.07%) treatments comparison with control soil.The Pb bioaccumulation decrease in SSBC (18.18-24.24%), SBBC (6.06-18.18%), RSBC (21.21-42.42%) and PNBC (12.12-54.55%). Zn bioaccumulation in turnip decrease by the addition of different biomass biochars SSBC (20.80-22.02%), SBBC (18.11-40.55%), RSBC (16.85-37.03%) and PNBC (13.59-22.00%)(See Table 7.12).

The concentration of As was significantly ($P \leq 0.05$) elevated in turnip grown on SSBC, SBBC, RSBC and PNBC amended soil, reaching 411.45-537.87$\mu g kg^{-1}$ as compared to the control (328 $\mu g kg^{-1}$). These levels of As exceeded the maximum permissible limits (0.05 $mg kg^{-1}$ dw) set for food by SEPA (2005). The addition of SSBC, SBBC ,RSBC and PNBC markedly decreased As concentration in turnip but this concentration still exceeded the limit set by SEPA(2005) (0.05 $mg kg^{-1}$dw).

The concentrations of Cd in turnip decreased in SSBC, SBBC, RSBC and PNBC amended soils, and exceeded the permissible limits (0.2 $mg kg^{-1}$ dw) set by SEPA (2005) for food. It should be noted that the soil used in this study had total Cd concentration that exceeded the MAL set by SEPA (1995)for agricultural practice (Table 7.2).

The concentration of Zn was also significantly ($P \leq 0.05$) elevated in turnip grownin SSBC, SBBC, RSBC and PNBC amended soil and within the MAL (100 $mgkg^{-1}$ dw) set for food by SEPA (2005).Total Zn concentration in the soil within the MAL (300 $mgkg^{-1}$) set by SEPA (1995) for agricultural practice (Table 7.2). Thus soil pollutedwith such high levels of As, and Cd may still cause a health risk through soil to food transfer of PTEs even after SSBC,SBBC,RSBC and PNBC amendments.

The concentrations of dissolved organic compounds (DOC)in SSBC, SBBC, RSBC and PNBC application to soil may have playedasignificant role in reducing bioavailability and bioaccumulation of PTEs. However, in the case of SS treatments, in our previous study PTE bioaccumulationincreased in spite of increased DOC. It is suggested that PTEsmay be bound more weakly to SS-DOC than to SSBC-DOC (see Table 7.2 for available metals) and a result they are as more easily released to soil solution for plant uptake even where DOC levels are high. The higherBET surface area, higher pore volume and smaller pore size in PNBC and SSBC, would also contribute to reducing PTE bioavailability. The biochars amendments improved S contents in soil (Table 7.3) which stronglyinterrelate with Asduring plant uptake, which influenceits bioaccumulation. Arsenateis aphosphate analog and taken up into plants through P transporters (Meharg and Macnair, 1992), hence, P associated withSSBC.SBBC, RSBC and PNBC might be responsible for the reduced in plantArsenic bioaccumulation. In contrast, the application of SSBC, SBBC, RSBC and PNBC to soil decreased As, Cu, Pb and Zn availability and succeeding uptake through combined effects attributed to (Uchimiya et al., 2011a): increasing soil pH; higher P and S availability; oxygen-functional-groups present on biochar, and; metal-complexation (Lehmann and Joseph, 2009). It is also probable thatchanges in microbialactions (Quilliamet al., 2012; Steinbeiss et al., 2009) would also have influenced PTEs bioavailability.

7.4 Conclusions

The findings of our research specified that theapplication of different biomass biochars was efficient in reducing PAHs and in decreasing the availability of PTEs such as As, Cd,Cu ,Fe,Pb and Zn The application of PNBC to contaminated soil was far more effective in reducing the availability of PAHs following by SBBC and in case of PTEs reduction PNBC are more effective following by SSBC and as a result PNBC was more effective in reducing contaminant transfer from soil to water and then to plant. In addition to reduced contaminant shifting from soil to plant it is worth mentioning that pathogens connected with organic biomass are exterminated during biochar preparation process. Thus, biochars suggest extra benefits in terms of alleviating pathogen.It should be highlighted that how well these findings can be extrapolated for other biochars, and other soil types is uncertain, and further research in this regard is required. However it can be concluded, from numerous viewpoints, that PNBC in case of PAHs removal whereas in the case of PTEs removal are more effective mostly where PAHs and PTEs concentrations are high in soil.

Table 7.2
Physical and chemical properties of soil, SSBC, SBBC, RSBC and PNBC and concentrations PTEs in turnip experiment. Mean values are shown±1 standard deviation (n =3).

Parameters	Soil	SSBC	SBBC	RSBC	PNBC
pH	7.35±0.07	7.43±0.03	10.06±0.04	10.05±0.03	9.68±0.02
ECmScm^{-1}	0.43±0.01	6.69±0.24	5.12±0.45	10.37±0.39	6.57±0.11
BET Surface area(m^{2}g^{-1})	2.34	4.07134	2.6267	2.2071	3.4548
Pore volume (cm^{3}g^{-1})	0.009	0.02	0.004470	0.003833	0.003300
Pore size (nm)	15.28	10.31	6.806775	6.94602	3.82040
Moisture content (%)	8.8	1.7	1.5	1.3	1.9
NH4-N (mg kg^{-1})	9.03±0.48	8.89±0.37	10.56±0.37	11.55±0.32	9.34±0.34
NO3-N (mg Kg^{-1})	55.69±1.04	3.39±0.51	3.39±0.51	6.65±0.54	3.63±0.56
TOC(mg kg^{-1})	435.55±62.23	283.05±16.59	2079±26.54	797.75±13.86	230.60±28.61
N (%)	0.14±0.01	2.83±0.03	1.72±0.02	1.65±0.07	1.58±0.01
C (%)	4.98±0.49	20.64±0.29	65.59±0.56	47.43±0.82	52.54±0.94
S (%)	0.05±0.005	2.25±0.09ls	0.13±0.02	0.40±0.044	0.43±0.01
			Total HMs concentration (mgkg^{-1})		
As	0.17±0.01	0.15 ±0.01	0.04 ±0.001	0.24±0.01	0.09±0.002
Cd	0.21 ±0.01	0.19± 0.005	0.04 ±0.003	0.09±0.004	0.03±0.0005
Cu	1.94 ±0.13	2.18 ±0.05	1.13± 0.01	0.71±0.03	1.34±0.07
Fe	21.05±1.06	30.71±0.61	3.93 ±0.03	0.44±0.02	1.18±0.06
Pb	8.27±0.07	2.03±0.01	0.38± 0.01	1.57±0.004	0.19±0.01
Zn	131.51±13.17	972.72±41.72	566.98±10.63	30.00±3.25	442.70±27.21

[b]Permissible limits set for soil pH>6.5 by the State Environmental Protection Administration(SEPA, 1995)
[c]Soil background values taken from Lu et al. (2007) for Fujian province, China

Table 7.3
Physical and chemical properties of soil and soil amended SSBC, SBBC, RSBC and PNBC in turnip experiment. Mean values are shown ±1 standard deviation (n =3).

	Soil	$SSBC_2$	$SSBC_5$	$SBBC_2$	$SBBC_5$	$RSBC_2$	$RSBC_5$	$PNBC_2$	$PNBC_5$
pH	7.35±0.07	7.36±0.05	7.39±0.02	7.73±0.02	7.80±0.07	7.64±0.14	8.66±0.14	7.18±0.03	7.24±0.05
EC $mScm^{-1}$	0.4248±0.01	0.52±0.03	0.87±0.03	0.61±0.04	0.61±0.02	0.98±0.01	1.63±0.11	0.51±0.04	0.54±0.03
NH_4-N (mg kg^{-1})	9.03±0.48	19.16±0.13	42.22±0.79	17.87±1.07	39.37±0.32	16.66±0.39	46.69±0.49	18.68±0.36	27.47±1.12
NO_3-N (mg Kg^{-1})	65.69±1.04	56.44±3.39	43.19±1.96	79.71±2.52	93.54±8.83	102.57±0.80	84.33±0.97	96.96±2.94	82.11±9.05
TOC(mg kg^{-1})	335.65±62.23	393.25±19.88	576±30.95	362.4±18.84	425.45±39.71	399.1±22.98	474.35±32.40	296.95±5.40	327.63±42.37
N (%)	0.14±0.01	0.17±0.01	0.43±0.17	0.18±0.01	0.23±0.01	0.17±0.03	0.19±0.03	0.18±0.02	0.21±0.01
C (%)	4.98±0.49	5.12±0.63	10.96±1.33	6.02±0.13	7.94±0.44	6.72±0.13	6.78±1.27	7.22±0.59	8.20±0.31
S (%)	0.05±0.005	0.10±0.01	0.35±0.12	0.07±0.01	0.07±0.0006	0.07±0.007	0.07±0.005	0.04±0.003	0.05±0.003
			Bioavailable Heavy metals Conc.($mgKg^{-1}dw$)						
As	0.37±0.01	0.16 ±0.003	0.15±0.01	0.31±0.01	0.26±0.004	0.31±0.003	0.23±0.01	0.21±0.01	0.19±0.01
Cd	0.37±0.02	0.32 ±0.002	0.23±0.002	0.31±0.02	0.23±0.003	0.36±0.02	0.32±0.01	0.31±0.01	0.21 ±0.01
Cu	3.94± 0.13	2.53±0.20	1.82 ±0.05	2.28±0.01	2.25 ±0.04	2.42±0.11	2.26±0.06	2.49±0.05	2.37±0.03
Fe	35.05±1.06	31.83±1.46	23.45±0.44	16.97±0.66	14.92±0.57	27.21±0.54	28.68±1.09	25.26±0.86	21.95±0.11
Pb	18.27±0.07	9.24 ±0.21	11.06±1.55	13.06±0.40	10.80 ±0.15	12.64±0.41	10.70 ±0.03	15.07±1.22	14.29±0.32
Zn	66.60±0.16	24.17±1.37	15.02±0.37	16.97±0.33	17.15±0.46	19.29±0.82	17.27±0.07	54.62±4.27	43.66±5.22

Table 7.4

PAH bioavailable Conc. in soil amended with different biochars (μgKg⁻¹).

Bioavailable PAH	Soil	$SSBC_2$	$SSBC_5$	$SBBC_2$	$SBBC_5$	$RSBC_2$	$RSBC_5$	$PNBC_2$	$PNBC_5$
Naphthalene	141.43	70.12	30.85	41.43	32.28	140.19	74.23	11.31	10.17
Acenaphthylene	29.65	26.7	16.28	13.63	13.02	28.24	7.69	8.18	7.12
Acenathphene	8.63	8.13	7.12	9.4	8.49	16.33	6.36	2.97	3.26
fluorene	13.57	10.66	7.68	10.35	4.68	39.57	7.16	2.16	2.47
Phenanthrene	170.22	149.44	111.98	161.88	46.07	149.43	86.71	48.42	27.06
Anthracene	14.19	13.56	13.3	13.86	4.57	31.46	5.88	4.65	2.71
Fluoranthene	173.27	99.85	72.98	88.98	72.1	143.71	129.07	82.39	48.07
Pyrene	118.49	105.96	85.41	87.03	58.71	112.87	55.79	58.78	37.01
Benzo(b)anthracene	65.37	43.01	33.43	22.36	10.04	34.48	20.48	10.99	4.58
Chrysene	55.38	43.01	46.75	30.11	10.04	34.48	51.46	12.57	16.3
Benzo(b)fluoranthene	46.1	37.64	31.4	23.33	9.22	21.96	31.81	14.76	11.52
Benzo(k)fluoranthene-2	46.09	37.63	31.39	23.33	17.49	28.09	31.8	22.9	11.51
Benzo(a)pyrene	27.15	29.49	16.63	24.05	14.09	16.05	8.93	16.24	3.46
Indeno(1,2,3-c,d)pyrene	13.44	11.25	8.15	1.23	0.86	6.77	2.55	2.58	1.64
Dibenzo(a,h)anthracene	13.61	9.52	5.62	3.67	1.75	3.64	1.78	1.79	0.84
Benzo(g,h,i)perylene	11.72	10.61	8.25	6.5	4.74	6.59	2.55	4.6	2.8
∑ of 16 PAH	948.31	707.58	527.22	561.14	308.15	813.86	524.25	305.29	190.52

Table 7.5

Sum of 16 PAH in different biomass biochars (µgkg^{-1}).

PAH	SSBC	SBBC	RSBC	PNBC
Naphthalene	223. 52	1199. 23	2230. 15	256. 96
Acenaphthylene	8	14. 3	16. 03	13. 52
Acenathphene	27. 51	31. 26	28. 76	20. 73
Fluorine	14. 05	181. 16	160. 11	147. 93
Phenanthrene	832. 45	737. 66	1420. 25	758. 57
Anthracene	45. 4	62. 45	238. 61	49. 25
Fluoranthene	138. 34	93. 59	417. 74	72. 97
Pyrene	158. 44	72. 69	466. 29	73. 79
Benzo (b) anthracene	23. 73	11. 67	194. 84	9. 03
Chrysene	74. 79	24. 47	126. 95	15. 99
Benzo (b) fluoranthene	53. 15	31. 49	139. 77	10. 53
Benzo (k) fluoranthene-2	49. 82	31. 49	139. 77	9. 53
Benzo (a) pyrene	0	37. 39	355. 16	42. 51
Indeno (1, 2, 3-c, d) pyrene	8. 06	6. 85	6. 64	6. 66
Dibenzo (a, h) anthracene	8. 8	8. 81	8. 69	. 8. 73
Benzo (g, h, i) perylene	5. 58	4. 08	47. 7	2. 65
∑ Sum of 16 PAH	1671.64	2548.59	5997.46	1499.35

Table 7.6
Fresh biomass of turnip roots and shoots (g).

	Control	$SSBC_2$	$SSBC_4$	$SBBC_2$	$SBBC_4$	$RSBC_2$	$RSBC_4$	$PNBC_2$	$PNBC_4$
Roots	193. 63±3. 52	287. 63±6. 26	154. 62±8. 94	235. 96±1. 07	176. 77±7. 96	278. 56±7. 46	187. 41±2. 83	249. 37±6. 53	161. 98±1. 77
Shoots	115. 04±2. 04	185. 14±5. 17	103. 32±0. 75	192. 4±5. 94	150. 31±4. 68	210. 33±12. 81	156. 93±13. 81	149. 95±6. 99	134. 97±3. 09
Leaves (%)									
N	2. 27±0. 140	2. 23±0. 092	2. 40±0. 092	2. 28±0. 033	2. 99±0. 029	2. 02±0. 129	2. 32±0. 064	2. 17±151	1. 89±0. 049
C	37. 46±0. 397	35. 76±0. 842	34. 577	35. 06±0. 738	34. 06±0. 100	34. 70±0. 768	35. 76±1. 801	35. 26±1. 121	35. 63±0. 421
S	0. 39±0. 020	0. 43±0. 058	0. 47±0. 023	0. 36±0. 058	1. 38±0. 127	0. 34±0. 009	0. 29±0. 028	0. 30±0. 014	0. 25±0. 013
Roots (%)									
N	1. 43±0. 046	1. 40±0. 005	1. 66±0. 018	1. 19±0. 017	2. 01±0. 254	1. 32±0. 327	1. 60±0. 019	1. 69±0. 365	1. 11±0. 039
C	35. 93±0. 123	36. 73±0. 063	36. 97±0. 406	36. 98±0. 354	36. 99±0. 196	36. 09±0. 645	36. 28±0. 186	35. 46±1. 230	36. 98±0. 866
S	1. 60±0. 179	0. 36±0. 023	0. 38±0. 037	0. 27±0. 023	0. 30±0. 023	0. 36±0. 006	0. 40±0. 005	0. 30±0. 024	0. 21±0. 017

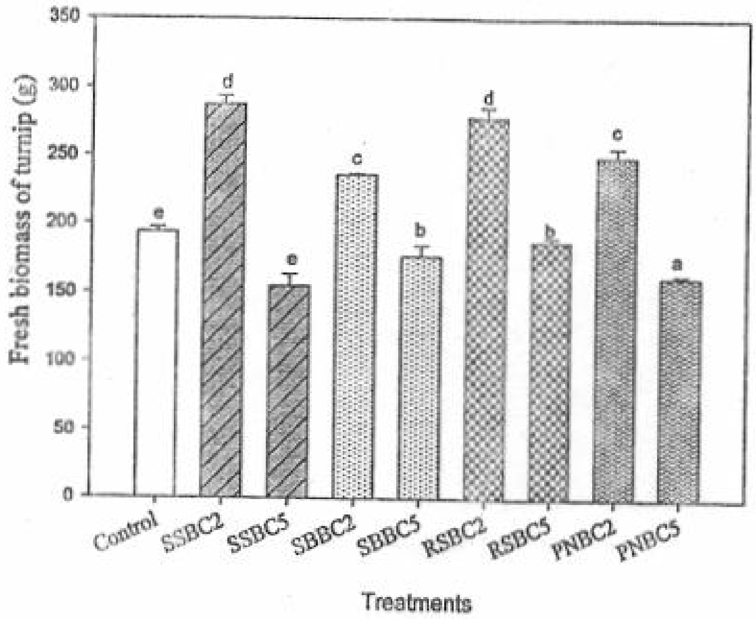


Fig.7.2 Fresh weight (g/pot) of turnip grown in control soil and soil amended with different biochars.Error bars represent standard deviations (n=4). Letters indicate significant difference (P≤0.05) from the control, while similar letters.

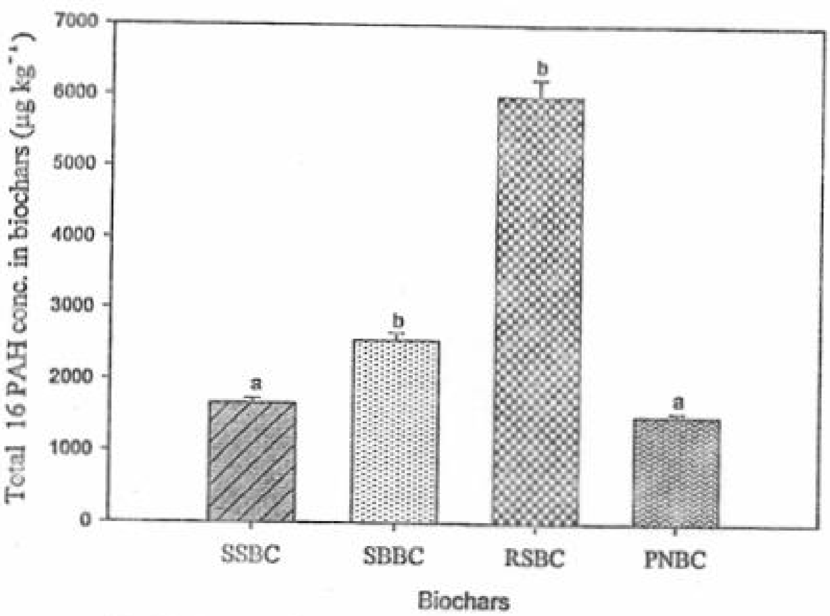


Fig.7.3 Sum of 16 PAH in different biochars.

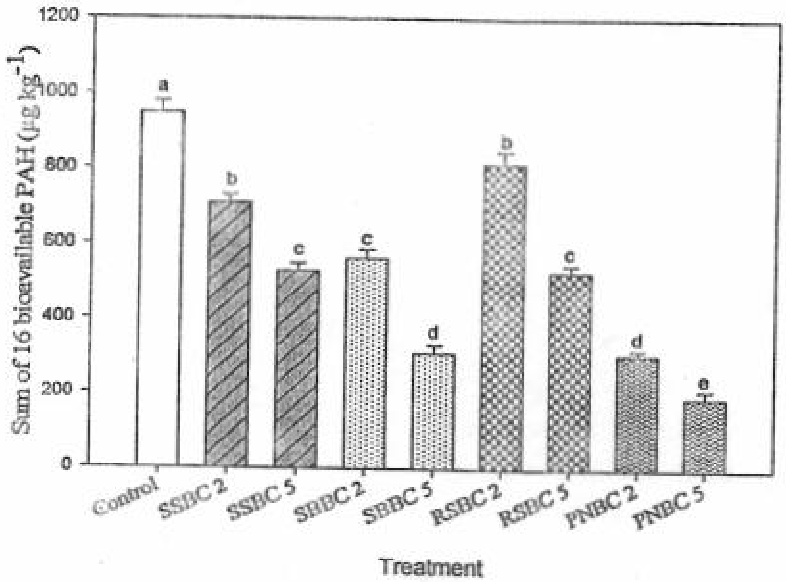


Fig.7.4 Sum of Bioavialable PAH in soil amended with different biochars.

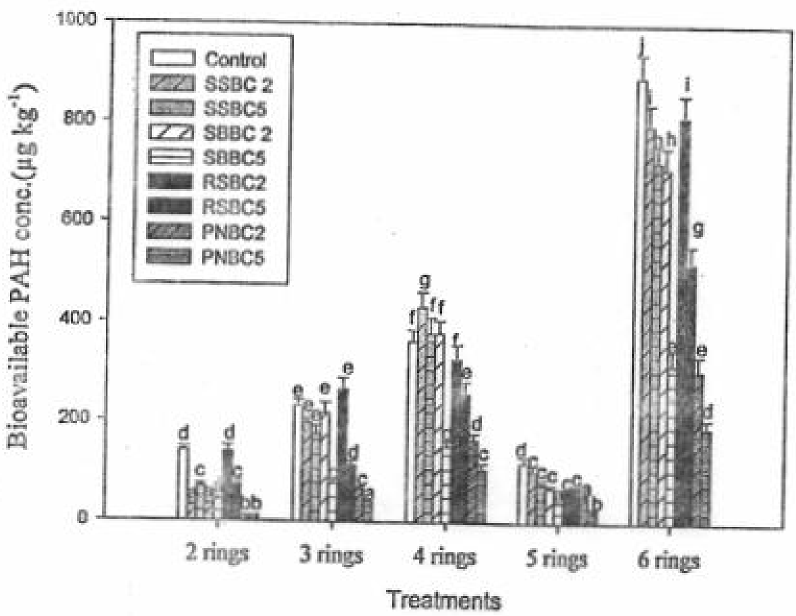
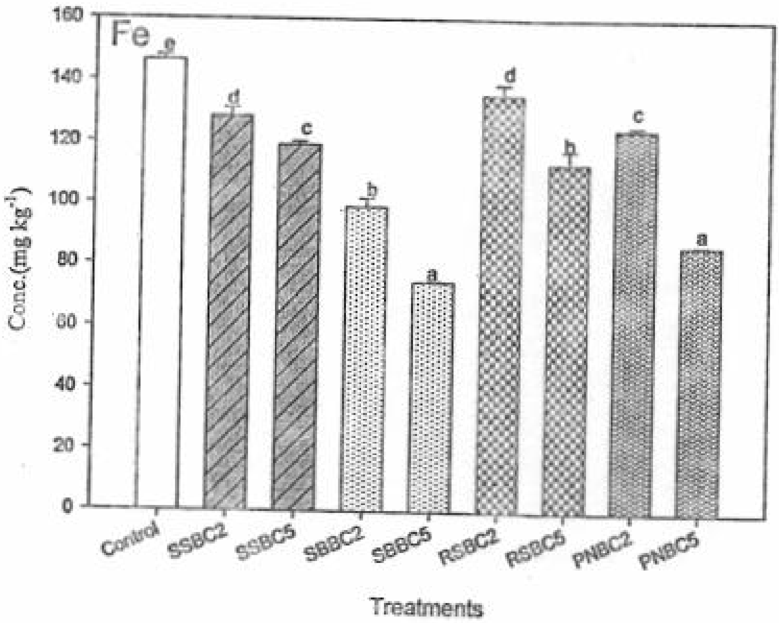
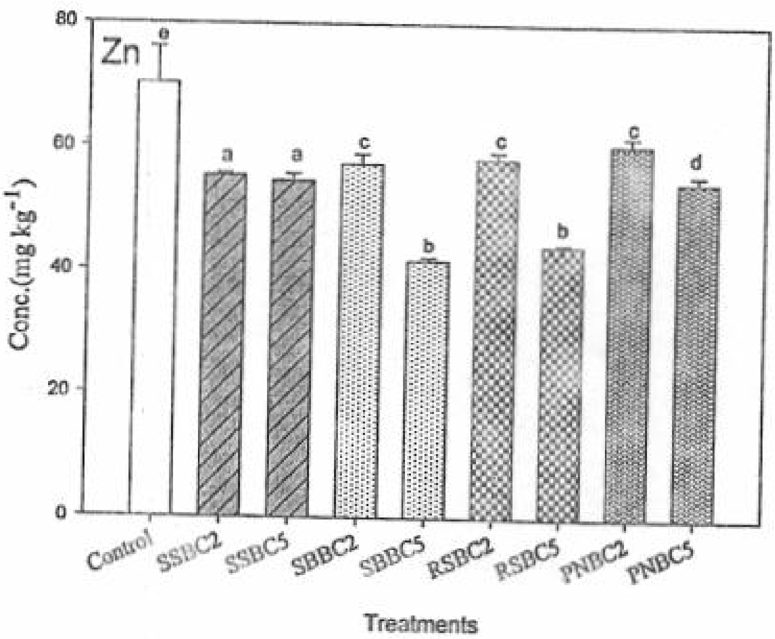
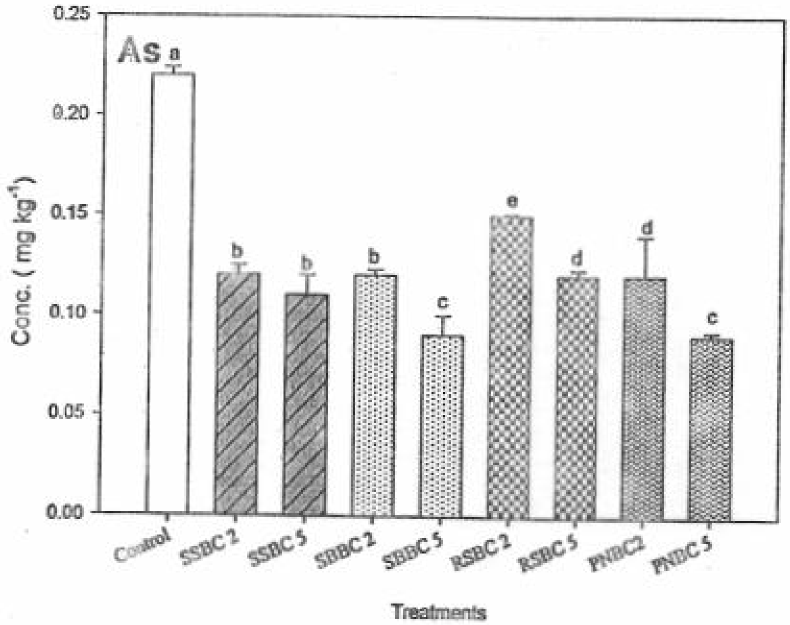
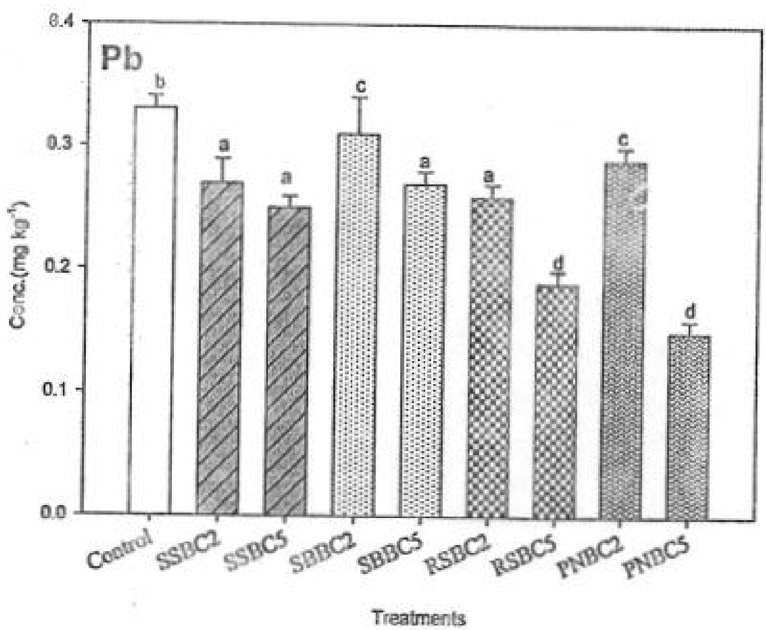


Fig.7.5 PAHs in the control soil and soil amended with SSBC, SBBC, RSBC and PNBCPAHs grouped by number of benzene rings (2-6). Error bars represent standard deviations (n=3). Different letters on the bars indicate significant difference (P≤0.05), while similar letters indicate no significant difference between the treatments.


Fe
Conc.(mg kg-1)
0
20
40
60
80
100
120
140
160
e
d
c
b
a
d
b
c
a
Control
SSBC2
SSBC5
SBBC2
SBBC5
RSBC2
RSBC5
PNBC2
PNBC5
Treatments


Zn
Conc.(mg kg-1)
0
20
40
60
80
e
a
a
c
b
c
b
c
d
Control
SSBC2
SSBC5
SBBC2
SBBC5
RSBC2
RSBC5
PNBC2
PNBC5
Treatments


As
Conc. (mg kg-1)
0.25
0.20
0.15
0.10
0.05
0.00
a
b
b
b
c
e
d
d
c
Control
SSBC 2
SSBC 5
SBBC 2
SBBC 5
RSBC 2
RSBC 5
PNBC2
PNBC 5
Treatments


Pb
Conc.(mg kg-1)
0.4
0.3
0.2
0.1
0.0
b
a
a
c
a
a
d
c
d
Control
SSBC2
SSBC5
SBBC2
SBBC5
RSBC2
RSBC5
PNBC2
PNBC5
Treatments

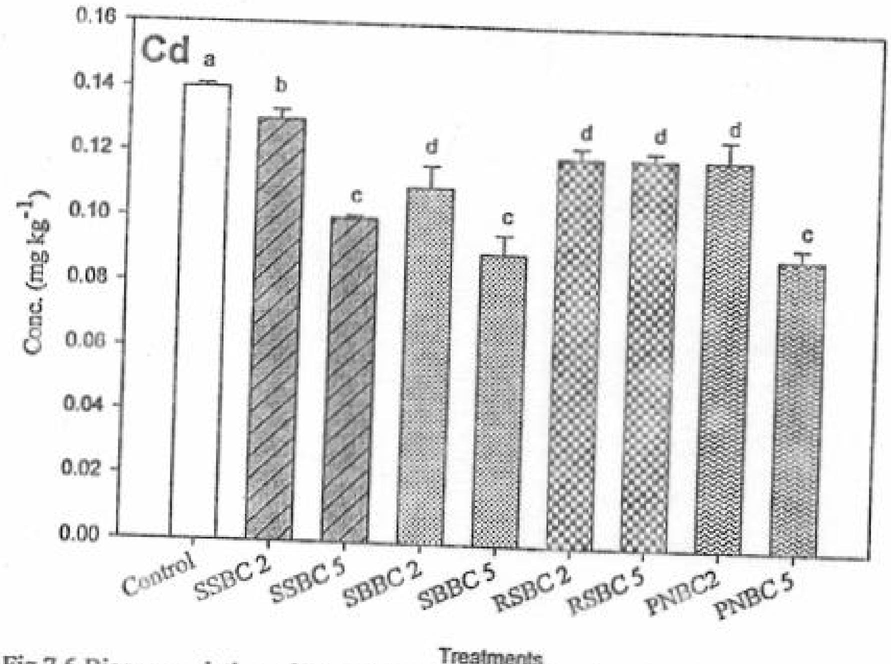


Fig.7.6 Bioaccumulation of PTEs in turnip grown in soil and soil amended with SSBC, SBBC, RSBC and PNBC. Error bars represent standard deviations (n=3). Different letters indicate significant difference (P≤0.05), while same letters indicate no significant difference.

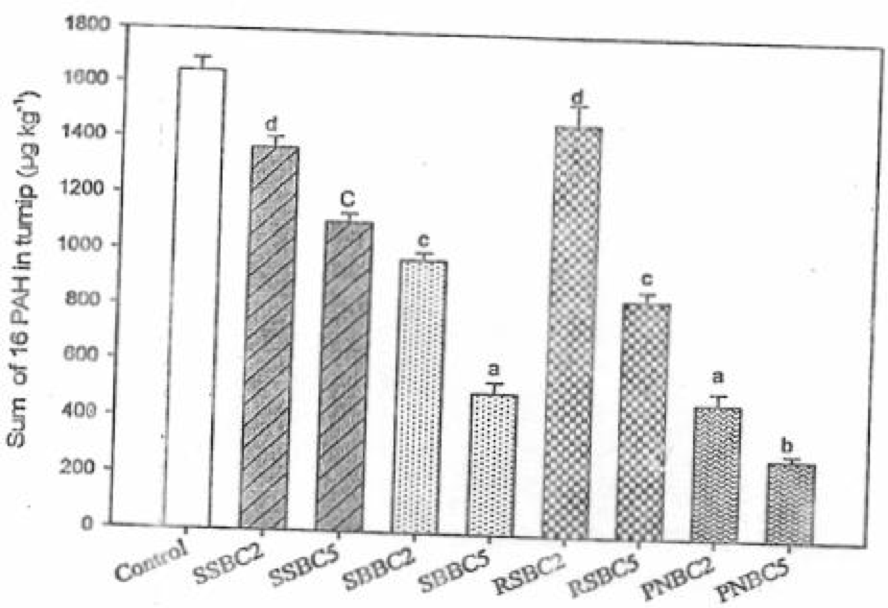


Fig.7.7 Sum of 16PAHs in turnip growincontrol soil and soil amended with SSBC, SBBC, RSBC and PNBC Error bars represent standard deviations (n=3). Letters indicate significant difference (P≤0.01) from the control, while similar letters indicate no significant difference between the treatments.

Table 7.7
Mean fresh weight (g/pot) of turnip yield (n=3) grown incontrol, SSBC RSBC, SBBC and PNBC amended soil. Increase changes, due to SSBC, SBBC, RSBC and PNBC addition, are given in percentage.

- show decrease and + indicate increase

	Turnip Biomass	
Treatments	Fresh weight (g/pot)	Changes (%)
Control	193.63	
SSBC 2	287.63	+48.546
SSBC 5	154.62	-20.147
SBBC 2	235.96	+21.861
SBBC 5	176.77	+11.439
RSBC 2	278.56	+43.862
RSBC 5	187.41	-3.212
PNBC 2	249.37	+28.787
PNBC 5	161.98	-16.346

Table 7.8
Mean concentrations of 2-3 rings PAH compounds (LMW-PAH) in turnip (n=3) on dry weight basis. Changes due to SSBC, SBBC, RSBC and PNBC amendments are given in percentage.

	Naphthalene		Acenaphthylene		Acenathphene		fluorene		Phenanthrene		Anthracene	
Treatments	Conc.(µg/kg)	Changes (%)	Conc.(µg/kg)	Changes (%)	Conc.(µg/kg)	Changes (%)	Conc.(µg/kg)	Change(%)	Conc.(µg/kg)	Changes (%)	Conc.(µg/kg)	Changes (%)
Control	301.5		33.52		32.46		62.16		271.69		60.88	
SSBC 2	234.20	-22.32	30.09	-10.23	28.77	-11.37	47.32	-23.87	240.37	-11.53	58.98	-3.12
SSBC 5	187.94	-37.67	18.32	-45.35	26.27	-19.07	36.47	-41.33	185.43	-31.75	51.56	-15.31
SBBC 2	88.32	-70.71	15.41	-54.03	35.36	8.92	47.41	-23.73	264.60	-2.61	59.46	-2.33
SBBC 5	68.81	-77.18	14.72	-56.09	31.93	-1.62	21.44	-65.51	75.30	-72.28	19.61	-67.79
RSBC 2	278.86	-7.51	31.93	-4.76	61.42	89.22	61.26	-1.45	244.25	-10.10	134.97	-42.55
RSBC 5	158.24	-47.51	8.69	-74.06	23.92	-26.30	32.80	-47.24	141.73	-47.83	25.23	-58.56
PNBC 2	24.11	-92.00	9.25	-72.41	11.17	-65.59	9.89	-84.08	79.14	-70.87	19.95	-67.23
PNBC 5	21.68	-92.81	8.05	-75.99	12.26	-62.22	11.31	-81.80	44.23	-83.72	11.63	-80.90

Table 7.9
Mean concentrations of 4 ring PAH compounds in turnip (n=3) on dry weight basis. Changes due to SSBC, SBBC, RSBC, PNBC amendments are given in percentage.

	Fluoranthene		Pyrene		Benzo(b)anthracene		Chrysene	
Treatments	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)
Control	87.63		86.96		138.55		99.95	
SSBC 2	76.43	-12.78	81.02	-6.83	90.51	-34.67	77.96	-22.00
SSBC 5	71.82	-18.04	88.09	1.30	74.43	-46.28	84.45	-15.51
SBBC 2	45.00	-48.65	63.87	-26.55	47.39	-65.79	54.34	-45.63
SBBC 5	36.46	-58.39	43.09	-50.45	21.28	-84.64	18.12	-81.87
RSBC 2	72.68	-17.06	82.84	-4.74	73.08	-47.25	62.23	-37.74
RSBC 5	65.28	-25.51	40.94	-52.92	43.41	-68.67	92.88	-7.08
PNBC 2	41.67	-52.45	43.14	-50.39	23.29	-83.19	22.69	-77.30
PNBC 5	24.31	-72.26	27.16	-68.77	9.71	-92.99	29.42	-70.57

Table 7.10
Mean concentrations of 5 ring PAH compounds in turnip (n=3) on dry weight basis. Changes due to SSBC, SBBC, RSBC and PNBC amendments are given in percentage.

	Benzo(b)fluoranthene		Benzo(k)fluoranthene-2		Benzo(a)pyrene	
Treatments	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)
Control	106.1		106.1		138.13	
SSBC 2	86.78	-18.21	96.78	-8.78	118.16	-14.46
SSBC 5	70.18	-33.85	73.18	-31.03	80.16	-41.97
SBBC 2	53.69	-49.39	53.71	-49.38	122.36	-11.42
SBBC 5	21.22	-80.00	40.26	-62.05	71.69	-48.10
RSBC 2	50.54	-52.36	64.66	-39.05	81.66	-40.88
RSBC 5	73.21	-31.00	73.20	-31.00	45.43	-67.11
PNBC 2	33.97	-67.98	52.72	-50.31	82.62	-40.18
PNBC 5	26.51	-75.01	26.50	-75.03	17.60	-87.26

Table 7.11 Mean concentrations of 6 ring PAH compounds in turnip (n=3) on dry weight basis. Changes due to SSBC, SBBC, RSBC and PNBC amendments are given in percentage.

	Indeno(1,2,3-c,d)pyrene		Dibenzo(a,h)anthracene		Benzo(g,h,i)perylene	
Treatments	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)	Conc.(μg/kg)	Changes (%)
Control	66.43		24.44		28.06	
SSBC 2	55.44	-16.54	19.44	-20.46	24.32	-13.33
SSBC 5	36.46	-45.12	9.45	-61.33	15.06	-46.33
SBBC 2	6.08	-90.85	6.59	-73.03	15.56	-44.54
SBBC 5	4.25	-93.60	3.14	-87.14	11.35	-59.56
RSBC 2	33.46	-49.63	6.54	-73.25	15.78	-43.77
RSBC 5	12.60	-81.03	3.20	-86.92	6.11	-78.24
PNBC 2	12.75	-80.80	3.21	-86.85	11.01	-60.75
PNBC 5	8.11	-87.80	1.51	-93.83	6.70	-76.11

Table 7.12 Concentration of toxic metals in turnip on DW basis. Increase or decrease changes due to SSBC, SBBC, RSBC and PNBC amendments are given in percentage.

	As		Cd		Cu		Fe		Pb		Zn	
Treatments	Conc.(mg/kg)	Changes (%)	Conc.(mg/kg)	Changes (%)	Conc.(mg/kg)	Changes (%)	Conc.(mg/kg)	Changes (%)	Conc.(mg/kg)	Changes (%)	Conc.(mg/kg)	Changes (%)
Control	0.22		0.14		3.51		146.41		0.33		70.13	
SSBC 2	0.12	-45.45	0.13	-7.14	3.28	-6.55	128.22	-12.42	0.27	-18.18	55.54	-20.80
SSBC 5	0.11	-50.00	0.10	-28.57	2.83	-19.37	119.04	-18.69	0.25	-24.24	54.69	-22.02
SBBC 2	0.12	-45.45	0.11	-21.43	3.13	-10.83	98.68	-32.60	0.31	-6.06	57.43	-18.11
SBBC 5	0.09	-59.09	0.09	-35.71	2.58	-26.50	74.35	-49.22	0.27	-18.18	41.69	-40.55
RSBC 2	0.15	-31.82	0.15	-21.43	2.69	-23.36	135.29	-7.60	0.26	-21.21	58.31	-16.85
RSBC 5	0.12	-45.45	0.12	-28.57	2.58	-26.50	112.93	-22.87	0.19	-42.42	44.16	-37.03
PNBC2	0.12	-45.45	0.12	-7.14	2.54	-27.64	124.33	-15.08	0.29	-12.12	60.60	-13.59
PNBC 5	0.09	-59.09	0.09	-42.86	2.40	-31.62	86.28	-41.07	0.15	-54.55	54.70	-22.00
SEPA limits	50		200		20				9		100	

Chapter- 8

Conculsions and suggestions

1. The research study was carry out to assess the concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in the soil and vegetable collected from 11 districts of Khyber Pakhtunkhwa Pakistan irrigated with wastewater. The sum of 16 total PAHs ranged from 222.6-928.6 μg kg^{-1} in the soils. The highest PAH concentration was observed in soil collected Peshawar District, while the lowest PAH concentration was recorded in Lakki Marwat's soil. The PAH concentrationsin vegetable was range from 51.6 to 402 μg kg^{-1}. The chrysene,benzo(b)fluoranthene, fluoranthene and pyrene were abundantly present in the soil samples whereasnaphthaene,fluoranthene,phenanthrene and pyrene were frequently observed in vegetable.The highest PAH concentrations were observed in leafy vegetable *L. sativa*L. > *S. oleracea* followingby underground bulb *A. cepa* ,*A. sativum*.The concentrations of high molecular weight polycyclic aromatic hydrocarbon compounds werelower in vegetable as compared to low molecular weight polycyclic aromatic hydrocarbon compounds.The highest total toxic B(a)P equivalent TEQ value was 4.1 for flouranthene followed by 3.8 for naphthalene in Khyber Pakhtunkhwa province.
2. The application of SS increased the uptake of PTEs and decreased theuptake ofPAHs in *C. sativa* L.grown in contaminated soil. However, the conversion of SS to SSBC significantly ($P \leq 0.01$) decreased PAH and available PTE concentrations. Uptake of PAHs in*C. sativa* L.was reduced by both SSBC (44.3-57.1%) as compared to SS (19.9-35.6%). The application of SSBC to soil significantly($P \leq 0.05$)decreasedavailable PTEs (except Cd), while SS significantly ($P \leq 0.05$) increased PTE availability as compared to SSBC significantly ($P \leq 0.05$)

reducedPTE bioaccumulation (except Cd), while SS increased PTE bioaccumulation.

3. The applications of biochar markedly increase the crop yield *of C. sativa* L.If the biochar are applied with the application rate of 5 %. In case of PAHs the Increase in biochar quantity will decrease the uptake of PAH, and 2 %and 5% biochars will effiecientetly sequester the PAH compounds.while incease in the concentration of Biochar will decrease the uptake of PTEs.
4. S. lycopersicum cultivated on wastewater irrigated soil.The mobility and uptake rate of toxic HMs and polycyclic aromatic hydrocarbons significantly reduced ($P \leqslant 0.01$) in the soil amended with SSBC and SS.uptake ofPAHs in S. lycopersicumwas decreased by both SSBC (48.0-62.4%) and SS (21.5-39.3%).The THMs reduced significantly ($P \leq 0.05$) in SSBC whereas significantly increased in SS amended soil.
5. The subsistence of pollutants such as PTEs and PAHs in the agriculture fields brings the consideration of scientists towards the application of various biomasses biochars to assess the effeteness in the removal of these contaminants.*B. rapa* L. was grown in PAHs and PTEs contaminated soil with the amendment of different biomass biochar to authenticate the bioavailability of these pollutants. The application ofdivergentbiomass biochars with different rates significantly ($p \leq 0.05$) reduced the PAH and PTEs concentration. The uptake of PAHs and PTEs in the *B.rapa*L. reduced upto 70.77-82.57% by addingPNBC whereas SBBC,SSBC and RSBC, decreased uptake by 40.46-69.43%, 10.24-48.51% and 16.91%-32.55 respectively. The biochars addition reduced significantly the accumulation of PTEsin *B. rapa*L..The efficiency of PNBC in reduction of PTEs are more significant ($p \leq 0.05$) in comparison with SSBC, RSBC , and SBBC biomass biochars. The trend of effecinecy of biochar is PNBC >SBBC>RSBC>SSBC in terms of reduction of PAHs/ PTEs.

6. Biochar application to soil improved crop yield and also sequester the organic pollutants such as PAhs and also reduce the loads of waste materials in the universe through converting of organic waste into biochars and kills the pathogens which is great environmental concerns with the direct use of organic biomass .Biochars as remediation can increase crop yield to eliminate pollutants to make the soil healthy. If the biochar is used in proper dose to soil, the results will be more fruitful and the uptake rate of PAH and HMs will be reduced, the health risks associated with the PAHs and HMs will be minimized in food crops. The results of our research summarize that peanut biochar is more effective in the minimization of PAHs uptake in food crops, if use in a correctmagnitude. The PAH bioaccumulation rate in biochars amended soilwere in order of PNBC >SBBC>RSBC>SSBC. Likewis HMs uptake can be reduced by increasing the application rate of different biochars. The bioaccumulation rate of HMs diverse because of the vegetables varieties, but all the biocharsfrom different biomassprovesimmenseefficiency in removal of HMs, the consequences depends on the application rate. With the increase of application rate more HMs will be entrained in the soil and less will be available for uptake in plants.

References

Ahmad, M., Lee, S.S., Yang, J.E., Ro, H.M., Lee, Y.H., Ok, Y.S., 2012.Effects of soil dilution and amendments (mussel shell, cow bone, and biochar) on Pb availability and phytotoxicity in military shooting range soil.Ecotoxicol. Environ. Saf. 79, 225-231.

Achakzai,A.K.K,Bazaid,Z.A.,2006.Phytoaccumulation of heavy metal in spinach(spinacea oleracea) irrigated with wastewater of Quetta city J.Chem.Soc.Pak.28,473-.478

Adhikari, B. K., Barrington, S., Martinez, J., and King, S., 2009.Effectiveness of three bulking agents for wood waste composting.Waste Manage. 29, 197-203.

Alber, L.J., Ravendra N., 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. J.Int.Biodeterioration & Biodegradation45, 57–88.

Alexander, M.,2000.Aging bioavailability and overestimation of risk from environmental contaminants Environ.Sci.Technol.34, 4259-4265.

Andrés, P., Mateos, E., Tarrasón, D., Cabrera, C., Figuerola, B., 2011. Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna.Appl. Soil Ecol. 48, 236-242.

Arias-Estevez, 2007.Sorption of PAHs to colloid dispersion of humic substances in water .McGraw Hill Higher Education.Fourth Edition. pp 315.

Arp, H.P.H., Azzolina, N.A., Cornelissen, G., Hawthorne, S.B., 2011. Predicting pore water EPA-34 PAH concentrations and toxicity in pyrogeniç-impacted sediments using pyrene content.Environ.Sci.Technol. 45, 5139-5146.

Atkinson,C. J., Fitzgerald, J. D., and Hipps, N. A.,2010. Potential mechanisms forachieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil, 337, 1-18.

Zohair, A., 2006. Levels of polycyclic aromatic hydrocarbons in Egyptian vegetables and their behavior during soaking in oxidizing agent solutions.World J. Agri. Sci. 2(1),90-94.

Berner, Jr., Bryner, N.P., Wise, S.A., Mulholland, G.H., Lao, R.C., Fingas, M.F., 1990. Polycyclic aromatic hydrocarbon emissions from combustion of crude oil on water.Environ. Sci. Technol. 24, 1418-1427.

Baran, S.,Oleszczuk, P., 2003.The concentration of polycyclic aromatic hydrocarbons in sewage sludge in relation to the amount and origin of purified sewage. Pol. J. Environ. Stud. 12, 523-529.

Baran, S., Bielin ska, E.J., Oleszczuk, P., 2004. Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons (PAH). Geoderma 118, 221-232.

Beesley, L., Moreno-Jiménez E., Gomez-Eyles J.,2010. Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environ.Pollut. 6, 2282–2287.

Beesley, L.,and Marmiroli, M.,2011. The immobilization and retention of solublearsenic, cadmium and zinc by biochar. Environ. Pollut.159, 474-480.

Bélanger, N.I.,Côté, B., Fyles, J.W., Chourchesne, F., Hendershot, W.H., 2004.Forest regrowth as the controlling factor of soil nutrient availability 75 years after fire in a deciduous forest of southern Quebec. Plant Soil 262, 363-372.

Bodzek, D.,Janoszka, B., Dobosz, C., Warzecha, L., Bodzek, M., 1997.Determination of polycyclic aromatic compounds and heavy metals in sludges from biological sewage treatment plants. J. Chromatogr. A 774, 177-192.

Borchard, N., Wolf, A., Laabs, V., Aeckersberg, R., Scherer, H. W., Moeller, A.,and Amelung, W., 2012.Physical activation of biochar and its meaning for soilfertility and nutrient leaching a greenhouse experiment. Soil Use Manage.28, 177-184.

Bosetti C., Boffetta B., La Vecchia C., 2007. Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to

2005. Ann. Oncol.18, 431-446.

Bossert, R. Bartha, 1984.Fate of hydrocarbons during oily sludge disposal in soil, Appl. Environ.Microbiol. 47, 763-767.

Bulter,T. A., Sikora, L. J., Steinhilber, P. M., and Douglass, L. W., 2001.Compost age and sample storage effects on maturity indicator of biosolids compost. J. Environ.Qual.30, 2124-2148.

Camargo MCR., Toledo MCF.,2003.Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control 14, 49-53.

Cao, X.and Harris, W., 2010.Properties of dairy-manure-derived biochar pertinentto its potential use in remediation.Bioresour.Technol.101, 5222-5228.

Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011.Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar.Environ.Sci.Technol.45, 4884-4889.

Caussy D, Gochfeld M, Gurzau E, Neagu C, Ruedele H, 2003. Lessons from case studies of metals: investigating exposure, bioavailability, and risk. Ecotoxicol. Environ. Saf. 56,45-51.

CEC, 2000. Council of the European Community, Working Document on Sludge, 3rd Draft, Brussels, 27 April, p. 20.

Chen, B., Yuan, M., 2011.Enhanced sorption of polycyclic aromatic hydrocarbons by soil amended with biochar. J. Soils Sediments. 11, 62-71.

Chen, B. L., Zhou, D. D., and Zhu, L. Z., 2008.Transitional adsorption and partition ofnonpolar and polar aromatic contaminants by biochars of pine needles withdifferent pyrolytic temperatures.Environ.Sci.Technol.42,5137-5143.

Chen, J., Zhu, D., Sun, C., 2007.Effect of heavy metals on the sorption of hydrophobic organic compounds to wood charcoal. Environ. Sci. and Technol. 41, 2536-2541.

Chen, L., Ran, Y., Xing, B., Mai, B., He, J., Wei, X., Fu, J., Sheng, G.,2005b.Contents and sources of polycyclic aromatic hydrocarbons and organochlorine pesticides in vegetable soils of Guangzhou, China.Chemosphere 60,879-890.

Chen, SC, Liao CM., 2006. Health risk assessment on human exposed to environmental polycyclic aromatic hydrocarbons pollution sources. Sci. Total Environ. 366, 112-123.

Chen, Y, Wang C, Wang Z., 2005.Residues and source identification of persistent organic pollutants in farmland soils irrigated by effluents from biological treatment plants. Environ.Int.31, 778-783.

Chen, B. L.,Yuan, M. X., and Qian, L. B., 2012.Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. J. Soils Sediments, 12, 1350-1359.

Chen, B. L., Chen, Z. M., and Lv, S. F., 2011.A novel magnetic biochar efficiently sorbsorganic pollutants and phosphate. Bioresour. Technol.102, 716-723.

Chen, B.L., and Yuan, M.X.,2011. Enhanced sorption of polycyclic aromatic hydrocarbons by soil amended with biochar. J. Soils Sediments, 11, 62-71.

Chun, Y., Sheng, G. Y., Chiou, C. T., and Xing, B. S., 2004.Compositions and sorptiveproperties of crop residue-derived chars. Environ.Sci.Technol.38, 4649-4655.

Cui, YJ., Zhu YG., Zhai RH., Chen DY., Huang YZ., Qiu,Y.,2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environ. Int.30, 785-791.

Clough,T.J., Condron,L.M., 2010.Biochar and the nitrogen cycle: Introduction.J. Environ. Qual. 39, 1218-1223.

Cornelissen, G., Gustafsson, O., 2004.Sorption of phenanthrene to environmental black carbon in sediment with and without organic matter and native sorbates.Environ.Sci, Technol. 38, 148-155.

Cornelissen, G., Gustafsson, O., 2005.Importance of unburned coal carbon, black carbon, and amorphous organic carbon to phenanthrene sorption in sediments. Environ. Sci.and Technol. 39,764-769.

Comunidad de Madrid, 2006. Plan Regional de Lodos de Depuradora (2006-2016) Comunidad de Madrid, Spain.

Chai, Y. Z.,Currie, R. J., Davis, J. W., Wilken, M., Martin, G. D., Fishman, V. N., 2012. The availability ron. Environ. Sci. and Technol.46, 1035-1043.

Cho, H. H., Wepasnick, K., Smith, B. A., Bangash, F. K., Fairbrother, D. H., andBall, W.P.,2010.Sorption of aqueous Zn[II] and Cd[II] by multiwall carbonnanotubes: the relative roles of oxygen-containing functional groups andgraphenic carbon.Langmuir 26, 967-981.

Cui, H. J., Wang, M. K., Fu, M. L., and Ci, E.,2011. Enhancing phosphorus availabilityin phosphorus-fertilized zones by reducing phosphate adsorbed on ferrihydriteusing rice straw-derived biochar, J. Soils Sediments 11, 1135-1141.

Dai, J., Xu, M.,Chen, J., Yang, X., Ke, Z., 2007. PCDD/F, PAH and heavy metals in the sewage sludge from six wastewater treatment plants in Beijing, China. Chemosphere. 66, 353-361.

Deluca, T. H., MacKenzie, M. D., and Gundale, M. J., 2009. Biochar effects on soilnutrient transformation, pp. 251-280, in: Lehmann, J. and Joseph, S. (Eds.),Biochar for environmental management: science and technology. Earthscan,London.

Dias, B. O., ilva, C. A.,Higashikawa, F. S.oig, A., and Sanchez- Monedero, M. A.,2010. Use of biochar as bulking agent for the composting of poultry manure: effect on organic matter degradation and humification.Bioresour. Technol. 101, 1239-1246.

Deenik, J. L., McClellan, T., Uehara, G., Antal, M. J., and Campbell, S., 2010.Charcoalvolatile matter conten influences plant growth and soil nitrogen trans- formations.Soil Sci. Soc. Am. J. 74, 1259-1270.

Dong, X. L., Ma, L. Q., and Li, Y. C., 2011.Characteristics and mechanisms of hex-avalent chromium removal by biochar from sugar beet tailing. J. Hazard.Mater. 190, 909-915.

Downie,A., Crosky A., and Munroe P., 2009. Physical properties of biochar, In Biochar for Environmentalmanagement: science and technology Eds. J. Lehmann and S. Joseph.Earthscan, London; Sterling, VA, pp. 13-32.

Edwards N.N., 1983. Polycyclic aromatic hydrocarbon (PAHs) in the terrestrial environment A review.J.Environ.Qual.12, 427-441.

Edwards, N.T., 1986. Uptake, translocation and metabolism of anthracene in bush bean (Phaseolus vulgaris L.). Environ.Toxicol.Chem. 5, 659-665.

Edwards, N. T., 1988. Assimilation and metabolism of polycyclic aromatic hydrocarbons by vegetation.An approach to this controversial issue and suggestions for future research. P. 211-229.

Elad, Y., David, D. R., Harel, Y. M., Borenshtein, M., Kalifa, H. B., Silber, A., andGraber, E. R.,2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent, Phytopathology, 100, 913-921.

Elad, Y., Cytryn, E., Harel, Y. M., Lew, B., and Graber, E. R., 2011. The biochar effect:plant resistant to biotic stresses Phytopathol. Mediterr.50, 335-349.

Eljarrat, E., Marsh, G., Labandeira, A., Barceló, D., 2008. Effect of sewage sludgescontaminated with polybrominated diphenylethers on agricultural soils. Chemosphere 71, 1079-1086.

Ensink JHJ, Mahmood T, van der Hoek W, Raschid-Sally L &Amerasinghe FP.,2004. A nation-wide assessment of wastewater use in Pakistan: Water Policy 6 197-206.

Eweis, J.B., S.J. Ergas, D. P. Y. Chang, and E.D. Schroeder. 1998. Bioremediation principles. McGraw-Hill, Boston, MA.

Fagbemi, L., Khezami, L., Capart, R., 2001. Pyrolysis products from different biomasses: application to the thermal cracking of tar. Appl. Energ. 69, 293-306.

Farooq, S., Ali-Musstjab-Akber-Shah Eqani, S., Malik, R. N., Katsoyiannis, A., Zhang, G., Zhang, Y., Li, J., Xiang, L., Jones, K. C., & Shinwari, Z. K., 2011.Occurrence, finger printing and ecological risk assessment of polycyclic aromatic hydrocarbons (PAHs) in the Chenab River, Pakistan. J.of Envirn. Monitor.13(11), 3207-3215.

FAO, 2002.Investment in Land and Water in Proceedings of the regional consultation Bangkok, Thailand 3-5 October, 2001. Publication 2002/09. FAO Regional Office for Asia and the Pacific, Bangkok.

FAO, 2011.Major food and agricultural commodities and producers-countries by commodity. Food and Agriculture Organization. http://www.fao.org. Retrieved on 2013-03-19.

Ferrarese,E.,G.Andre Ottola, and I.A.Opera 2008 Remediation of PAH-contaminated Sediments by chemical oxidation. J. of hazard Mater.152, 128-139.

Feng,Y.,Xu, Y.,Yu, Y.,Xie, Z.,Lin, X., 2012.Mechanisms of biochar decreasing methane emission from Chinese paddy soils.Soil Biol. Biochem.46, 80-88.

Fismes, J., Perrin-Ganier C., Empereur-Biosoonnet P., Morel JL., 2002.Soil to Root transfer and translocation of polycyclic aromatic hydrocarbons by vegetables grown on industrial contaminated soils. J Environ. Qual. 31, 1649-1656.

Fonts, I., Azuara, M., Gea, G., Murillo, M.B., 2009. Study of the pyrolysis liquids obtained from different sewage sludge. J. Anal. Appl. Pyrol. 85, 184-191.

Freddo, A., Cai, C., Reid, B.J., 2012.Envirmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environ.Pollut. 171, 18-24.

Fryer, ME and Collins, CD.,2003.Model intercomparison for the uptake of organic chemicals by plants. Environ. Sci. Tech.; 37:1617-1624.

Fytili, D., Zabaniotou, A., 2008.Utilization of sewage sludge in EU application of old and new methods-A review. Renew. Sust. Energ. Rev. 12, 116-140.

Gao, Y., Zhu L., 2004.Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55, 1169-1178.

Gascó,G., Blanco, C.G., Guerrero, F., Méndez, A., 2005b.The influence of organ matter on sewage sludge pyrolysis. J. Anal. Appl. Pyrol. 74, 413-420.

Ge, KY., 1992.The status of nutrient and meal of Chinese in the 1990s.Beijing People's hygiene press. p. 415-34.

Glaser, B., Lehmann, J., Zech, W., 2002.Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal e a review. Biology and Fertility of Soils 35, 219-230.

Gomez-Eyles,J. L., Sizmur, T.,Collins, C. D.,and Hodson, M. E.,2011. Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements.Environ. Pollut.159,616-622.

Harvey, R.R.G., 1997.Polycyclic Aromatic Hydrocarbon; Johan Wiley & sons; New York.

Harmsen, J., 2007. Measuring bioavailability: From a scientific approach to standard methods. J.Environ. Qual. 36, 1420-1428.

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvist, K. L., Arp, H. P. H., Cornelissen, G., 2012.Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars.Environ.Sci. Technol.46,2830-2838.

Hernández-Apaolaza, L.,Gascó, J.M., Guerrero, F., 2000. Initial organic matter transformation of soil amended with composted sewage sludge. Biol. Fert. Soils 32, 421-426.

Harel, Y. M., Elad, Y., Davide, D. R., Borenstein, M., Shulchani, R., Lew, B., and Graber, E. R.,2012.Biochar mediates systemic response of strawberry to foliar fungal pathogens. Plant Soil, 357, 245-257.

Budzinski, H., Jones, I., Bellocq, J., Piérard, C., Garrigues, P. 1997, Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. Mar.

Chem. 58 85-97.

Soclo, H.H., Garrigues, P.H., EWALD, M. 2000 Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas, Mar. Pollut. Bull. 40, 387-396.

Hilber, I.,Blum, F., Leifeld, J., Schmidt, H-P., Bucheli, T.D., 2012. Quantitative determination of PAHs in biochar: A prerequisite to ensure its quality and safe application. J. Agric. Food Chem. 60, 3042-3050.

Hiller, E.,Fargasova, A., Zemanova, L., Bartal, M., 2007. Influence of wheat ash on the MCPA immobilization in agricultural soils. Bulletin of Envirn.Contam. Toxicol.78,345-348.

Howsam, M., Jones, K.C., Ineson, P., 2001. PAHs associated with the leaves of three dediduous tree species. II: uptake during a growing season. Chemosphere 44, 55-164.

Hua, J., Wua, s., Cao, z., Lin,X., Wang,M.H.,2013. Bioaccessabiliy, dietary exposure and human risk assessment of heavy metal from market vegetables in the Hong Kong revealed with an in vitro gastro intestinal model.Chemosphere 91, 455-461.

IBI ,2012. The International Biochar Initiative standardized product definition and product testing guidelines for biochar that is used in soil. Final version (IBI-STD-01: Revision date 15 May 2012).Retrievedon 31.07.2012. http://www.biochar-international.org/sites/default/files/Guidelines_for_Biochar_That_Is_Used_in_Soil_Final.pdf

IBI, 2010.International Biochar Initiative: http://www.biochar-international.org/pressImran M., Khan S., Khokhar K.M., and Hussain S.I.,2011.Growing Turnips for Feeding Livestock Science, Technology & Development Vol. 30, No. 1 .

Inyang, M., Gao, B., Ding, W. C., Pullammanappallil, P., Zimmerman, A. R.,and Cao, X. D., 2011. Enhanced lead sorption by biochar derived from anaerobicallydigested sugarcane bagasse. Sep.Sci. Technol. 46, 1950-1956.

Jan, FA., Ishaq M., Ihsanullah I., Asim SM,. 2009. Multivariate statistical analysis of heavy metals pollution in industrial area and its comparison with relatively less polluted area: A case study from the City of Peshawar and District Dir Lower. J. Hazard. Mater.76, 609-616.

Jacobson, M.Z., 2010. Short-term effects of controlling fossil-fuel soot, biofuel sootand gases, and methane on climate, arctic ice, and air pollution health. J.Geophys. Res. 115,D14209, doi:10.1029/2009JDO13795.

Jiang, J., Xu, R. K., Jiang, T. Y., and Li, Z., 2012. Immobilization of Cu (II), Pb (II) and Cd(II) by the addition of rice straw derived biochar to a simulated polluted Ultisol.J. Hazard.Mater. 229-230, 145-150.

Jiang, J., Xu, R-K., 2013. Application of crop straw derived biochars to Cu(II) contaminated Ultisol: Evaluating role of alkali and organic functional groups in Cu(II) immobilization,Bioresour.Technol.doi:http://dx.doi.org/10.1016/j.biortech.2013.01.161.

Jian, Y, Yong L, Peter PF, Yu HT., 2004.Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. Mutat Res. 557, 99-108.

Neff, J.M., 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates and Biological Effects, Applied Science Publishers, London.

Jones, K.C., Alcock, R.E., Johnson, D.L., Northcott, G.L., Semple, K.T., and Woolgar, P.J., 1996.Organic chemicals in contaminated land: analysis, significance and research priorities. Land Contam. Reclam. 4, 189-197.

Jones, K.C., J.A. Stratford, K.S.Waterhouse, and N.B. Vogt. 1989a.Organic contaminants in Welsh soils: polynuclear aromatic hydrocarbons. Environ. Sci. Technol. 23,540-550.

Jose, L.,Gomez-Eyles, Tom Sizmur, Chris D. Collins, Mark E. Hodson 2011.Effects of biochar and the earthworm Eisenia fetida on the bioavailability of polycyclic aromatic hydrocarbons and potentially toxic elements.Environ.Pollut. 159, 616-622.

Kapusta, LA., 2004.Establishing eco-ssls for PAHs: lessons revealed from a review of literature on exposure and effects to terrestrial receptors. Human Ecolog Risk Assess.10, 185-205.

Karami, N., Clemente, R., Jimenez, E. M., Lepp, N. W., and Beesley, L.,2011.Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J. Hazard.Mater. 191, 41-48.

Keech, O., Carcaillet, C., Nilsson,M.C., 2005. Adsorption of allelopathic compounds bywood-derived charcoal: the role of wood porosity. Plant and Soil 272, 291-300.

Keiluweit, M.,Kleber, M., Sparrow,M.A., Simoneit,B.R.T., Prahl, F.G., 2012. Solvent-extractable polycyclic aromatic hydrocarbons in biochar:Influence of pyrolysis temperature and feedstock.Environ.Sci.Technol.46, 9333-9341.

Keith,A., Singh,B.,Singh,B.P., 2011.Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil.Environ.Sci.Technol.45,9611-9618.

Khan, A., Ishaq, M., , Murad,K.A.,2008 .Effect of vehicle exhaust on the quality of PAHs in soil.J. of Envirn. Monit and Assess.137,363-369.

Khan, S., Aijun, L., Zhang, S., Hu, Q., Zhu, Y-G., 2008. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. J. Hazard.Mater. 152, 506-515.

Khan, S., Lin AJ., Zhang, S., Hu, Q, Zhu, YG., 2007. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. J. Hazard.Mater.152, 506-515.

Khan S., Wang, N., Reid, B.J., Freddo, A., Cai, C., 2013. Reduced bioaccumulation of PAHs by Lactuca satuva L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar. Environ. Pollut.175, 64-68.

Keiluweit, M., Kleber, M., Sparrow, M.A., Simoneit, B.R.T., Prahl, F.G., 2012. Solvent-extractable polycyclic aromatic hydrocarbons in biochar: Influence of pyrolysis temperature and feedstock Environ.Sci.Technol.46, 9333-9341.

Keith, A., Singh, B., Singh, B.P., 2011.Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil.Environ.Sci.Technol.45, 9611-9618.

Kipopoulou, A.M., Manoli, E., Samara, C., 1999. Bioconcentration of PAHs in vegetables grown in an industrial area. Environ.Pollut. 106, 369-380.

Kong, H. L., He, J. Gao, Y. Z., Wu, H. F., and Zhu, X. Z.,2011 Cosorption of phen- anthrene and mercury from aqueous solution by soybean stalk-based biochar.J. Agric. Food Chem., 59, 12116-12123.

Kookana, R.S., Sarmah, A.K., Van Zwieten, L., Krull, E., Singh, B., 2011. Biochar Application to Soil: Agronomic and Environmental Benefits and Unintended Consequences. Adv. Agron. 112, 103-143.

Kuo, C.Y., Hsu, Y.W., and Lee H.S., 2003. Study of human exposure to particulate PAHs using Personal air samplers. Archives of Environmental Contamination and Toxicology 44, 454-459.

Kwon,S., and Pignatello, J. J., 2005.Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): pseudo pore blockage by model lipid components and its implications for N-2- probed surface properties of natural sorbents. Environ.Sci.Technol.39, 7932-7939.

Klasson K T.,Wartelle, L. H., Rodgers,J. E., III, and Lima, I. M., 2009.Copper(II) adsorption by activated carbons from pecan shells effect of oxygen level during activation. Ind. Crop Prod.30, 72-77.

Kumar, S., Loganathan, V. A., Gupta, R. B., and Barnett, M. O.,2011. An assessment of U (VI) removal from groundwater using biochar produced from hydrothermal carbonization.J. Environ. Manage. 92, 2504-2512.

Lal R.,1995.The role of residue management in sustainable agricultural systems. J Sustain Agric.5, 51-78.

Lal R., 2005.World crop residues production and implications of its use as a biofuel.Envirn. Intern.31, 575-584.

Larson, WE., Swan, JB., Pierce, FJ., 1982. Agronomic implications of using crop residues for energy. In: Lockertz W, editor.Agriculture as a producer and consumer of energy. AAAS Selected Symposium.78,91-122.

Latawiec, A.E., Reid B.J., 2009. Beyond contaminated land assessment: On costs and benefits of bioaccessibility prediction. Environ. Int. 35, 911-919.

Lehmann, J., 2007.A handful of carbon, Nature, 447, 143-144.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology.Earthscan, London,1-12.

Lehmann, J., Gaunt, J., and Rondon, M., 2006.Bio-char sequestration in terrestrialecosystems ea review. Mitig. Adapt.Strat. Gl., 11, 403-427.

Lehmann, J., Rillig, M. C.,Thies, J.,Masiello, C.A. Hockaday, W. C., andCrowley, D.,2011.Biochar effects on soil biota ea review.Soil Biol. Biochem. 43,1812-1836.

Li,Y.T., Li, F.F., Chen J.J, Yang, G.Y., and Wan H.Y., 2008. The Concentrations, distribution and sources of PAH in agriculture soil and vegetables from Shunde, Guang dong,China.Environ.Monit.Assess.139,61-76.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Thies, J.O., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70, 699-709.

Luo, L.,Lou, L. P., Cui X. Y., Wu, B. B., Hou, J., Xun, B., Xu, X. H., andChen, Y. X., 2011.

Sorption and desorption of pentachlorophenol to black carbon ofthree different origins.J. Hazard Mater.185, 639-646.

L.Z. Zhu, Y.Y. Chen, R.B. Zhou 2008.Distribution of polycyclic aromatic hydrocarbons in water, sediment and soil in drinking water resource of Zhejiang Province China. J. Hazard. Mater.150,308-316.

Lu, H. L., Zhang, W. H., Yang, Y. X., Huang, X. F., Wang, S. Z., and Qiu, R. L. ,2012. Relative distribution of Pb2+ sorption mechanisms by sludge-derived biochar.Water Res., 46, 854-862.

Liang, B., Lehmann, J., Solomon, D., Kinyang, J., Grossman, J., O'Neill, O.,Skjemstad, J. O., Thies, J., Luizao, F. J., Petersen, J., and Neves, E. G., 2006. Blackcarbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J., 70,1719-1730.

Maliszewska-Kordybach, B.,1996. Polycyclic aromatic hydrocarbons in agricultural soils in Poland: preliminary proposals for criteria to evaluate the level of soil contamination. Appl.Geochem.11, 121-127.

Mansour. S.A., Belal M.H., Abou-Arab A.A.K., Gad M.F., 2009. Monitoring of pesticides and heavy metals in cucumber fruits produced from different farming systems. Chemosphere, 75, 601-609.

Martorell, G. Perelló, R. Martí-Cid, J.M. Llobet, V. Castell, J.L. Domingo 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend.

Marris, E., 2006.Putting the carbon back: black is the new green, Nature, 442, 624-626.

Meharg, A.A.,Macnair, M.R., 1992.Suppression of the high-affinity phosphate-uptake system- a mechanism of arsenate tolerance in Holcus lanatusL.J. Exp. Bot.43,519-524.

Méndez, A., Gascó, G., Freitas, M.M.A., Siebielec, G., Stuczynski, T., Figueiredo, J.L., 2005.

Preparation of carbon-based adsorbents from pyrolysis and air activation of sewage sludges. Chem. Eng. J. 108, 169-177.

McNaughton, I.H. 1976. Turnip and relatives. In: Evolution of crop plants. N.W. Simmonds (ed.). Longman, London and New York.

Méndez, A., Gómez, A., Paz-Ferreiro, J., Gascó, G., 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere,89, 1354-1359.

MEP, 2007.Standard of soil quality assessment for exhibition sites.Ministry of Environmental Protection, the People Republic of China. (HJ 350-2007) (in Chinese).Accessed on 21.03.2013.

Micheal, D., Aitken, Shu-hwa Chen,Chikoma kazunga and Randal,B.,Marx 2004. Bacterial, Biodegradation of High Molecular Weight Polycyclic Aromatic Hydrocarbon, "SUPERFUND BASIC RESEARCHPROGRAMME (http://cmr.sph.unc.edu/SBRPI).

Mielke, H.W., Wang, G.D., Gonzales, C.R., Powell, E.T., Le, B., Quach, V.N., 2004. Distributions of polycyclic aromatic hydrocarbons in surface PAHs and metals in the soils of inner-city and suburban New Orleans, Louisiana, USA. Environ.Toxicol. Pharm 18, 243-247.

Menzie, C.A., Potocki, B.B., and Santodonato J., 1992.Exposure to carcinogenic PAHs in theenvironment. Environ. Sci. and Technol. 26(7),1278-1284.

Mackay, D., andCallcott D., 1998. Partitioning and physical chemical properties of PAHs.

A.H. Neilson (ed.) The handbook of environmental chemistry: PAHs and relatedcompounds. Springer, New York, NY. 325-346.

Maliszewska-Kordybach, B., 1993.The persistence of polycyclic hydrocarbons in soils.Institute of Soil Science and Plant Cultivation, Pulawy (in Polish).

Mendez, A., Gomez, A., Ferreiro, J. P., and Gasco, G., 2012. Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere, 89, 1354-1359.

Muel B., Saguem S., 1985.Determination of 23 polycyclic aromatic hydrocarbons in atmospheric particulate matter and of Paris area and photolysis by sunlight.Environ.Sci.Technol.19, 111-131.

Nguyen, B. T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S. J., andEngelhard, M. H., 2009.Long-term black carbon dynamics in cultivated soil.Biogeochemistry, 92,163-176.

Neary, D.G., Klopaket, C.C., DeBano, L.F., Folliot, P.F., 1999. Fire effects of belowground sustainability: a review and synthesis. Forest Ecology and Management 122, 51-71.

Nicholson, FA., Smith, SR., Alloway, BJ., Carlton-Smith, C., Chambers, BJ., 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci. Total Environ. 311, 205-219.

Novak, J. M., Busscher, W. J., Watts, D. W., Laird, D. A., Ahemdna, M. A., andNiandou, M.A. S.,2010.Short-term CO2 mineralization after additions of biocharand switchgrass to a Typic Kandiudult.Geoderma, 154, 281-288.

Nguyen, B. T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S. J., andEngelhard, M.H., 2009.Long-term black carbon dynamics in cultivated soil,Biogeochemistry, 92 163-176.

Noguera,D., Barot, S., Laossi, K. R., Cardoso, J., Lavelle, P., and Cruz deCarvalho, M. H., 2012. Biochar but not earthworms enhances rice growth throughincreased protein turnover, Soil Biol. Biotech.52, 13-20.

Oleszczuk,P.,Baran,S.,2004a.The concentration of mild-extracted polycyclic aromatichydrocarbons in sewage sludges. J. Environ. Sci. Heal. A 39, 2799-2815.

Oleszczuk, P., Hale, S.E., Lehmann, J., Cornelissen, G., 2012. Activated carbon and biochar amendments decrease pore-water concentrations of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge. Bioresour. Technol. 111, 84-91.

Papadopoulos, A., Paton, G.I., Reid, B.J., Semple, K.T., 2007. Prediction of PAH biodegradation in field contaminated soils using a cyclodextrin extraction technique. J. Environ. Monitor. 9, 516-522.

Peng,X.,Ye,L.L.,Wang,C.H., Zhou,H., Sun,B.,2011.Temperature and durationdependent rice straw-derived biochar: characteristics and its effects on soilproperties of an Ultisol in southern China. Soil Till. Res.112, 159-166.

Peter, C., Metre V., Barbara, J., Mahler, Furlong A., 2000 Urban Sprawl Leaves It's PAH Signature.Environ.Sci.Technol.34, 4064-4070.

Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sorensen, J.,Madsen, T., Petersen, J., Gron, C., 2003.Recycling of sewage sludge and household compost to arable land, fate and e□ects of organic contaminants and impact on soil fertility. Soil Till. Res. 72, 139-152.

Pierzynski, G.M., Sims, J.T., and Vance G.F., 2000. Soils and environmental quality, SecondEdition. CRC Press, Boca Raton, FL.

Peng, Y. S., Brown, M. A., Wu, J. P., Liu, Z., 2010. Different oilseed supplements alter fatty acid composition of different adipose tissues of adult ewes. Meat Science, 85 (3), 542-549.

Qiu,Y.,Zheng,Z.,Zhou,Z.,andSheng,G.D.,2009.Effectivenessandmechanisms ofdye adsorption on a straw-based biochar, Bioresour. Technol.,100, 5348-5351

Quilliam,R.S.,Marsden,K.A.,Gertler,C.,Rousk,J., DeLuca,T.H., Jones,D.L., 2012. Nutrient dynamics microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric. Ecosyst. Environ. 158, 192-199.

Rayment, G.E., Higginson, F.R.,1992.Australian Laboratory Handbook of Soil and Water Chemical Methods.Inkata Press, Melbourne, Australia.

Raton,FL., Stokes, J.D.,Paton G.I., and Semple K.T., 2006. Behavior and assessment of bioavailability oforganic contaminants in soil: relevance for risk assessment and remediation. Soil Use Manage. 21, 475-486.

Rayment, G.E., Higginson, F.R., 1992.Australian Laboratory Handbook of Soil and Water Chemical Methods.Inkata Press, Melbourne, Australia.

Regmi, P., Moscoso, J. L. G., Kumar, S., Cao, X. Y., Mao, J. D., and Schafran, G., 2012. Removal of copper and cadmium from aqueous solution using switchgrassbiochar produced via hydrothermal carbonization process.J. Environ. Manage.109, 61-69.

Reilly, M., 2001.The case against land application of sewage sludge pathogens. Canadian Journal of Infectious Diseases 12, 205-207.

Reid,B.J., K.C. Jones, and K.T. Semple 2000a.Bioavailability of persistent organic contaminants in soils and sediments - a perspective on mechanisms, consequences and assessment.Environ.Pollut.108,103-112.

Ritter,L.,Solomon,K.R,Forget.J,Stemeroff,M.,O'leary,C.,1995.persistant organic Pollutants. Prepared for the international programme on chemical safety (IPS) within the framework of the inter-organization program me for the sound management of chemicals (IOMC) united nation environmental programme.

Roca-Pérez, L., Martínez, C., Marcilla, P., Boluda, R., 2009.Composting rice straw with sewage sludge and compost effects on the soil-plant system. Chemosphere 75, 781-787.

Romanyà, J., Rovira, P., 2011.An appraisal of soil organic C content in Mediterranean agricultural soils. Soil Use Manage. 27, 321-332.

Salanitro, J. P., Dorn, P. B., Huesemann, M. H., Moore, K. O., Rhodes, I. A., Jackson, L. M. R., 1997.Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. Environ. Sci. Technol. 31, 1769-1776.

Samsoe-Peterson, L., Larsen , E.H., Larsen, P.B., Bruun, P., 2002. Uptake of trace elements and PAHs by fruit and vegetables from contaminated soils.Envion.Sci.Technol.3, 3057-3063.

Sadia, R.T., Munir, H.S., Shaheen, N. A., Khlique, A., Manzoor, S., Jaffar,M.,2006.Multivariate analysis of heavy metal level in tannery effluents in relation to soil and water. J. Environ. Manage. 79, 20-29.

Sanders, M.,Sivertsen, S., Scott, G., 2002 Origin and distribution of polycyclic aromatic hydrocarbons in surficial sediments from the Savannah River. Arch. Environ.Contam. Toxicol. 43, 438-448.

Sanchez,M. P., andUtrilla,J. R.,2002. Adsorbenteadsorbate interactions in theadsorption of Cd(II) and Hg(II) on ozonized activated carbons, Environ.Sci.Technol.36, 3850-3854.

Sánchez Monedero, M.A., Mondini, C.D., De Nobili, M., Leita, L., Roig, A., 2004. Land application of biosolids: soil response to different stabilization degree or treated organic matter. Waste Manage. 24, 325-332.

Sánchez, M.E., Menéndez, J.A., Domínguez, A., Pis, J.J., Martínez, O., Calvo, L.F., Bernad, P.L., 2009. Effect of pyrolysis temperature on the composition of the oils obtained from sewage sludge. Biomass Bioenergy 33, 933-940.

Sander, M.,and Pignatello, J.J., 2005. Characterization of charcoal adsorption sites for aromatic compounds: Insights drawn from single and bi-solute competitive experiments. Environ. Sci. Technol. 39, 1606-1615.

SEPA, 1995.Environmental quality standard for soils. State Environmental Protection Administration, China.GB15618-1995.

SEPA, 2005.The Limits of Pollutants in Food. State Environmental Protection Administration, China.GB2762-2005.

Shackley, S., Sohi, S.P. , 2010. An assessment of the benefits and issues associated with the application of biochar to soil. Report to the Department for Environment, Food and Rural Affairs and the Department of Energy and Climate Change, London

.Sheng, G., Yang, Y., Huang, M., and Yang, K., 2005. Influence of pH on pesticide sorption by soil containing wheat residue-derived char. Environ.Pollut.134, 457-463.

Smith, S.R., 2009. Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. Philos. T. R. Soc. A 367, 4005-4041.

Sneath, H.E., Hutchings, T.R., de Leij, F.A.A.M., 2013. Assessment of biochar and iron filing amendments for the remediation of a metal, arsenic and phenanthrene co-contaminated soil. Environ.Pollut. 178, 361-366

Sohi, Reid, B.J., Jones, K.C., and Semple K.T., 2000a. Bioavailability of persistent organiccontaminants in soils and sediments - a perspective on mechanisms, consequences andassessment. Environ. Pollut.108, 103-112.

S.P.; Krull, E.; Lopez-Capel, E. & Bol, R.,2010.A review of biochar and its use and function in soil. In: Advances in Agronomy, Publisher Elsevier Academic Press Inc., ISSN 0065-2213, San Diego, CA-92101-4495, and USA. PP. 47-82.

Spinosa, L., 2011. Wastewater Sludge: a Global Overview of the Current Status and Future Prospects. IWA Publishing Ltd, Alliance house 12 Caxton St, London, pp. 28.

Steinbeiss, S., Gleixner, G., Antonietti, M., 2009.Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41, 1301-1310.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G., Jones, K.C., 2003. PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks and polychlorinated n-alkanes in UK sewage sludge, survey results and implications. Environ. Sci. Technol. 37, 462-467.

Salanitro, J. P., Dorn, P. B., Huesemann, M. H., Moore, K. O., Rhodes, I. A., Jackson, L. M. R., 1997.Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. Environ. Sci. Technol. 31, 1769-1776.

Scott, A. C., Zarazoa J. A., and Levine G., 2000.Urban-wastewater reuse for crop roduction in the water short Guanajuato river basin, Mexico. IWMI Research report 43. Colombo, Sri-Lanka: International Water Management Institute.

Singh, A.,Rajesh, k.S.,Madhoolika, A.,Fiona.,M.M., 2010.Health risk assessment of heavy metals via dietary intake of food stuffs from the wastewater irrigated site of a dry tropical area of india. Food chem. Toxicol.48, 611-619.

Singh, K.P., Mohan, D., Sinha s., dalwani, r., 2004.impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants to health agricultural and environmental quality in the wastewater disposal area.chemosphere 55,227-255.

Scott, A.C., J. A., andLevine G., 2000. Urban wastewater reuse for crop production in the water short Guanajuato river basin, Mexico. IWMI Research report 43. Colombo, Sri-Lanka: International Water Management Institute.

Simonich, S.,Hites, R.A.,1995.OrganicPollutantsAccumulation in Vegetation.Environ.Sci.Technol.29, 2905-2914.

Song, YF., Wilke, BM., Song, XY., Gong, P, Zhou, QX., Yang, GF., 2006.Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (HMs) as well as their genotxicity in soil after long-term wastewater irrigation.Chemosphere, doi:10.1016/j.chemosphere.2006.03.076.

Sort, X., Alcañiz, J.M., 1999. Effects of sewage sludge amendment on soil aggregation. Land Degrad. Dev. 10, 3-12.

Stevens, D., Unkovich,M., Kelly, J., and Ying, G., 2004. Impacts on soil, groundwater and surface water from continued irrigation of food and turf crops with water reclaimed from sewage, Australian Water Conservation and Reuse Research Program.

Stout BA., 1984.Energy use and management in agriculture.North Scituate, MA7 Breton Publishers.

Shi,Z., Tao,S., Pan ,B., Fan, W., He, X.C., Zuo, Q., Wu, S.P., Li, B.G., Cao,J., Liu, W.X., Xu, F.L., Wang, X.J., Shen, W.R., Wong, P.K., 2005.Contamination of rivers in Tianjin, china by polycyclic aromatic hydrocarbons. Environ. Pollut.134, 97-111.

USEPA 2002.Polycyclic organic matter. Environmental Protection Agency, Washington, 2002. http://www.epa.gov/ttn/atw/hlthef/ polycycl.html. Accessed 10 June 2011

Uyttebroek, M., A. Spoden, J. Ortega-Calvo, K. Wouters, P. Wattiau, L. Bastiaens, and D. Springael. 2007. Differential responses of eubacterial, Mycobacterium, and Sphingomonas communities in polycyclic aromatic hydrocarbon (PAH)-contaminated soil to artificially induced changes in PAH profiles. J. Environ. Qual. 36, 1403-1411.

Tsai, P.J., Shieh H.Y., Lee, W.J., Lai S.O., 2001. Health risk assessment for workers exposed to polycyclic aromatic hydrocarbon (PAHs) in carbon black manufacturing industry. Sci.Total Environ.278137-150.

Uchimiya, M., Chang, S., Klasson, K.T., 2011a. Screening biochars for heavy metals retention in soil: Role of oxygen functional groups. J. Hazard.Mater. 190, 432-441.

Uchimiya, M., Lima, I. M., Klasson, K. T. Chang, S., Wartelle, L. H., andRodgers, J. E., 2010.Immobilization of heavy metal ions (Cu□, Cd□, Ni□, and Pb□) bybroiler litter-derived biochars in water and soil. J. Agric. Food Chem.8,5538-5544.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Fortier, C. A., and Lima, I. M.,2011.Influence of pyrolysis temperature on biochar property and function as a heavymetal sorbent in soil. J. Agric. Food Chem.59, 2501-2510.

Uchimiya, M.,Bannon, D.I., and Wartelle, L. H.,2012.Retention of heavy metals by carboxyl functional groups of biochars in small arms range soil. J. Agric. FoodChem., 60, 1798-1809.

Uchimiya,M., Wartelle, L.H., and Boddu, V.M.,2012.Sorption of triazine andorganophosphorus pesticides on soil and biochar. J. Agric. Food Chem.60,2989-2997.

USEPA, 1995.Process design manual, land application of sewage sludge and domestic septage.United States Environmental Protection Agency, Office of Research and Development, Washington, DC 20460, EPA/625/R-95/001.

Uzoma, K.C., Inoue, M., Andry, H., Fujimaki, H.,Zahoor,A.,andNishihara, E., 2011.Effect of cow manure biochar on maize productivity under sandysoil condition. Soil Use Manage.27, 205-212.

Verheijen, F. G. A., Jeffery, S., Bastos, A. C., Van der Velde, M., and Diafas, I., 2010.Biochar application to soilsa critical scientific review of effectson soilproperties, processesand functions.EUR 24099 EN.Office for the OfficialPublications of the European Communities, Luxembourg.

Wang, X., Sato, T., Xing, B., Tao, S., 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci. Total Environ. 350, 28-37.

Wang, X., Chen, T., Ge, Y., Jia, Y., 2008.Studies on land application of sewage sludge and its limiting factors. J. Hazard.Mater. 160, 554-558.

Wang, YC., Qiao, M., Liu, Y-X., Arp, HPH., Zhu, Y-G.,2011 Comparison of polycyclic aromatic hydrocarbon uptake pathways and risk assessment of vegetables from waste-water irrigated areas in northern China. J. Environ. Monit. doi:10.1039/c0em00098a.

Wang,X.J., Xu, X.M., Liang, X. , Wang, Y., Liu, M., Wang, X., Xia, S.Q., Zhao, J.F., Yin, D.Q., Zhang, Y.L., 2011.Adsorption of copper (II) onto sewage sludge-derived materials via microwave irradiation. J. Hazard.Mater. 192, 1226-1233

Wen, B., Li, R. J., Zhang, S. Z., Shan, X. Q., Fang, J., Xiao, K., and Khan, S. U.,2009.Immobilization of pentachlorophenol in soil using carbonaceous materialamendments. Environ.Pollut. 157, 968-974.

Wenzl, T.,Simon, R.,Anklam, E.,Kleiner, J., 2006.Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union Trends. Anal. Chem. 25 (7),716-725

Westeman, P. W. and Bicudo J. R., 2005.Management considerations for organicwastes use in agriculture, Bioresour. Technol.96, 215-221.

Wild, E., dent, J., Barber, JL., Thomas, GO., Jones, KC., 2004 A novel analytical approach for visualizing and tracking organic chemicals in plants. Environ. Sci.Technol.38,4195-4199.

Wang, XL., Tao S., Dawson RW., Xu FL., 2002. Characterizing and comparing risks of polycyclic aromatic hydrocarbons in a Tianjin wastewater-irrigated area. Environ Res 90,201-206.

Wang, X.,Sato,T., and Xing, B., 2006.Competitive sorption of pyrene on wood chars. Environ.Sci.Technol.40, 3267-3272.

Wang, Y., Luo, C.L., Li, J., Yin, H., Li, X.D., G., 2011. Zhang Characterization and risk assessment of polychlorinated biphenyls in soils and vegetations near an electronic waste recycling site, South China.Chemosphere 85,344-350.

Waqas M, Khan S, Qing H, Reid BJ, Chao C (2014) The effects of sewage sludge and sewage sludge biochar on PAHs and potentially toxic element bioaccumulation in Cucumis sativa L., Chemosphere 105:53-61

WHO/UNICEF, 2000.Global Water Supply and Sanitation Assessment 2000 Report. Joint Monitoring Programme for Water Supply and Sanitation: Geneva, Switzerland.

WHO, 2006.Guidelines for Safe Use of Wastewater, Excreta and Grey water Volume 2.

Wild, S.R., andJones K.,1995. Polynuclear aromatic hydrocarbons in the United KingdomEnvironment: a preliminary source inventory and budget. Environ.Pollut. 88, 91-108.

Wilcke,W., 2000.Polycyclic aromatic hydrocarbons (PAHs) in soil – a review. J. Soil Sci. Plant Nutr. 163, 229.

Wilson, S.C., and JonesK.C., 1993. Bioremediation of soil contaminated with polynuclearAromatic hydrocarbons (PAHs): a review. Environ. Pollut.81, 229-249.

Wu,S., Gschwend, P.M.,1986.Sorption kinetics of hydrophobic organic contaminants to natural sediments and soils. Environ. Sci. Technol. 20,717-725.

Xing, B., Pignatello, J.J., 1996.Mechanisms of slow sorption of organic chemicals to natural particles. Environ. Sci. Technol. 30, 1-11.

Yang, Y N., Sheng, G Y., 2003.Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. Environ. Sci. Technol. 37(16), 3635-3639.

Yao, Y., Gao, B., Chen, H., Jiang, L., Inyang, M., Zimmerman, A. R., Cao, X.,Yang, L., Xue, Y., and Li, H.,2012.Adsorption of sulfamethoxazole on biochar and its impact on reclaimed water irrigation.J. Hazard.Mater. 209-210,408-413

Yao, Y., GAO, B., Inyang, M., Zimmerman A. R., Cao, X., Pullammanappallil, P., and Yang, L. Y., 2011.Biochar derived from anaerobically digested sugar beet tailings: characterization and phosphate removal potential. Bioresour.Technol.102, 6273-6278.

Wang, Y.C., Qiao, M., Liu, Y.X., Arp,H.P.H.,Zhu,Y.G., 2011.Comparison of polycyclic aromatic hydrocarbon uptake pathways and risk assessment of vegetables from wastewater irrigated areas in northern china.J.ofEnvirn. Monit,13(2) , 433-439.

Yu, Y., Xu, J., Wang, P., Sun, H., Dai, S., 2009.Sediment-porewater partition of polycyclic aromatic hydrocarbons (PAHs) from Lanzhou Reach of Yellow River, China. J. Hazard.Mater. 165, 494-500.

Yu, J., 2011.The foreground analysis of sewage sludge agriculture application in China. Adv. Mater. Res. 335-336, 1316-1320.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., Zhang, G., 2011.The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ.Pollut. 159, 84-91

Zhu, B., Fan,T., Zang, D.,2008. Adsorption of copper ions from aqueous solution by citric acid modified soya bean straw. J.hazard mater.153, 300-308

Zhu, H., Aitken, M.D., 2010. Surfactant-enhanced desorption and biodegradation of polycyclic aromatic hydrocarbons in contaminated soil.Environ.Sci.Technol.44, 7260-7265.

Zheng, R.L., Cai, C., Liang, J.H., Huang, Q., Chen, Z., Huang, Y.Z., Arp, H. P. H., Sun, G.X., 2012.The effects of biochars from rice residue on the formation of iron plaque and the accumulation of Cd, Zn, Pb, As in rice (Oryza sativaL.) seedlings.Chemosphere.7,856-862.

Zhang, H.B., Luo, Y.M., Wong, M.H., Zhao, Q.G., Zhang, G.L.,2006.Distributions and concentrations of PAHs in Hong Kong soils. Environ. Pollut.141, 107-114.

Zhang, Y.C., C.N. Long, W.B. Rossow, and E.G. Dutton, 2010: Exploiting diurnal variations to evaluate the ISCCP-FD flux calculations and radiative-flux-analysis-processed surface observations from BSRN, ARM and SURFRADJ. Geophys. Res.,115, D15105, doi: 10.1029/2009JD012743.

Shi,Z., Tao,S., Pan ,B., Fan, W., He, X.C., Zuo, Q., Wu, S.P., Li, B.G., Cao,J.,Liu, W.X., Xu, F.L., Wang, X.J., Shen, W.R., Wong, P.K., 2005.Contamination of rivers in Tianjin, china by polycyclic aromatic hydrocarbons. Environ. Pollut.134, 97-111.

Zhu, D., and Pignatello, J.J., 2005.Characterization of Aromatic Compound Sorptive Interactions with Black Carbon (Charcoal) Assisted by Graphite as a Model. Environ. Sci. Technol. 39, 2033-2041.