Toxic Chemicals in the Environment: from understanding pollution and its impact to removal and verification techniques

OPCW
Organisation for the Prohibition of Chemical Weapons

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A compendium of articles from research projects supported by the OPCW

OPCW – 2020

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The Technical Secretariat of the Organisation for the Prohibition of Chemical Weapons (OPCW) is pleased to announce its first compilation of original research papers summarizing the work of scientists who have received support from the Organisation and the International Foundation for Science (IFS) between 2012 and 2017.

Support for research on the peaceful applications of chemistry in various fields were supported based on the Organisation's mandate to promote the technological and economic development of Member States under the provisions of Article XI of the Chemical Weapons Convention.

This publication highlights projects focused on chemical analysis and environmental safeguards and projects that are connected to the development of methods to monitor and mitigate the environmental impact of toxic chemicals. Toxic chemicals are an issue of global concern, regardless of whether they are released as remnants of war or through exposure to humans and the environment. Mitigating these concerns is a complex multidisciplinary task, requiring the participation of stakeholders with wide-ranging expertise. Toxic chemicals, including their environmental impact, are a central theme of the Organisation's programmes to build capacity and support scientific research.

A general objective of the international community, including international organisations, is a paradigm away from the exploitation of resources and to prevent and solve the problems that this has caused in the past. By supporting the development of chemistry for peaceful purposes, the OPCW prioritizes the principles of safety and security in the concept of sustainable chemistry practices, which is a key provision of the Organisation's mandate. From this perspective, it is essential to support relevant research activities because science and technology form the foundation of economic development through industrialization.

This publication aims to raise awareness of the efforts of the OPCW to support the research community in using the science of chemistry to make the world safer and more secure. The articles presented in this book contribute to the constantly growing body of scientific knowledge and informs potential new partners and beneficiaries about the expanding role of the OPCW in supporting scientific research. The OPCW provides an international forum for cooperation among scientists, industry and policymakers on issues that include chemical safety and security, and chemistry education. The Convention is underpinned by science and technology, with scientists playing a critical role in the implementation of the Convention. In the support of science, the OPCW runs a multitude of programmes, which are described on our website (www.opcw.org) in the International Cooperation section.

We hope you find the scientific content presented in this document interesting and informative, and we welcome you to our community of practitioners of peaceful and responsible chemistry.

Introduction to the Programmes Offered by the OPCW in Support of the Peaceful Uses of Chemistry

Article XI of the Chemical Weapons Convention

The *Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction* (hereinafter the Convention) entered into force on 29 April 1997, four years after it opened for signature to the governments of the world. The Convention is the world's most successful disarmament treaty, involving 193 States Parties and encompassing 98% of the global population. The Organisation for the Prohibition of Chemical Weapons (OPCW) was created upon entry into force as the implementing body of the Convention. In recognition of the OPCW's objectives to achieve a world free of chemical weapons, the Organisation received the 2013 Noble Peace Prize for its extensive efforts to eliminate chemical weapons. At the beginning of 2017, through major efforts of the OPCW, 98% of the world's declared stockpiles of warfare chemicals have been verifiably destroyed.

The implementation of the Convention and the related work of the OPCW are based on four pillars: destruction, non-proliferation (as well as preventing the re-emergence of chemical weapons), assistance and protection, and international cooperation. Each pillar is discussed in specific articles on the Convention, and a well-balanced set of measures, obligations, and opportunities is presented. Destruction and non-proliferation refer to the obligations of Member States to destroy all chemical weapons in their possession, and to declare industrial activities related to the production and transfer of various types of chemicals. Assistance and protection, and international cooperation provide opportunities for chemical defense and the use of chemistry for peaceful scientific developments.

International Cooperation at the OPCW: A Focus on Science

International cooperation to promote the peaceful applications of chemistry, which is the key element of Article XI of the Convention, facilitates development in all areas of chemistry for the technological and economic development of the Member State. Implementation of Article XI is enabled by capacity building through science and technology. The Article XI capacity building programmes are categorized into three domains as illustrated in Figure 1.

Integrated Chemicals Management	Enhancing Laboratory Capabilities	Promoting Chemical Knowledge
• Associate Programme • Regional Seminars on Chemical Safety & Security Management • Courses on Chemical Safety & Security Management (Voluntary Fund) • Workshop on Developing Tools on Chemical Safety & Security Management • Workshop on Responsible Care® Programme • Executive Programme on Integrated Chemicals Management • Workshop on Green Chemistry • Forum on Peaceful Uses of Chemistry	• Analytical Skills Development Courses • Analytical Chemistry Course • Specialised Analytical Chemistry Courses • Courses on Proficiency Testing • Customs Laboratory Services • Basic Analytical Chemistry Course for Women Chemists • Twinning Lab • Equipment Exchange Programme	• Symposium for Women on Chemistry • Course on Policy and Diplomacy for Scientists • Fellowship Programme on peaceful use of Chemistry • Conference Support Programme • Programme for support of Research Projects • Information Service and e-learning materials

Figure 1. Strategic mapping of the programmes and initiatives implemented within the framework of Article XI of the Chemical Weapons Convention

Integrated Chemical Management

The Integrated Chemicals Management domain focuses on chemical industry and its coordination and cooperation with the National Authorities of OPCW Member States. The domain objectives are to promote and adopt sustainable practices for chemical production, and a culture of chemical safety and security involving industrial chemicals.

Associate Programme

The Associate Programme is the flagship capacity building programme of the OPCW. This annual 9-week-long programme contributes to building capacities in Member States with developing and transitioning economies in areas related to the chemical industry. It offers training in Convention-relevant skill sets, including chemical engineering. The programme provides valuable opportunities for scientists and engineers to master state of the art practices in the chemical industry with an emphasis on chemical safety and security.

Chemical Safety and Security Management

The Chemical Safety and Security Management Programme promotes awareness among OPCW Member States on chemical risks and threats. The programme facilitates knowledge sharing, building national capacity on chemical safety and security, and creating frameworks for cooperation at national, regional, and international levels to prevent chemical incidents, whether accidental or intentional.

Green and Sustainable Chemistry

In 2016, an initiative to promote the substitution of toxic industrial chemicals, including green chemistry methodologies, was established. This initiative focuses on raising awareness and providing a forum for stakeholder discussion, including chemical industry, academia, government agencies, and educators, on innovative approaches and best practices for safe and sustainable chemical production. Activities

to support green chemistry for purposes that align with the objectives of the Convention include the awareness raising, stimulating cooperation among stakeholders and the discussion of the OPCW specific support actions in the field in the format of experts groups and dedicated workshops. Some specific activities also included a dedicated call for research projects, and a Green Chemistry fellowship (2016–2017).

Enhancing Laboratory Capabilities

The enhancing laboratory capabilities domain focuses on capacity building in analytical chemistry, which is a scientific discipline critical to the effective implementation of the Convention. This domain includes training and equipment exchange programmes.

Analytical Chemistry Courses

Courses in analytical chemistry are offered by the OPCW in cooperation with specialized institutions worldwide. These courses are designed to enhance the national capacities in OPCW Member States in the field of chemical analysis relevant to the Convention. Further, the courses provide training to laboratory personnel from academic, research, and specialized governmental institutions, including customs, forensic, and military.

The courses are conducted for a period of up to two weeks and are adapted for different levels of experience, analytical methods, regions, and language groups. The standard Analytical Skills Development Courses (ASDC) are geared toward participants with a medium to high level of experience, focusing mainly on Gas Chromatography/Mass Spectrometry (GC/MS) and related methods of sample preparation. Other courses include topics, such as Nuclear Magnetic Resonance (NMR), Liquid Chromatography/Mass Spectrometry (LC/MS), quantitative MS, Laboratory Quality Management, and others.

The OPCW's Designated Laboratory network forms a critical aspect of the verification regime of the Convention by providing laboratories with proven expertise in off-site analyses of Convention-related samples. This can include the analysis of samples from investigations of the alleged use of chemical weapons. To qualify for the OPCW designation, laboratories are required to pass a series of challenging Proficiency Tests conducted by the OPCW Laboratory. The proficiency testing scheme is aimed at maintaining a recognized and transparent procedure for the continued assessment. Courses to assist in the preparation for OPCW Proficiency Tests are typically held at the OPCW Training Facility in Rijswijk.

Laboratory Twinning and Assistance

In 2016, the OPCW initiated a "twinning of laboratories" initiative to enhance the capabilities of the analytical chemistry laboratories in Member States that lack a designated laboratory. This initiative was further merged with the Laboratroy Assistance Programme and is now providing a comprehensive and targeted support to laboratories aspiring to the OPCW designation by facilitating their partnerships with more advanced laboratories that currently hold (or previously held) designated status and wish to share their experience. The programme can include mentorship visits, individual or group training, the support of collaborative research and coordination workshops, exchange of equipment, and assistance for laboratories who wish to participate in the OPCW testing schemes, such as Proficiency Tests.

Equipment Exchange Programme

This programme facilitates the transfer of used equipment from an institution in one Member State to another; the recipient institution is typically located in a developing or transitioning economy. Support is provided in the form of grants to finance the transportation and insurance of the equipment from door to door. The OPCW facilitates a process for matching donors and recipients with appropriate equipment for exchange. The costs of installation and training at the receiving institution can also be facilitated under separate agreements to this programme.

Promoting Chemical Knowledge

Finally, within the third domain of Promoting Chemical Knowledge, the Convention offers pathways to support scientific research, mobility of researchers, and exchange of scientific information via the provision of grants and fellowships. The OPCW also organizes specialized events to raise awareness regarding the Convention, build cooperative relationships between scientists, and to facilitate the engagement of scientists with other stakeholders, including industry and policy makers. Some of these programmes are described below.

Programme for Support of Research Projects

The Programme for Support of Research Projects focuses on the generation of scientific and technical knowledge for peaceful applications of chemistry. These applications include sustainable industrial production and chemistry that is beneficial to agriculture, food, health and medicine, energy, water, and the environment. Specific attention is focused on the topics that directly relate to the work of the OPCW, such as the destruction and analysis of toxic chemicals, development of safer chemical processes and products, toxicological research to produce more effective treatments and therapies for the victims of chemical exposure, and materials for protection against chemical threats.

Funding is provided to a limited number of projects in research groups or institutions based in developing countries or with transitioning economies in OPCW member States. It covers auxiliary costs, such as consumables and disposables, sampling and analysis, access to scientific literature, and other minor expenditures. Projects typically last for a duration of one to three years and include small-scale activities that may already be supported by the existing infrastructure and other resources.

Since the inception of the programme, the OPCW has supported hundreds of projects, both directly funded by the OPCW and co-funded with other organizations such as the Stockholm-based International Foundation of Science (IFS).

The OPCW-funded research includes projects for destroying toxicants, analytical chemistry methodologies, studies of novel materials alternative to toxic chemicals, environmental monitoring and clean-up, renewable resources, bio-catalyzed synthetic pathways, drug discovery, and chemistry for health and medicine (Figure 2). The findings of the completed projects remain the property of the funded institutions and are published in scientific literatures.

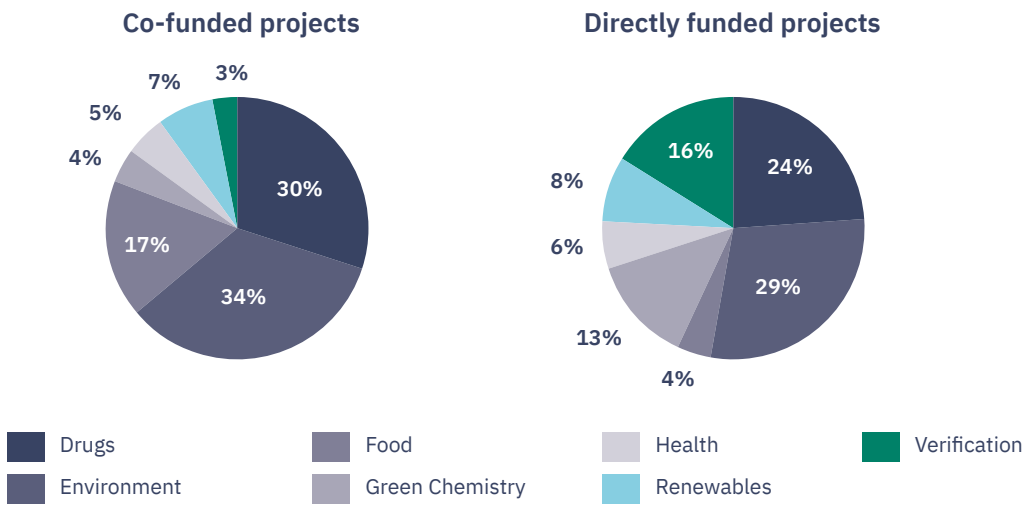

Figure 2. Thematic distribution of research projects under various thematic areas. The charts represent 388 co-funded and 74 directly funded projects supported by the OPCW from 2004–2017.

Fellowship Programme

The Fellowship Programme enables scientists and engineers working in research institutions, universities, and publicly funded specialized laboratories from OPCW Member States with developing or transitioning economies to work for a limited duration in advanced laboratories or facilities in other Member States. The programme seeks to enhance the skills of technical staff (scientists, engineers and technicians), particularly young professionals, while simultaneously facilitating the exchange of scientific and technical knowledge. This exchange facilitates the establishment of links between the two institutions after termination of the fellowship, thereby strengthening the South-South and South-North cooperation.

Similar to the Programme for the Support of Research Projects, the thematic coverage of projects undertaken by the fellowships includes a variety of fields of the peaceful applications of chemistry. In addition, a number of fellowships are dedicated to specific projects in areas of significant relevance to the Convention.

The programme is open to individuals affiliated with institutions in the Member States. The host institution must be located in a different Member State, and should be proposed by the candidate. Specific projects necessitate that calls for applications be published separately through an agreement of the OPCW with the host institution. Such agreements have been implemented with host laboratories, including VERIFIN, the Spiez Laboratory in Switzerland, and TU Delft in the Netherlands. Funding under this programme covers travel and living expenses for fellowships that typically range from three to six months.

The OPCW supports nearly 10 fellows each year from across the regional groups of the OPCW; majority are hosted in universities and research institutions in the European Union and the United States of America. We have recently noted an increasing number of fellowships hosted by institutions in Africa, which is indicative of the strengthening of the South-South and regional cooperation.

Conference Support Programme

The Conference Support Programme provides sponsorship for participants attending scientific conferences, workshops, and seminars on the peaceful applications of chemistry. Without precluding the possibility of funding for events in other fields, the scientific areas that were typically supported in the past include: natural-products chemistry and chemistry for the valorization of renewable resources; analytical chemistry methods and monitoring techniques for the detection of chemical hazards and toxicants; chemical and technological aspects of the destruction of toxic materials; applications of green chemistry for the development of safer and more sustainable products and production processes; chemistry applications in nanotechnology; toxicology, prophylaxis, and treatment of intoxications; risk assessment and management with respect to toxic chemicals and related safety and security aspects for chemical enterprises.

Support under this programme is provided to institutions in Member States hosting conferences, and may consist of: travel grants for participants or resource personnel (who must be citizens of an OPCW Member State); core grants to cover administrative costs, such as the costs of publishing the proceedings conferences, photocopying, and translating the proceedings into one of the official languages of the Organisation. The resource personnel or conference organizers, or both, must be based in a Member State with developing or transitioning economies.

Women in Chemistry

The Women in Chemistry Initiative was launched to foster the advancement of female chemistry professionals and the need for an improved gender balance of participants in the international cooperation programmes of the OPCW. Under this initiative, the OPCW organized symposia on women in chemistry and basic courses for female analytical chemists at the OPCW Training Facility on a yearly basis starting from 2016. The OPCW will continue to build on this initiative and hold future events to promote gender mainstreaming.

Science Diplomacy

Within the implementation framework of Article XI programmes and in accordance with its education and outreach initiative, the OPCW has held in 2016, 2017, and 2019 a series of workshops on policy and diplomacy for scientists targeting young PhD level chemists and biolochemists. The workshops, organized in cooperation with The World Academy of Sciences (TWAS), included lectures and interactive sessions intended to raise awareness within scientific communities in areas of policy and diplomacy that intersect with science, particularly in areas where the OPCW and other similarly focused international organizations are prioritized. These areas included the implementation of Chemical and Biological Weapons Conventions, science in the UN Sustainable Development Goals, safety and security in the chemical and life sciences, education, and scientific ethics and responsibility.

Part I.
Environmental Monitoring and Exposure to Toxicants

Concentration of Brominated Flame Retardants in Indoor Dust from Homes and Offices in Developing Countries: A Case Study of Potential Implications on Humans in South Africa and Nigeria

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Abstract

There are limited data on polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs) in indoor dust in South Africa and Nigeria, thereby making it necessary to conduct this study. Dust samples were collected from offices and houses using glass wool and a vacuum cleaner. The samples were sieved, extracted using acetone-hexane Soxhlet extraction, concentrated, and the extract cleaned using a Pasteur pipette column. The cleaned extract was then concentrated and analyzed using GC-EI-MS with ZB-5 and DB-5 columns. BB-209, BDE-47, 66, 85, 99, 153, and 209 were detected in both the office and house dust samples. The mean PBB concentrations detected in the office and house dust samples were 38.2 and 4.6 ng g^{-1}, respectively. In contrast, the mean PBDE concentrations for the office and house dust samples were 169 and 51.1 ng g^{-1} (dw), respectively. A positive correlation between ΣPBB and ΣPBDE was observed for the office samples, suggesting similar pollution sources. However, no correlation was observed between the electronic materials and ΣPBBs or ΣPBDEs. The estimated exposure rate for toddlers and adults via the ingestion of BDE-209 in house dust ranged from 0.05–0.18 and 0.61–2.44, respectively. Conversely, the estimated exposure rate for toddlers and adults via the ingestion of ΣPBDEs in house dust ranged from 0.02–0.05 and 0.24–0.61 ng day^{-1}, respectively. The daily dust ingestion exposure rates estimated in this study were 1–2 and 2–3 orders of magnitude lower than those in the developed countries for toddlers and adults respectively. The electronic equipment treated with PBDEs are potentially the main emission sources in indoor dust. Based on this study, South Africans residing in Pretoria are exposed to lower concentrations of PBDEs in dust samples in their houses than offices compared to Nigerians with higher exposure levels.

Keywords: BFRs, concentrations, indoor dust, offices, homes, South Africa, Nigeria

1. Introduction

New technologies, processes, and applications introduce new sources of fire hazards such as welding sparks and short circuits [1]. Modern fire-fighting techniques, equipment, and building designs have reduced the destruction caused by fires. However, high fuel loads in residential or commercial buildings can offset even the best building construction [2]. Notably, fires also occur in cars, buses, ships, airplanes, and trains. Methods to enhance the flame retardance of consumer goods have been developed to provide additional protection from fires and to increase the escape time when a fire occurs. Flame retardants are chemicals that are added or applied to materials such as plastics, textiles, furniture, electronic equipment, and other polymer products to increase their fire resistance [3]. Based on a previous report [4], the different groups of flame retardants include:

- Inorganic chemicals (such as antimony oxides);
- Phosphorus-containing organic or inorganic compounds, e.g., phosphoric acid;
- Nitrogen-based compounds; and
- Organo-halogenated compounds, e.g., chlorinated or brominated organic compounds.

Flame retardants are classified into two major categories according to their method of production: reactive and additive flame retardants. Reactive flame retardants are chemically bonded to polymers during polymerization through the formation of weak covalent bonds. Therefore, they are less likely to leach out of the matrix and into the environment until the product is decomposed or burnt; typical examples are tetrabromophthalic anhydride and tetrabromobisphenol A [5]. By contrast, additive flame retardants are often incorporated into plastics during or following polymerization when they are blended with the polymer constituents along with other additives like plasticizers. The blend is then applied to the substrate as a spray in a coating formulation. They are not permanently bonded to the polymer. Consequently, they have the tendency to leach out of the polymer matrix prior to, during, or after its operational life. Additive flame retardants include PBDEs, hexabromocyclododecane (HBCD), bis(tribromophenoxy)ethane, magnesium hydroxide, and aluminum hydroxide [6].

The use and application of the abovementioned groups of flame retardants are determined by the type of material, costs, and level or fire safety standard to be achieved. However, organo-halogenated flame retardants exhibit a greater interference with the combustion process through the release of effective halogen halidese (HX), the efficiency of which depends on the halogen used. Flame retardants containing bromine are more effective than those containing fluorine, chlorine, and iodine, and requires a lower loading of materials [5]. Brominated flame retardants (BFRs) are a structurally diverse group of compounds, including aromatic, cyclic, phenolic derivatives, aliphatics, and phthalic anhydride derivatives with various numbers of bromine atoms. The chemical structures of the most common BFRs are shown in Figure 1.1.

These compounds exhibit characteristics and properties similar to prohibited organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) because of their persistence in the environment. PBDEs and PBBs like PCBs each have a total of 209 possible congeners divided into ten homologous groups based on the degree of bromination. Each congener, though similar, has different effects on various biological systems.

BFRs comprise approximately 25% of the volume of flame retardants used on a global scale and are used in applications or resins requiring high flame retarding performance or flame retarding active ingredients in the gas phase, respectively [7]. These are the chemicals of choice, owing to their low cost and high-performance flame retarding properties. In 2001, the global production of technical PBDE mixtures, i.e., penta-, octa-, and deca-BDEs was 67,440 tons (BSEF, 2006). Of the total BFRs produced in the USA, Europe, Middle East, and Asia, 40% was distributed to Northern America, 30% to the Far East, and 25% to Europe [8]. Brominated flame retardants are considered toxic pollutants because of their significant bioaccumulation in animals and humans and subsequent detection in increasing concentrations in human samples. Consequently, the European Union (EU) proposed a ban on the use of penta-BDE that became effective in 2004 [9]. This has led to a tremendous decline in the consumption of penta-BDE and TBBPA in Europe [10]. Penta-BDEs and octa-BDE have been listed in Stockholm Convention among other persistent organic pollutants (POPs).

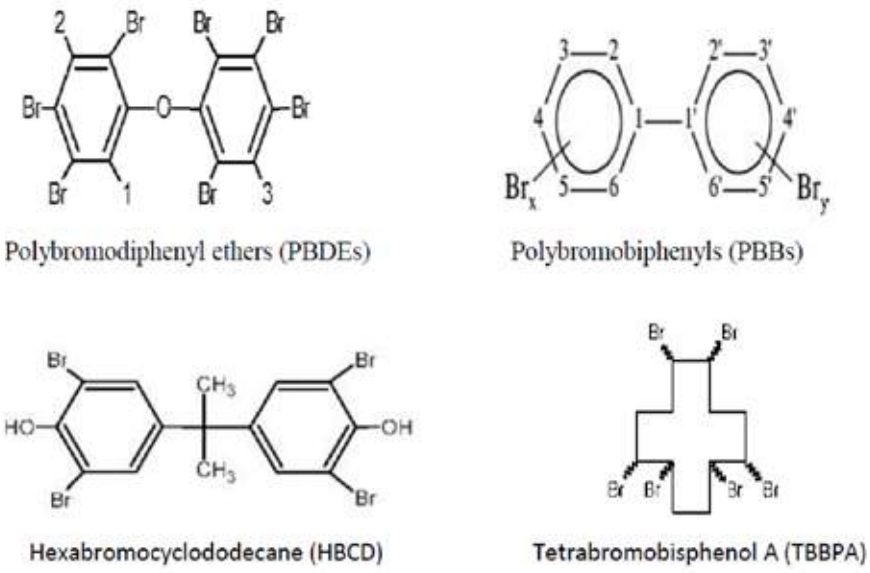

Figure 1.1. Chemical structures of common brominated flame retardants (BFRs).

Thus far, there is limited information on the production, use, and distribution of BFRs in and around African countries, including South Africa and Nigeria. However, it is safe to assume that products containing these materials are being exported to African countries. Furthermore, studies conducted in the last 15 years have mainly focused on PBDEs, particularly the congeners that are within the penta-BDE mixtures, i.e., BDE-47, BDE-99, and BDE-100 [11-17].

Brominated flame retardants are currently not on any list of hazardous materials in Africa and there is no official information regarding BFRs such as PBDEs, despite ratification of the Stockholm Convention in September, 2002 [18] (DPWM, 2005). Studies on the occurrence and concentrations of PBDE in landfill leachates in the city of Tshwane [14] and on PBDE and HBCD in the eggs of South African birds [13] confirm their presence in the South African environment. In Nigeria, e-waste plastics (computer monitor and TV casings) were screened [19], confirming that they contained various amounts of BFRs, depending on their origin (USA, EU, or Asia). Considering the general approach to solid waste management [20] and the e-waste disposal regime in Nigeria [21-22], coupled with the importation of a large number of used cars that may have been treated with BFRs, it is reasonable to assume that these substances ultimately end up in the open environment. These reports indicate the strong need for further investigation of BFRs in the environment, particularly in dust.

Additive brominated flame retardants such as PBBs and PBDEs lack binding sites to be chemically bonded to the host materials [23]. Consequently, household and workplace materials containing PBBs and PBDEs can readily release these toxic pollutants into the environment during product use and disposal. The existence of the aforementioned in humans, wildlife, household dust, water, sediments, and biological materials has been proven. These toxic pollutants can impact human and animals by causing cancer, endocrine disruption, neurobehavioral and developmental effects, and memory retardation [6, 24, 25]. The findings of this study are presumably useful to the Department of Environmental Affairs of South Africa and the Ministry of Environment in Nigeria who are in the process of updating their National Implementation Plans on POPs. Furthermore, PBDEs are among the new POPs on the Stockholm Convention list earmarked for elimination. Also, PBBs are among the chemicals previously mentioned; however, there is scarce information available on PBBs in the South African and Nigerian environment. Therefore, knowledge of the types and amount of these flame retardants in house and office dust can assist members of the society in taking the necessary precautions and aid the relevant authorities in taking the necessary measures to enforce the elimination of these chemicals in domestic materials.

The objectives of the study were to: (1) identify and quantify congeners of PBBs and PBDEs in house and office dust from South Africa and Nigeria to provide information on the exposure levels of these emerging pollutants, and (2) compare the results obtained with those reported in developed countries to evaluate the extent of exposure in South Africa and Nigeria via dust.

2. Materials and methods

2.1 Chemicals and reagents

The house dust standard reference material (SRM 2585) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Each certified standard solution (1.2 mL, 50 mg L^{-1}) of the sixteen PBDE congeners (BDE-3, 15, 17, 28, 47, 66, 77, 85, 99, 100, 126, 138, 153, 154, 183, and 209) and sixteen PBB congeners (BB-1, 2, 4, 10, 15, 26, 29, 30, 31, 38, 49, 80, 103, 153, 155, and 209) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The isotopic labeled internal standards (1.2 mL, 50 mg L^{-1}), i.e., $^{13}C_{12}$-BDE-139 and $^{13}C_{12}$-BDE-209, were purchased from Cambridge Isotope Laboratories (CIL, Andover, MA, USA). The commercial decabromodiphenyl ether mixture containing > 95% decabromodiphenyl ether (BDE-209) (Fluka Chemie GmbH, CH - 9471 Buchs, EC No: 2146049, Switzerland) was purchased. Copper powder (99.98% purity, Saarchem (Pty) Ltd., Muldersdrift, South Africa), silica gel (100–200 mesh), sodium sulphate (99.9% purity), glass wool, and HPLC grade solvents (acetone, hexane, dichloromethane, ethanol, and toluene) were purchased from Sigma Aldrich (Chemie GmbH, Steinheim, Germany). Nonane (50 mL, 99.8% purity, Sigma Aldrich, Switzerland) was purchased from Industrial Analytical Pty. Ltd. Midrand, Gauteng, South Africa.

2.2 Sampling and pre-sample preparation

Dust samples were collected from the offices and homes of several staff members at the Tshwane University of Technology Pretoria. The selection of offices and homes was based on the willingness of the staff members, particularly, in the case of the houses. Brief descriptions of the locations and methods employed for dust sample collections are given below.

2.2.1 Office dust

To achieve better characterization, two dust sampling methods were used: surface wiping and suction with a vacuum cleaner. Surface wiping methods have been utilized since the early 1970s. The sampler types were mainly glass fibers, cotton swabs, Whatman filter papers, or glass filter papers [26-28]. Vacuum cleaners were commonly used. Dust samples were collected from offices at the Faculty of Science (Arcadia) and main campus of the Tshwane University of Technology, Pretoria, South Africa between October 2012 and February 2013. The dust samples were obtained by wiping all the material surfaces in the offices (e.g., computers, chairs, tables, fans, and air conditioning units) using glass wool prebaked at 450 °C for 12 h. The glass wool was preweighed, after carefully wiping the surface. Then, the glass wool with the dust was weighed to determine the amount of dust collected. The samples were collected from a total of twenty-one offices.

The mass of dust samples collected using the above methods varied from 0.10–3.7 g. Owing to the extremely low mass of dust collected from several offices, the dust samples collected from two or three offices were pooled together based on the similarity of electronic materials available in the office where the collection was done. Hence, a total of 12 samples were analyzed (six non-pooled and pooled samples). In addition, dust samples were collected from the same offices by surface wiping and also from additional offices using 1000 watts portable standard vacuum cleaner (Model: 601SA, made in China) equipped with a dust collection bag.

Exhaustive dust analyses from each office were not attempted because one of the main objectives of this research was to determine the overall average concentration. Instead, after the identification of additional offices, four pooled sample categories were sorted depending on the similarity and proximity of the offices with the one collected by glass wool. Accordingly, dust samples were collected from the entire floor of twenty-six offices in Arcadia (one pooled sample) and fifty-five offices at the Pretoria West campus (three pooled samples).

The vacuum cleaner was thoroughly cleaned and a new bag was inserted between sampling. All the pooled dust samples were sieved separately with precleaned and dried stainless-steel sieves of 250 µm. Thereafter, the sieved dust was homogenized thoroughly and stored in 50 mL precleaned amber glass at room temperature until extraction.

In May 2012, eleven dust samples were collected from different offices in a similar manner. The selected offices were North Bank, UAM North Core, UAM South Core, High level and low level, respectively, in Benue State situated in the Middle Belt region of Nigeria. In this region, the climate is tropical and subhumid, with a mean annual temperature of 32.5 °C. Samples were collected by wiping the surfaces of all materials available in the offices such as televisions, computers, chairs, fans, air conditioners, and chairs using a glass wool pre-baked at 450 °C for 12 h. Carpet dust was collected using a portable standard vacuum cleaner (Model: 601SA, made in China) of approximately 1000 Watts equipped with a dust collection bag. Details of the electronic items and characterization of the interior of the homes were recorded. Samples were transferred from the vacuum cleaner to the solvent-rinsed foils, wrapped, then transferred to the laboratory where they were sieved and stored in amber glass bottles at a temperature of -20 °C prior to extraction and further analyses.

2.2.2 House dust

Indoor dust samples were collected from the living rooms of 31 houses using the same vacuum cleaner used for office dust collection. The collection was performed in October 2012 to April 2013. During collection, the number and type of electronics, as well as the furniture, floor type, ventilation system, and other suspected materials that were potentially treated with flame retardants were recorded. To avoid cross-contamination, new or cleaned dust bags were used for each house. Further, all the collected dusts were sieved, homogenized, and stored until extraction, under the same conditions as that for office dust.

2.2.3 Hotel, office, and computer room dust

During the application of developed methods on GC-ECD for BDE-209, three pooled dust samples from three different microenvironments were used. Two dust samples were taken from a normal vacuum cleaner used daily for cleaning purposes. The dust samples (n=37 rooms) and (n=42 offices) were taken from a hotel in Pretoria and offices at the Faculty of Science, Tshwane University of Technology, Pretoria, South Africa, respectively. More dust samples were obtained by wiping the surfaces of computers, chairs, and tables available in the Pharmacy department of the abovementioned university computer classroom (n=32 computers).

3. Apparatus and instrumentations

3.1 Soxhlet extraction and ultrasonication

The extraction of all dust samples collected from homes and offices in South Africa were conducted using Soxhlet extraction. In each case, the dry dust samples were weighed and activated copper powder (0.25 g) was added to remove sulphur. Subsequently, the mixture was homogenized, transferred to a cellulose extraction thimble (internal diameter (ID): 19 mm and length: 90 mm), covered with glass

wool, placed inside the Soxhlet apparatus, and extracted with *n*-hexane:acetone (250–270 mL, 2:1 v/v) for 8 h. Thimbles containing the activated copper powder and glass wool that represented the blank were also extracted under the same condition as the samples. In contrast, the dust samples collected from different offices in Nigeria were extracted using ultrasonication. In this case, approximately 100 mg of fine sieved dust from each of the samples was weighed and extracted. The use of ultrasonication was deemed less labor-intensive than the Soxhlet technique. Prior to extraction, the dust samples were spiked with 3 µL of the labeled BDE-139 and BDE-209 standards to monitor the recoveries. Numerous techniques have been reported in the literature such as Soxhlet and accelerated solvent. However, ultrasonication-assisted solvent extraction provides fast and efficient separation of target analytes from dust samples and have been recently employed for the extraction of PBDEs from dust samples (Abb, Stahl & Lorenz, 2011; Stasinska *et al.*, 2013).

3.2 Rotary evaporator

All dust extracts were cooled and reduced to approximately 2 mL in a rotary evaporator (RotaVapor R-210, BÜCHI Labortecnik AG, Switzerland) under a fume cupboard. The temperature of the water bath of the rotary evaporator was adjusted to 40 °C to reduce the loss of target analytes.

3.3 Pasteur pipette column

The extracts obtained from the dust samples collected from South Africa were purified using a modified clean-up technique. A Pasteur pipette column (ID: 5 mm) was plugged with glass wool at the base and packed with pre-prepared silica gel and sodium sulphate from bottom to top in the following order: neutral silica gel (0.2 g), basic silica gel (0.2 g), neutral silica gel (0.2 g), acidic silica gel (0.2 g), and sodium sulphate (0.2 g). To enhance cleaning, glass wool was used to partition each packed chemical. Each of the packed disposable Pasteur pipette columns was first eluted with 20 mL of *n*-hexane: dichloromethane (5:1 v/v) mixture, then the extract was transferred onto it. Subsequently, it was eluted with 2 × 10 mL of *n*-hexane:dichloromethane (5:1 v/v) mixture. The extract was further concentrated under a gentle flow of nitrogen to about 50 µL. Finally, the concentrated extract was diluted to 200–250 µL by a mixture containing *n*- nonane: toluene (9:1 v/v*)*.

A fairly different clean-up technique was employed for purifying the dust samples collected from offices in Nigeria. This was achieved by sequentially packing into Pasteur pipettes (230 mm) containing approximately 0.16 g silica, 0.06 g pesticarb, 0.16 g silica, and finally topped with 0.5 g sodium sulphate. The Pasteur pipette was plugged at the base with glass wool and was used to separate each layer of material for enhanced cleaning. Prior to introducing 1 mL of the reduced extract, the packed column was eluted to saturation with 12 mL toluene/dichloromethane (1:1 v/v). A sample was introduced into the column before the solvent reached the bed of the sodium sulphate plugged with glass wool and was further eluted with 4 mL combined solvent. Thereafter, nitrogen gas was bubbled into the eluant to concentrate it to 200 µL. The internal standard (10 µL, 2.5 ng μL^{-1}, BDE-118) was added as a quantification internal standard. Then, the extract (1.0 µL) was injected into the GC-MS under optimized instrumental conditions. Silica gel was activated at 450 °C overnight.

3.4 Solvent choice

Commercial decabromodiphenyl ether is not soluble in most organic solvents. Its solubility in ethanol, toluene, hexane, acetone, and their mixtures was tested. The deca- mixture was determined to be readily soluble in toluene and the ethanol:toluene (1:1 v/v) mixture. However, in the presence of ethanol, the prepared standard was less stable than that in the presence of toluene. Therefore, toluene was used to dissolve the commercial deca- mixture. Hence, the selection of an appropriate solvent is essential for successful extraction. The extraction efficiency of different solvent mixtures, such as hexane:acetone (2:1 v/v), hexane:acetone (3:1 v/v), hexane:dichloromethane (2:1 v/v), and hexane:toluene (2:1 v/v), were evaluated by extracting 1.0 g of SRM 2585. Based on the recovery obtained, appropriate solvent mixtures were selected. Prior to extraction of the dust samples collected from Nigeria, the yield and extraction

efficiencies of several of the selected solvents, namely *n*-hexane, toluene, and dichloromethane used individually and as a mixture, were evaluated. Dichloromethane yielded the optimum recovery for majority of the target analytes. The observed values for *n*-hexane/toluene (1:1 *v/v*), *n*-hexane/toluene (2:1 *v/v*), DCM/toluene, DCM/hexane, and DCM ranged from 23–103, 28–130, 25–96, 39–130, and 75–101%, respectively.

3.4.1 Decabromodiphenyl ether

The Zebron capillary column (ZB-5, length: 15 and 30 m, ID: 0.25 mm, and d_f: 0.25 µm, Phenomenex, Torrance, California, USA) with a composition of 5% phenyl and 95% dimethylpolysiloxane, TRACE GC Ultra (Thermo Electron S.P.A, Rodano, Milan, Italy) coupled with an electron capture detector (ECD, ^{63}Ni) and equipped with digital pressure and gas flow control was employed. A glass liner for splitless injection (ID: 3 mm) was tapered at the bottom and high purity nitrogen (99.999%) was used as a makeup and carrier gas throughout the experiment. In addition, to facilitate comparison, helium was also used as a carrier gas. One micro- liter of 20 ng μL^{-1} BDE-209 solutions in toluene was injected into the GC. The GC parameters, injection temperature, final oven temperature, splitless time, and flow rate were varied until a set of optimum conditions were obtained as discussed below.

3.4.2 Optimum chromatographic conditions for BDE-209 analysis

The optimum working conditions were determined by investigating the impacts of the four main chromatographic parameters on the determination of decabromodiphenyl ether (BDE-209); the details are given below.

3.4.3 Injection temperature

Injection temperatures of 250, 270, 290, 300, and 310 °C were studied under the following temperature program: 90 °C at 30 °C min^{-1} for 1 min, then to 300 °C at 10 °C min^{-1} for 5 min, then to 310 °C for 20 and 5 min hold for the long and short columns, respectively. The ECD temperature was maintained at 320 °C and the flow rate of the carrier gas was 2.5 mL min^{-1}. Three consecutive injections were used for the study at each individual temperature.

3.4.4 Final oven temperature

Five final oven temperatures (290, 300, 310, 320, and 330 °C) were studied with triplicate injections at each temperature using the following oven temperature program: 90 °C at 30 °C min^{-1} for 1 min to each final oven temperature mentioned above with final hold times of 47, 33, 23, 20, and 12 min for the 30 m column and 17, 12, 9, 7, and 6 min for the 15 m column. The ECD temperature remained at 330 °C for all except the last final oven temperature of 330 °C, which was 335 °C at a flow rate of 3 mL min^{-1}.

3.4.5 Splitless time

The influence of four splitless times (0.5, 1, 1.5, and 2 min) on the sensitivity of BDE-209 was investigated. This was undertaken to obtain the optimum conditions for the analysis of BDE-209 under the following oven temperature program: 90 °C at 30 °C min^{-1} for 1 min hold, then to 300 °C at 10 °C min^{-1} for 5 min hold, then to 310 °C with final hold times of 20 and 6 min for the 30 and 15 m columns, respectively. The ECD and injection temperatures were 320 °C and 290 °C, respectively, with a carrier gas flow rate of 2 mL min^{-1}.

3.4.6 Flow rate

The investigated flow rates were 1.0, 1.5, 2.0, 2.5, and 3.0 mL min^{-1} under the following oven temperature program in each case: 90 °C at 30 °C min^{-1} for 1 min hold, then to 300 °C at 10 °C min^{-1} for 5 min, then to 310 °C and different hold times based on the elution of BDE-209, an ECD temperature of 320 °C, and an injection temperature of 290 °C.

3.4.7 Environmentally relevant polybromobiphenyls and polybromodiphenyl ether congeners

The suitability of the developed optimum working conditions for the analysis of the major congeners of PBBs and PBDEs on different types of nonpolar GC capillary columns were evaluated. The following nonpolar GC columns were used during the method development: ZB-5 and DB-5ms (30 m, ID: 0.25 mm, d_f: 0.25 µm), ZB-5, DB-5 (30 m, ID: 0.25 mm, d_f: 0.1 µm), ZB-5 (15 m, ID: 0.25 mm, d_f: 0.25 µm), and HP-5MS (30 m, ID: 0.25 mm, d_f: 0.25 µm). To determine the best column for analysis, a mixture (1 µL, 0.1 ng $µL^{-1}$) of 32 standards was injected under the same condition.

3.4.8 Determination of LOD and LOQ

Throughout this work, signal-to-noise ratios (S/N) of 3:1 and 10:1 were used to determine the LOD and LOQ, respectively. However, BDE-209 was detected in the blank samples during the application of the optimized method on the real sample analysis. Hence, the blank determination methods were used to estimate the LOD and LOQ values.

3.4.9 Quality control/quality assurance

Several quality control methods were assessed to obtain reliable data for each analysis. Glass wool, silica gel, and sodium sulphate were baked in the oven to remove any volatile organic compounds and some other impurities. Silica gel and sodium sulphate were stored in a glass jar that was precleaned and rinsed with n-hexane:acetone solvent and then sealed. Glass wool was wrapped with aluminum foil and maintained within a desiccator to prevent the absorption of moisture. All the glassware used in this study was cleaned with ultrapure water, dried, and finally rinsed with *n*-hexane:acetone mixture. Furthermore, the developed methods were validated by extracting the SRM 2585 organic contaminants in house dust.

The calibration curves for each standard ranged from 0.02–1.00 ng $µL^{-1}$, exhibiting good linearity in the ranges considered with regression coefficient ($r^2 \geq 0.99$). Furthermore, none of the congeners were detected in the solvent and method blanks. Therefore, the LOD was calculated by extrapolating the concentration that would yield a signal-to-noise ratio of 3 by injecting the extracted spiked sample of lowest concentration.

The LOD values for the PBB and PBDE congeners ranged from 0.3–0.5 ng g^{-1}, except for BB-209 and BDE-209 with LOD values of 0.8 ng g^{-1} and 1.2 ng g^{-1}, respectively. Excellent recoveries of the SRM-2585 indicated the high quality of the method. The precision of the method could be observed from the low standard deviation obtained for majority of the congeners (< 10%), exhibiting enhanced repeatability.

3.4.10 Decabromodiphenyl ether

Quantification was performed using a five point calibration curve ($r > 0.99$) prepared from the certified standard solution of BDE-209 in toluene through dilution (three replicate average calibrations were used for each level). After triplicate injections of each sample, a middle calibration standard was injected to control any change in retention time and concentration. Before injection of a new sample, column

cleaning was done at least twice using toluene. The precision of the method was also evaluated at two different concentration levels by calculating the relative standard deviation of the three replicate analyses of each level.

3.4.11 Polybromodiphenyl ethers and Polybromobiphenyls

During the analysis of the office dust, quantitative analyses were conducted using external standard methods with five level calibration curves. As stated earlier, one method blank was extracted along with the set of actual samples using similar procedures. The solvent blank was injected after three samples to control any carryover contamination; none of the congeners were detected in the solvent and method blanks. Similar quality control procedures were used during the PBDE and PBB analyses from house dust. The only difference was that the reported concentrations of each PBDE and PBB congener were calculated based on the spiked amount of internal standards. Hence, the analyte loss or increment of concentration due to some interference was minimized.

3.4.12 Statistical analysis

Unless otherwise stated, all the descriptive statistics were computed to characterize the PBB and PBDE concentrations in the sample using Microsoft Office Excel 2007. Nonparametric methods, Spearman's rank correlation, Pearson correlation, and t-test were applied between the summation of BFR concentrations for testing the correlation of common sources of pollution and relevance of the number of electronic materials with concentration detected, all statistical significances were set at an alpha value of 0.05.

3.5 Analysis of indoor dust

In all analysis, the same GC oven program was used throughout the analysis with different instruments and columns; and these are stated below under each section. Helium at a flow rate of 1.5 mL min^{-1} was employed as a carrier gas, splitless time of 1 min, injection temperature of 290 °C and oven program of 90 °C for 1 min, ramped by 30 °C min^{-1} to 300 °C for 5 min, 10 °C min^{-1} to 310 °C for 1 min were used on GC- MS for analyses of all congeners except BB-209 and BDE-209. When $^{13}C_{12}$- BDE-209 was not used, these two congeners were analyzed on GC-ECD.

3.5.1 PBDE and PBB analysis in office dust

The dust extracts were analyzed using an Agilent 7890A GC system (serial number: US 92023178, made in USA). Aliquots (1 µL) of the extracted sample were injected into a split/splitless injection port on the DB-5 GC column (30 m, ID: 0.25 mm, d_f: 0.10 µm) using an Agilent 7693A automatic liquid sampler (Agilent Technologies). The GC was coupled to an Agilent 5975C inert MSD with a triple axis detector operated in EI mode. The operating conditions were as follows: ion source of 250 °C, and transfer line of 300 °C. Identification was performed using full scan mode by monitoring the presence of the mass spectra of molecular ion and two qualifier ions of each congener at the elution retention time. Each congener was quantified against five level external standard calibration curves. BB-209 and BDE-209 were analyzed separately using a ZB-5 GC column (15 m, ID: 0.25 mm, d_f: 0.25 µm) with similar oven programs, except that the final hold time was changed to 3 min. Nitrogen was used as a carrier and makeup gas with flow rates of 2.5 and 30 mL min^{-1}, respectively.

In contrast, the clean extracts obtained from the dust samples collected from Nigeria were analyzed using the Shimadzu model 2010 plus gas chromatograph coupled with the QP 2010 Ultra mass spectrometer (Shimadzu, Japan) using electron ionization. The instrument was equipped with a Shimadzu A0C-20i autosampler. The operation mode used was selected ion monitoring (SIM). A 15 m ZB-5 column (ID: 0.25 mm, d_f: 0.25 µm) was used for separation. The method was validated with the recovery of surrogate standards ($^{13}C_{12}$ BDE-139 & BDE-209) and were both observed to be 90 and 81%, respectively.

3.5.2 PBDE and PBB analysis in house dust

The same instrumental conditions described for office dust were also applied. The only differences were: (1) the Agilent technologies 7890A GC system was made in USA, (2) the HP-5MS GC column (30 m, ID: 0.25 mm, d_f: 0.25 µm d_f), and (3) identifications were conducted using the SIM mode. Each congener was quantified against five level external standard calibration curves and internal standards (BDE-77, $^{13}C_{12}$-BDE-139). The BB-209 and BDE-209 analyses were conducted under similar conditions, but using ZB-5 GC columns (15 m, ID: 0.25 mm, d_f: 0.1 µm), and quantified by internal standard $^{13}C_{12}$- BDE-209.

4. Results and discussion

4.1 Method development

4.1.1 Selection of Column

The relative retention times of BDE-47 and BDE-183 obtained from the different columns investigated are shown in Table 3.1. The columns with film thickness (d_f: 0.25 or 0.1 µm d_f) provided better resolutions relative to the other columns. There was no significant difference in retention time observed between the ZB-5 (15 m, ID: 0.25 mm, d_f: 0.25 µm) and DB-5 (30 m, ID: 0.25 mm, d_f: 0.1 µm) columns. However, poor response and prolonged retention times were observed with DB-5ms, HP-5ms, and ZB-5 columns (30 m, ID: 0.25 mm, d_f: 0.25 µm), particularly for higher BDE congeners. With respect to the base line, retention time, response, and resolution of each chromatogram obtained on different columns, the ZB-5 and DB-5 columns (30 m, ID: 0.25 mm, d_f: 0.1 µm) were determined to be optimal for the analysis of all congeners except for BB-209 and BDE-209. For BB-209 and BDE-209, the response almost tripled on the shorter column. BB-209 and BDE-209 were analyzed on GC-ECD, owing to the low sensitivity of EI-MS for higher congeners [4]. The following co-elution was observed: BB-4 and BB-10; BB-15 and BDE-15; BB-26 and BB-29; BDE-47, BB-80, and BB-103; BDE-100 and BB-155; BB-153 and BDE-154, irrespective of the column length and film thickness. The co-elution of BB-153 and 154 on DB-1, DB-5, and CP-Sil 19 columns has also been previously reported [31]. Therefore, when using GC-ECD, which is exclusively dependent on the retention time of the standards for identification, special attention should be placed on any environmental sample that is expected to contain PBBs. The chromatograms of 32 standards with a concentration of 0.5 ng µL^{-1} and a nitrogen flow rate of 1.5 mL min^{-1} on GC-ECD using a ZB-5 (30 m, ID: 0.25 mm, d_f: 0.1 µm) column are shown in Figure 3.1.

Table 3.1. Relative retention time (RRT) database relative to the sum of BDE-47 and BDE-183 retention times.

d_f (µm)	0.1	0.25	0.1	0.1	0.25	0.25
Column type	ZB-5	ZB-5	ZB-5	DB-5	DB-5ms	HP-5ms
Length (m)	30	15	15	30	30	30
Flow rate (mL min^{-1})	1.5	2.5	1.5	1.5	1.5	1.5
BB-1	0.259	0.243	0.248	0.261	0.227	0.249
BB-2	0.278	0.264	0.269	0.281	0.246	0.266
BDE-3	0.282	0.269	0.274	0.285	0.250	0.269
BB-4	0.302	0.292	0.294	0.303	0.267	0.286
BB-10	0.302	0.292	0.296	0.305	0.268	0.288

BB-15	0.342	0.336	0.342	0.346	0.306	0.321
BDE-15	0.342	0.344	0.343	0.346	0.307	0.322
BB-30	0.350	0.344	0.351	0.354	0.312	0.343
BB-26	0.368	0.364	0.371	0.372	0.329	0.343
BB-29	0.368	0.364	0.371	0.372	0.329	0.344
BB-31	0.372	0.368	0.376	0.376	0.333	0.347
BDE-17	0.379	0.377	0.385	0.383	0.339	0.353
BDE-28	0.385	0.383	0.392	0.389	0.345	0.358
BB-38	0.390	0.389	0.397	0.395	0.350	0.363
BB-49	0.399	0.399	0.408	0.404	0.358	0.370
BDE-47	0.425	0.428	0.438	0.430	0.385	0.395
BB-80	0.425	0.428	0.438	0.430	0.385	0.394
BB-103	0.425	0.428	0.438	0.430	0.385	0.396
BDE-66	0.430	0.434	0.444	0.435	0.393	0.400
BDE-77	0.439	0.443	0.454	0.443	0.404	0.410
BDE-100	0.454	0.460	0.471	0.459	0.422	0.430
BB-155	0.454	0.459	0.471	0.459	0.422	0.429
BDE-99	0.463	0.469	0.481	0.467	0.436	0.441
BDE-85	0.483	0.486	0.498	0.486	0.466	0.467
BDE-126	0.485	0.488	0.500	0.488	0.471	0.470
BDE-154	0.493	0.495	0.507	0.495	0.479	0.481
BB-153	0.493	0.495	0.507	0.496	0.481	0.481
BDE-153	0.509	0.510	0.520	0.510	0.508	0.504
BDE-138	0.536	0.536	0.537	0.556	0.556	0.546
BDE-183	0.575	0.572	0.562	0.570	0.615	0.605
BB-209	1.011	1.015	0.861	1.035	1.349	1.430
BDE-209	1.261	1.247	1.040	1.291	1.816	1.931
BDE-47*	7.685	6.793	6.592	7.696	8.55	8.65
BDE-183*	10.392	9.073	8.457	10.211	13.66	13.272
BDE-209*	22.782	19.787	15.644	23.121	40.33	42.334

*Retention time on the corresponding columns

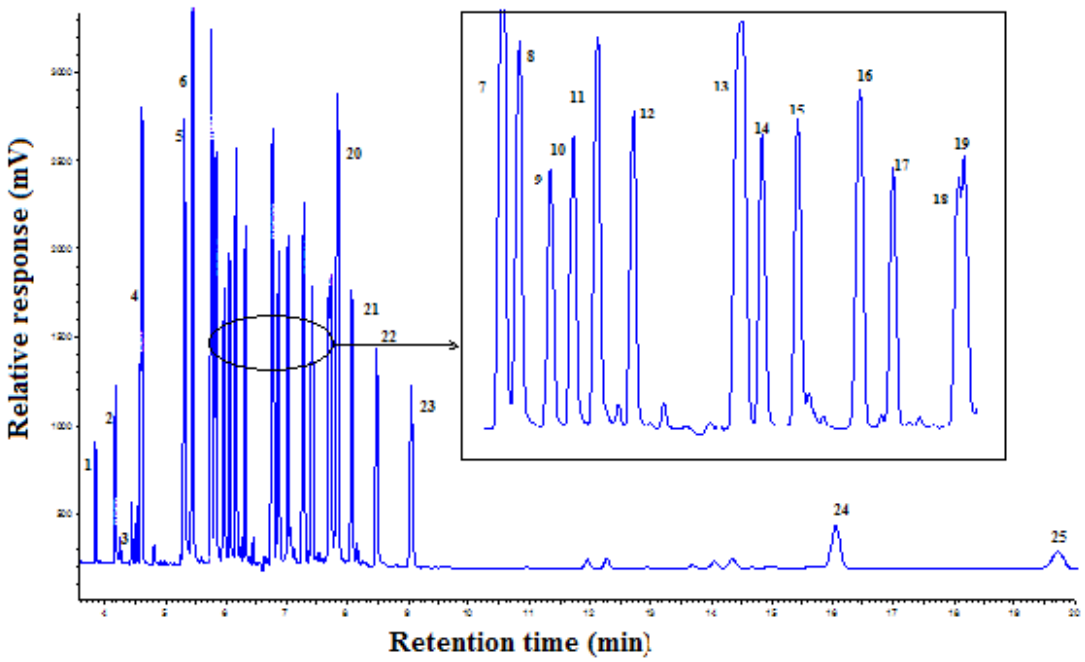

Figure 2.1. GC-ECD chromatograms of 32 standards on the ZB-5 column (30 m, ID: 0.25 mm, df: 0.1 µm): 1) BB- 1; 2) BB-2; 3) BDE-3; 4) BB-4 and 10; 5) BB-15 and BDE-15; 6) BB-30; 7) BB-29 and 26; 8) BB-31; 9) BDE-17; 10) BDE- 28; 11) BDE-38; 12) BB-49; 13) BDE-47; BB-80; and BB-103; 14) BDE-66; 15) BDE-77; 16) BDE-100 and BB-155; 17) BDE-99; 18) BDE-85: 19) BDE-126: 20) BDE-154 and BB-153; 21) BDE-153; 22) BDE-138; 23) BDE-183; 24) BB-209; 25) BDE-209.

4.1.2 Solvent choice and method performance evaluation

The solvents used for extraction were selected based on the recoveries obtained from the extraction of the SRM 2585 house dust standard reference material (1.0 g). There were no significant differences between the different combinations of HPLC grade solvents (Table 3.2). However, a good recovery of BDE-209 was obtained with hexane:acetone (2:1 *v/v*), whereas hexane:toluene (1:1 *v/v*) yielded a very low recovery of the same congener. Hexane:acetone (2:1 *v/v*) exhibited a recovery of ~104%. Therefore, hexane:acetone (2:1 *v/v*) was used throughout this study. Generally, ~96% of the congeners were recovered, irrespective of the type of solvents used. This demonstrated good correlation between the certified and measured values. Higher recoveries of BDE-17, 28, and 66 were obtained. This was partially attributed to the debromination of a higher concentration of congeners during extraction, analysis, or breakthrough in the silica gel column during clean-up.

Table 3.2. Mean percentage recoveries ± SD of BDE congeners in a house dust certified reference material relative to the certified values with different solvents

PBDE congener	Hexane:acetone (2:1 v/v)	Hexane:acetone (3:1 v/v)	Hexane:toluene (2:1 v/v)	Hexane:dichloromethane (2:1 v/v)
BDE-17	137 ± 7.9	106 ± 5.3	134.3 ± 25	108 ± 3.8
BDE-28	132 ± 15	108 ± 11	132 ± 11	103 ± 1.6
BDE-47	89 ± 11	94 ± 11.6	80 ± 10	84.3 ± 10
BDE-66	131 ± 22	103 ± 1	102.9 ± 10.2	111 ± 13

PBDE congener	Hexane:acetone (2:1 v/v)	Hexane:acetone (3:1 v/v)	Hexane:toluene (2:1 v/v)	Hexane:dichloromethane (2:1 v/v)
BDE-85	104 ± 14	94.4 ± 4.8	83.6 ± 1.5	105 ± 14
BDE-99	85 ± 2.6	90 ± 3.6	85.3 ± 5.9	89 ± 7
BDE-100	90 ± 5.0	82 ± 7.8	90.6 ± 1	92.3 ± 1
BDE-138	97 ± 38	101 ± 37	72 ± 15	88 ± 28
BDE-153	102 ± 4.7	96.4 ± 7.2	102.4 ± 12	103 ± 3.3
BDE-154	98 ± 3.4	91 ± 16	98 ± 26	102 ± 21
BDE-183	94 ± 1	87 ± 20	66 ± 2.3	86 ± 30
BDE-209	84 ± 5.7	80.8 ± 5.7	55 ± 1.8	75 ± 3.4

4.2 Concentrations and profiles of the PBBs and PBDEs in office dust collected from South Africa

Of the 32 target congeners measured (BDE-3, 15, 17, 28, 47, 66, 77, 85, 99, 100, 126, 138, 153, 154, 183, 209 and BB-1, 2, 4, 10, 15, 26, 29, 30, 31, 38, 49, 80, 103, 153, 155, and 209), only BDE-47, 66, 85, 99, 153, 209 and BB-2, 4, 30, 153, 209 were detected. The summary of all 11 detected individual congeners are shown in Table 3.3. From the results, only BDE-47 and BDE-99 exhibited a median concentration above the detection limit; these congeners were detected in more than 50% of the samples. The frequency of detection was dominated by BDE-99 (81.3%), followed by BDE-47 (62.5%). The ΣPBDEs detected ranged from 21.4–578.6 ng g^{-1} with mean and median concentrations of 169 and 162 ng g^{-1}, respectively. Irregular distributions of PBDEs were observed, indicated by the high value of SD (144.5 ng g^{-1}) after the summation of the six congeners. This observation may be ascribed to the different electronic materials sampled, floor type, frequency of cleaning, and ventilation conditions of each office. BDE-209 exhibited the highest concentration (578.6 ng g^{-1}) in one of the dust samples collected from a dusty office with old computers, sofas, padded chairs, and other electronic materials. The most frequently detected PBB congener was BB-4 (43.8%), followed by BB-2 (31.3%).

The ΣPBBs detected ranged from <dl–196 ng g^{-1} with mean and median concentration of 38.2 and 11.4 ng g^{-1}, respectively. Generally, the concentrations of PBBs detected were relatively very low, probably due the limited use and ban of PBB in products compared to PBDEs. Penta-mixture mainly comprises five congeners, i.e., BDE-99, 47, 100, 153, and 154 in the ratio 12:9:2:1:1 [32]. Hence, BDE-99 and BDE-47 are approximately 48% and 36% of the penta-mixture, respectively. However, different percentage compositions of BDE-47 and BDE-99 in the penta-mixture have been reported [33]. Sjodin et al. [34] reported 37% BDE-47 and 35% BDE-99, as well as 51% (BDE-47) and 34% (BDE-99). The variable percentages reported reveal that the composition of these products may vary with manufacturer or between batches of production [34]. In this study, the BDE-47 concentration detected was lower than the BDE-99 concentration. The BDE-99 and BDE-209 concentrations were dominant in office dust [35]. Frequent cleaning, adequate ventilation, and the frequent use of fan in the offices may have contributed to the nondetection of BDE-3, 15, 17, and 28. The nondetection of some higher PBDEs could be attributed to their low concentrations in the samples below the detection limits of the instrument. Table 3.3 shows samples 13, 14, 15, and 16, which were dust samples collected using a vacuum cleaner; the remaining samples were collected by wiping the surfaces with glass wool. The average concentrations of PBBs in the dust samples collected by a vacuum cleaner (82.7 ng g^{-1}) were higher than those collected using glass wool (23.4 ng g^{-1}). This was attributed to the high concentration of BB-209 detected in sample number 14. A slightly higher average concentration of ΣPBDEs was observed in the dust samples collected using glass wool (171 ng g^{-1}) compared to vacuum cleaner (163 ng g^{-1}). The low concentration of BFRs in the dust collected by the vacuum cleaner may have been due to the dilution of the congeners by higher dust loadings. This suggests that it is more likely to detect lower concentrations of PBDE congeners at a higher dust loading of BFRs. It is pertinent to mention that besides lower dust loading,

dust collection by wiping with glass wool tends to only target the surface of materials such as computers, printers, fans, air conditioning units, chairs, and tables, where the sources of BFRs are expected and easier to collect. Therefore, this may contribute to an increase in the BFR concentration. Notably, in contrast to the offices where the dust samples were collected by glass wool, the average concentrations detected by both methods were comparable in the case of PBDEs, regardless of the additional offices.

Table 3.3. Summary of PBB and PBDE concentrations (ng g–1) detected in office dust collected from South Africa.

Sample no.	BB-2	BB-4	BB-30	BB-153	BB-209	ΣPBBs	BDE-47	BDE-66	BDE-99	BDE-85	BDE-153	BDE-209	ΣPBDEs
1	<dl	32.0	<dl	<dl	<dl	32.0	53.0	35.0	88.9	40.3	<dl	<dl	217.2
2	31.7	45.7	<dl	<dl	<dl	77.4	<dl	<dl	<dl	7.6	<dl	571	578.6
3	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	21.4	<dl	<dl	21.4
4a	22.2	<dl	<dl	<dl	<dl	22.2	44.0	<dl	50.0	<dl	<dl	2.8	96.8
5	<dl	<dl	<dl	<dl	126	126	44.0	44.9	89.9	<dl	<dl	142	320.8
6	<dl	<dl	<dl	<dl	<dl	<dl	78.9	<dl	127.7	44.7	<dl	<dl	251.3
7	<dl	<dl	15.3	<dl	<dl	15.3	<dl	<dl	<dl	44.4	12.5	<dl	56.9
8a	<dl	<dl	<dl	<dl	<dl	<dl	14.8	<dl	75.0	<dl	<dl	<dl	89.8
9a	<dl	<dl	<dl	<dl	<dl	<dl	62.7	<dl	82.8	<dl	<dl	<dl	145.5
10b	<dl	<dl	<dl	<dl	<dl	<dl	46.9	<dl	78.1	<dl	<dl	87.5	212.5
11b	<dl	7.5	<dl	<dl	<dl	7.5	<dl	<dl	32.5	<dl	<dl	<dl	32.5
12b	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	31.6	<dl	<dl	<dl	31.6
13c	7.9	34.2	<dl	5.4	<dl	47.5	61.8	<dl	119.7	<dl	<dl	<dl	181.5
14c	26.5	26.5	<dl	<dl	143	196	76.7	<dl	109.8	<dl	<dl	<dl	186.5
16c	<dl	1.3	<dl	<dl	<dl	1.3	<dl	<dl	28.8	<dl	<dl	<dl	28.8
Σ16PBB/ PBDE	121.6	180.5	15.3	24.8	269.0	611.2	564.7	79.9	1035.6	170.9	12.5	842.3	2706
Mean	7.6	11.3	1.0	1.6	16.8	38.2	35.3	5.0	64.7	10.7	0.8	52.6	169.1
SD	12.8	16.6	3.8	4.9	46.0	56.8	32.4	13.8	44.9	17.2	3.1	144.0	144.5
Median	<dl	<dl	<dl	<dl	<dl	11.4	44	<dl	76.5	<dl	<dl	<dl	162.2
Min	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	<dl	21.4
Max	33.3	45.7	15.3	19.4	143	196	81.9	44.9	127.7	44.7	12.5	571	578.6
%ΣPBB/ PBDE	19.9	29.5	2.5	4.1	44.0		20.9	3.0	38.3	6.3	0.5	31.1	
n> dl	5	7	1	2	2		10	2	13	6	1	5	
%det.fre	31.3	43.8	6.3	12.5	12.5		62.5	12.5	81.3	37.5	6.3	31.3	

<dl: less than detection limit, det. Fre: detection frequency, [a] pooled samples collected from three offices, [b] pooled samples collected from two offices, [c] samples collected using vacuum cleaner

Based on observations, the amount of dust sample used for analysis, the instrumental detection limits, and the dilution factors can influence the analysis results. For example, for samples containing low concentrations of BFRs, the extraction of ≥ 1.0 g of dust is important to validate the presence or absence of BFRs in the sample. However, when the dust samples contain extremely low BFR concentrations, the concentrations will be below the detection limit and may lead to the wrong conclusion if a limited amount of dust is extracted. Similarly, increasing the dilution factor of the concentrated extract may lead to nondetection of analytes in the sample. Therefore, to confirm the presence or absence of BFRs in environmental samples with low contaminants, it is essential to carefully determine the amount to be extracted and the dilution factors.

The sources of BFRs for both the PBBs and PBDEs Spearman's rank correlation coefficients were computed between the number of electronic materials used in the office and the concentrations of Σ_5PBBs and Σ_6PBDEs detected. Almost no correlation was observed for both Σ_5PBBs (r_s = -0.26, p = 0.07) and Σ_6PBDEs (r_s = 0.07, p = 0.0004). Similarly, studies on the PBDE concentrations in dust and residential characteristics (i.e., the number of televisions, computers, and other electronics in the household) revealed no significant correlations [36-38]. Comparison of the analysis results against information recorded during dust sample collection exhibited non-uniform results. Therefore, it was a difficult task to conclusively identify the main sources. However, it is highly probable that the BFRs may have originated from the materials (computers, printers, carpets, sofas, and other electronic) found in offices. From the Spearman's rank correlation, i.e., the calculation between the concentration of Σ_5PBBs and Σ_6PBDEs (r_s = 0.55, p = 0.003), a significant positive correlation was observed, indicating a common pollution source for both BFRs. Similarly, a significant strong correlation (r_r = 0.92, p = 0.04) between the two frequently detected congeners, i.e., BDE-47 and BDE-99, was observed, which also supports the common emission sources. As shown in Table 3.4, few congeners have significant Spearman's correlation coefficients.

Table 3.4. Spearman's rank correlation coefficients and the corresponding p-value for the analyzed PBBs and PBDEs

Congeners	BB-4	BB-30	BB-153	BB-209	BDE-47	BDE-66	BDE-99	BDE-85	BDE-153	BDE-209
BB-2	0.68* 0.49	-0.16 0.06	0.53 0.09	0.20 0.45	0.31 0.00	-0.23 0.58	0.13 0.00	-0.19 0.57	-0.16 0.05	0.47 0.23
BB-4		-0.18 0.49	0.45 0.06	0.07 0.09	0.27 0.45	0.06 0.00	0.22 0.58	0.03 0.00	-0.18 0.57	0.50 0.05
BB-30			-0.08 0.71	-0.10 0.19	-0.29 0.00	-0.10 0.27	-0.38 0.00	0.52 0.04	1.00 0.89	-0.10 0.17
BB-153				-0.12 0.21	0.44 0.00	-0.12 0.36	0.42 0.00	-0.02 0.06	-0.08 0.60	-0.05 0.18
BB-209					0.31 0.20	0.45 0.34	0.31 0.01	-0.24 0.62	-0.10 0.18	0.04 0.36
BDE-47						0.15 0.00	0.92 0.04	0.08 0.01	-0.2 9 0.00	-0.23 0.64
BDE-66							0.21 0.00	0.16 0.31	-0.10 0.25	0.07 0.21
BDE-99								-0.02 0.00	-0.38 0.00	-0.31 0.75
BDE-85									0.52 0.04	-0.11 0.26
BDE-153										-0.10 0.17

* each cell contains the Spearman's correlation coefficient and p-value

5. PBDE Concentrations in office dust samples collected from Nigeria

The mean and median concentrations of ΣPBDE in all 11 office dust samples were 79.84 ng g^{-1} dw and 62.63 ng g^{-1}, respectively. These results are slightly higher than our previous findings on office dust in Pretoria [39]. This was found to be about one order of magnitude lower (BDE-209 median: 622 ng g^{-1}) than that reported in office dusts in Germany [29] and Australia (Σ_7PBDE median: 571 ng g^{-1}) [30]. A total Σ_7PBDE concentration of 7008.23 ng g^{-1} was determined in all 11 samples. All target congeners were detected in sample 1, except BDE-183. The concentrations ranged from nd–428.64 ng g^{-1} (BDE-209). The frequency of detection of the congeners in all the dust samples, mean, median, maximum, and 10th and 90th percentiles are presented in Table 3.5. The highest median concentrations were reported for BDE-209, while BDE-100, BDE-99, and BDE-154 exhibited relatively similar median values. BDE-209 was the dominant congener in all samples, with percentage contribution of ~41%, followed by BDE-153 (11. 6%) and BDE-183 (11.4%). They were ranked in ascending order as follows: BDE-47 (8.1%) < BDE-100 (9%) < BDE-99 (9.5%) < BDE-154 (10.1%) < BDE-183 (11.4%) < BDE-153 (11.6%) < BDE-209 (40.5%). A comparison of the findings in this study with other related studies in different regions was undertaken and the details presented in Table 3.6.

Table 3.5. Summary of ∑7PBDEs concentrations (ng g^{-1}) in office dust from Nigeria

Congener	Mean	Median	10%	90%	% DF	Max	%Contribution
BDE-47	51.62	45.95	37.36	81.35	100	84.76	8.1
BDE-100	56.06	51.11	41.58	84.76	100	92.69	9
BDE-99	60.55	54.15	44.67	95.67	100	99.34	9.5
BDE-154	64.65	58.62	48.21	94.94	100	110.58	10.1
BDE-153	74.28	67.13	59.34	110.23	100	118.34	11.6
BDE-183	72.14	71.90	57.57	111.24	90	124.29	11.4
BDE-209	179.56	139.45	115.94	261.36	100	428.64	40.5

The results of this study were compared to the PBDE levels reported in the literature by researchers from other regions around the world. Sjödin et al. [40] reported a median level of 74 ng g^{-1} and a range of 17–550 ng g^{-1} for dust samples from Germany. A median level of 1200 ng g^{-1} and a range of 500–13000 ng g^{-1} were reported for Australian dust samples. The United States and Great Britain reported median concentrations of 4200 ng g^{-1} (range: 950–54000 ng g^{-1}) and 10000 ng g^{-1} (range: 950–54000 ng g^{-1}), respectively [30,40]. These previous results were higher than the median (62.63 ng g^{-1}) and range (<dl–7008.23 ng g^{-1}) in this study. However, higher median values were observed for majority of the lower congeners in this study compared to the other studies, except for the samples from the United States.

A one-way ANOVA was performed to test the level of significance in the different mean concentrations recorded for the seven congeners, considering a number of influencing factors such as the presence of materials treated with flame retardants, smaller space, ventilation, and climatic conditions. Benue State is located in the middle belt of Nigeria, where the average temperature is 32.5 °C throughout the year. Higher temperatures can lead to higher emission rates of PBDEs from household products [41]. The test results exhibited a significant difference in the PBDE levels determined in each sample. The contributions of the various commercial mixtures (Figure 3.4) were ranked as follows: pentaBDE > decaBDE > octaBDE. PentaBDE is used in polyurethane foams, which are found in most homes and in circuit boards. Conversely, decaBDE comprising mainly BDE-209 is used in the hard casings of televisions and computers, electrical, electronic equipment, and plastics, which are also common features in many homes and offices. The mean PBDE concentrations in the eleven samples are shown in Figure 3.5.

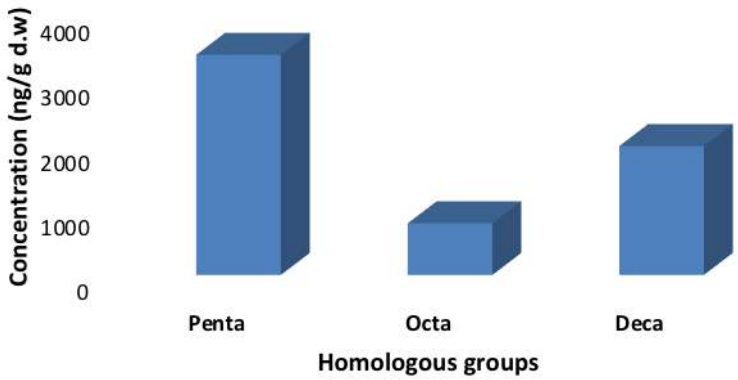

Figure 3.3. Contributions of the representative congeners of various commercial mixtures in the analyzed dust samples.

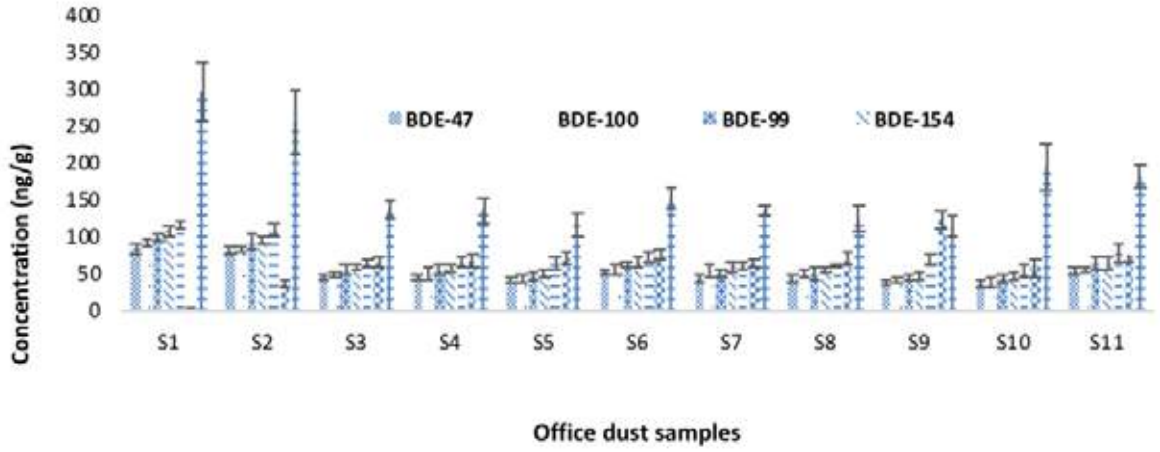

Figure 3.4. Mean concentrations of PBDE congeners in office dust samples.

Figure 3.6 shows the concentration of ΣPBDEs in each of the eleven samples. Sample S1 yielded the highest ΣPBDE concentrations, followed by S2, S11, and S6 respectively. Samples S3–S5 and S7–S10 yielded relatively similar concentrations. However, this was not surprising because the samples with higher PBDE concentrations (S1, S2, and S11 and S6) correspond to the micro environments with computers and other office electronics, except for S4.

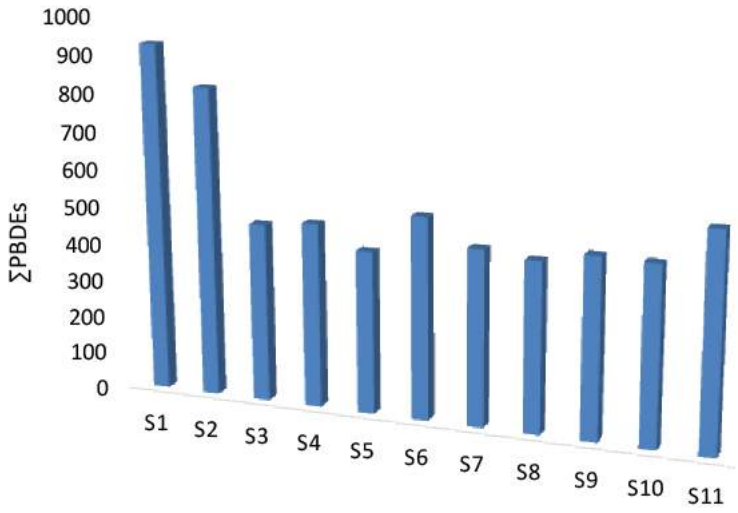

Figure 3.5. Observed ΣPBDEs per sample in office dust.

Table 3.6 presents a summary of the concentrations of nine PBDE congeners in office dust samples from South Africa, Nigeria, and other selected regions. Table 3.6 reveals that the order of average PBDE congener concentrations in different countries is: South Africa < Nigeria < Tokyo Japan < Michigan USA < Birmingham UK. The high average concentrations observed for Michigan, USA and Birmingham, UK is an indication of the use of large quantities of PBDEs as flame retardant in products. It is most likely that the major and probably the only source of PBDEs in the South African and Nigerian environment may have originated from imported products treated with PBDEs, since neither country produces these chemicals. A higher concentration was observed for Benue Nigeria than South Africa, which indicates the importation of PBDE treated electrical and electronic products.

Table 3.6. Summary of the concentrations (ng g^{-1}) of common PBDE congeners in office dust samples from South Africa, Nigeria, and other selected regions.

Location and references	Statistical Parameters	PBDE congeners								
		BDE-47	BDE-66	BDE-85	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209
Makurdi, Nigeria, n=11 (This study)	Average	51.62	na	na	56.06	60.55	64.65	74.28	72.14	179.56
	Median	45.95	na	na	51.11	54.15	58.62	67.13	71.90	139.45
	Min	35.81	na	na	38.08	43.07	46.69	51.68	1	112.09
	Max	84.76	na	na	92.69	99.34	110.58	118.34	124.29	428.64
Pretoria, South Africa, n=16 (Kefeni, 2012)	Average	35.30	5	10.70	na	64.70	na	0.80	na	52.60
	Median	44.00	<dl	<dl	na	76.50	na	<dl	na	<dl
	Min	<dl	<dl	<dl	na	<dl	na	<dl	na	<dl
	Max	10	2	6	na	127.70	na	1	na	5

Location and references	Statistical Parameters	PBDE congeners								
		BDE-47	BDE-66	BDE-85	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209
Michigan, USA, n= 18 (Batterman *et al.*, 2010)	Average	1,650	860	650	5,900	3,310	430	230	840	110,000
	Median	978	49	130	1,200	1,760	190	110	220	190
	Min									
	Max	46,000	13,000	7,000	78,000	79,000	1,300	790	7,600	66,000
Birmingham, UK, n=18 (Harrad *et al.*, 2008a)	Average	67	na	na	16	120	10	16	11	30,000
	Median	23	na	na	3.20	65	5.10	8.70	8.30	6,200
	Min	2.60	na	na	<dl	4.20	<dl	<dl	<dl	620
	Max	380	na	na	79	490	38	99	24	280,000
Tokyo, Japan, n=14 (Suzuki, 2006)	Average	110	7.80	8.70	na	170	na	34	na	2,400
	Median	30.50	1.60	2.10	na	38	na	15.50	na	1,100
	Min	4.30	0.32	<0.0025	na	3.10	na	3.30	na	150
	Max	580	64	43	na	810	na	100	na	17,000

na = not analyzed, <dl = less than detection

5.1 Levels and profiles of PBBs and PBDEs in house dust collected from South Africa

Of the 31 samples of house dust analyzed, PBDEs were only detected in 21 samples. This amounted to 67.7% and 32.3% of samples in which PBDEs were detected and nondetected, respectively. The number of congeners detected in each sample varied from 1 to 5 of the 15 target congeners considered for identification. Of the 15 PBDE congeners considered for identification, only BDE-47 and BDE-99 exhibited median values. BDE-17, 28, 126, 138, and 183 were not detected at all. BDE-3, 15, 66, 100, 154, and 153 were detected in less than four samples, whereas BDE- 85 and BDE-209 were detected in nine and eleven samples, respectively. The ΣPBDEs detected ranged from <0.3–234 ng g^{-1} dw of dust with median and mean values of 18.3 and 51.1 ng g^{-1} dw, respectively (Table 3.7). The concentration and type of congener detected in each sample was non-uniform. Such non-uniform distribution of PBDE congeners has also been reported previously [42, 43]. Variations in the PBDE concentrations by more than approximately two orders of magnitude, ranging from 9.8–1700 ng g^{-1} with a median of 1200 ng g^{-1}, was observed for eight floor dust samples obtained from a Japanese commercial hotel that was assumed to have many flame-retardants. This was possibly because concentration differed among floors, suggesting that the localization of source products was associated with flame retardants in dust [43]. Allen et al. [44] corroborated this reasoning after observing a non-uniform distribution of BFRs in home dust. Similar to this study, a report on landfill sites in the same area revealed that the PBDE congeners detected varied significantly from the patterns of commercial mixture [14]. Therefore, it was not surprising to detect some different congeners in the house and office samples. It is possible that environmental conditions may have facilitated the degradation of higher congeners to lower congeners. The frequency of detection was extremely low in office and house environments, except for BDE-47 and BDE-99. In part, this is an indication of a low contamination of house dust, which suggests the presence of a few emission sources. The three congeners (BDE-47, 99, and 209) constituted an average concentration of 84.7% to the PBDEs measured in this study. The orders of concentrations detected in these congeners were as follows: BDE-209 > BDE-99 > BDE-47, corresponding to the total percentage of 31.8, 29.7, and 23.3, respectively. The congener profiles detected in house dust were almost similar to that detected previously from office and other micro environments such as hotels and departmental stores [39, 45].

In the case of PBBs, of the 16 targeted congeners considered for identification, only three congeners, BB-4, BB-10, and BB-209, were detected in seven, one, and two samples, respectively. In one sample, all three were detected, and were also observed in office dust samples. The detection frequencies and concentrations of these congeners were lower in house dust than in office dust. BB-4 and BB-10 were not environmentally relevant congeners and were not detected in technical PBBs. Furthermore, the PBBs detected from environmental samples typically do not correlate with that in technical products; consequently, their origin is expected to be from the reductive debromination of the technical bromobiphenyls [46]. Generally, both types of BFRs were detected in low concentrations in environmental samples. The mean, median, 95th percentile, and maximum value detected (ng g^{-1}) are presented in Table 3.7. For all the samples, the 5th percentile and minimum values were below the detection limits; therefore, they were not included in Table 3.7.

Table 3.7. Concentration of PBDEs and PBBs (ng g–1) detected in 31 South African houses.

Congener	Mean	Median	95th percentile	%DF	Maximum
BB-4	3.2	<dl	16.2	22.6	21.3
BB-10	0.3	<dl	0.0	3.2	9.6
BB-209	1.1	<dl	7.2	6.5	20.4
BDE-3	0.4	<dl	0.0	3.2	10.9
BDE-15	0.6	<dl	4.8	9.7	8.4
BDE-47	11.9	2.60	45.6	54.8	48.2
BDE-66	0.9	<dl	6.5	12.9	10.5
BDE-100	1.1	<dl	8.1	6.5	16.6
BDE-99	15.2	2.60	53.2	54.8	71.1
BDE-85	2.7	<dl	13.7	29.0	19.7
BDE-154	1.2	<dl	7.2	9.7	23.1
BDE-153	1.0	<dl	1.9	6.5	26.6
BDE-209	16.2	<dl	69.7	35.5	78.9
ΣPBDEs	51.1	15.21	62.28		234
ΣPBBs	4.60	<dl	15.3		21.3

<dl = blow detection limit, %DF = % detection frequency

5.2 Comparison of PBDE concentration in indoor dust

The BFR concentrations detected in office and house dust samples are summarized in Figure 3.7. The average concentration of PBDEs detected in office dust was 1.6 times that detected in house dust.

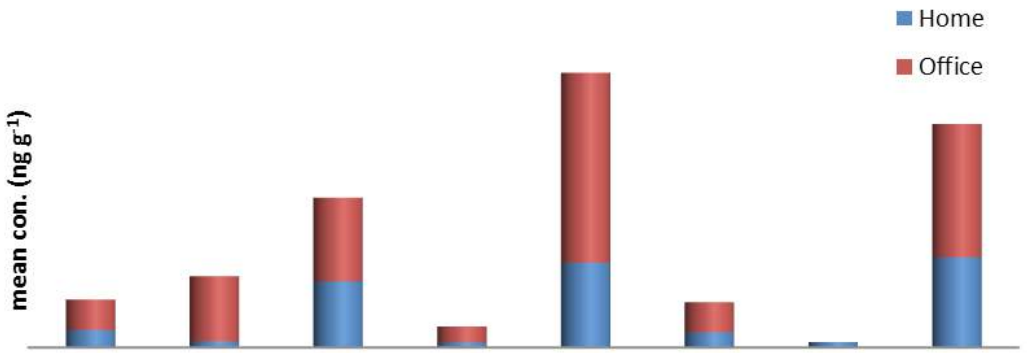

Figure 3.6. Mean concentration of PBDEs detected in both offices and houses collected from South Africa.

The higher contamination of office dust correlated with most of the research reports [47, 48]; however, the observed concentrations were still extremely lower than the concentrations reported in developed countries.

5.3 Comparison to other studies

Table 3.8 summarizes the six PBDE congeners detected in office dust in this study and compares them with similar results from different studies. Generally, the concentrations of PBDEs detected in this study are lower than those reported from developed countries. From Table 3.7, the average concentration of BDE-209 detected in this study was 2–3 orders less than the reported values from UK [47], Japan [48], and USA [49].

Table 3.8. Summary of the concentration (ng g^{-1}) of six PBDEs congeners detected in office dust in this study and other selected studies used for comparison

Location and references	Statistical parameters	PBDE Congeners					
		BDE-47	BDE-66	BDE-99	BDE-85	BDE-153	BDE-209
Pretoria, South Africa, this study n = 16	Average	35.3	5	64.7	10.7	0.8	52.6
	Median	44	<dl	76.5	<dl	<dl	<dl
	Min	<dl	<dl	<dl	<dl	<dl	<dl
	Max	81.9	44.9	127.7	44.7	12.5	571
	n> dl	10	2	13	6	1	5
Michigan, USA n = 10 (Batterman et al., 2010)	Average	1650	26	3310	113	126	6930
	Median	978	6	1760	48	48	1

Location and references	Statistical parameters	PBDE Congeners					
		BDE-47	BDE-66	BDE-99	BDE-85	BDE-153	BDE-209
Birmingham, UK n = 18 (Harrad et al., 2008a)	Average	67	na	120	na	16	30000
	Median	23	na	65	na	8.7	6200
	Min	2.6	na	4.2	na	<dl	620
	Max	380	na	490	na	99	280000
Japan, Tokyo n = 14 (Suzuki et al., 2006)	Average	110	7.8	170	8.7	34	2400
	Median	30.5	1.6	38	2.1	15.5	1100
	Min	4.3	0.32	3.1	<.0025	3.3	150
	Max	580	64	810	43	100	17000

na = not analyzed, <dl = less than the detection limit

Large differences between the mean and median are indicators of skewed distributions. For skewed data, the median is a better comparator than the mean. Accordingly, the median concentrations of BDE-47 and BDE-99 in South Africa are similar to those in UK and Japan, but are one to two orders of magnitude lower than those in the USA. On the other hand, the average Σ_6PBDEs detected in this study were lower by far; for instance, the individual average concentration of BDE-209 (dominant congener) reported from Japan, USA, and UK are about 14, 41, and 178 times greater than the average values of the Σ_6PBDEs in this study, respectively.

Furthermore, majority of the targeted BFRs congeners analyzed were below the detection limits, particularly BDE-28, 77, 100, 154, and 183, which have been reported in other studies [35, 48, 49, 50]. These BFRs were also in a few samples at low concentrations. Generally, besides the low concentration that was detected, few congener types were detected in office dust samples from Pretoria, South Africa with non-uniform distribution. There is no published evidence of the production of BFRs in South Africa. Therefore, the detected PBDEs may have originated from imported electronics and furniture in the offices where the samples were taken. There was a slight correlation between the detected congeners and the composition of commercial products, particularly in the case of PBDEs. The research from the landfill sites in the Pretoria area revealed similar variations of PBDE congeners from the patterns of commercial mixtures [14]. For PBB congeners, no comparison was possible, owing to limited reports on the analysis results in office dust.

The mean, median, and range of concentrations (ng g^{-1}) for the PBDE congeners detected in dust samples collected from the houses measured in this study and other studies are given in Table 3.8. For lower PBDE congeners, the mean and median concentrations in South Africa, Kuwait, Pakistan, UK, and Germany are < 100 ng g^{-1} dw of dust. The PBDE concentrations in house dust samples from different countries was in the following order: Pakistan < Kuwait < South Africa < Germany < UK. Similar mean and median BDE-209 concentrations were observed in this study with Pakistan and Kuwait. Further, the concentration detected was ranked in the following order: South Africa < Pakistan < Kuwait. Generally, irrespective of the type of congener, the mean and median PBDE concentrations in South Africa was 1–3 orders of magnitude less than that detected in the USA and Canada. Furthermore, the result demonstrated lower contamination rates of home dust from South Africa, Kuwait, and Pakistan. Due to the decreasing trend of PBDE concentration as a result of banning the penta- and octa- commercial PBDEs, such a comparison will be most reliable for analyses performed in the same year or more recently. In a study of 10 dust samples taken at monthly intervals, the 400-fold variations were observed in the concentration of PBDEs [47].

5.4 Human exposure rates

Human exposure studies typically focus on dietary pathways as the main route of contamination of toxicants in humans and animals. However, the house dust in indoor environments has recently been found to be an important non-dietary exposure pathway. The inhalation of house dust in some cases may be more important than food consumption as a major source of exposure [51]. For adults, next to food ingestion, dust ingestion is the second exposure pathway to BFRs, whereas for toddlers and children, the contribution from dust is nearly similar to that from food ingestion [52, 53]. Exposure to these pollutants in the indoor environment has been associated with numerous adverse health effects including allergies and weakened immune systems, compromised respiratory, cardiovascular, and nervous systems, irritation to the skin and mucous membranes, cancer, and reproductive effects [54]. An increasing number of studies have highlighted the importance of indoor dust exposure [52, 55-58].

A rough estimation of the daily intake of PBDEs in young children and adults via house dust in terms of the average concentration of the total PBDEs measured in house dust can be made based on low and high dust ingestion scenarios. However, in addition to the high uncertainty in the estimation of exposure rates, there are several limitations of comparing the exposure rates with different published data. These limitations could be attributed to three main reasons. First, there is no available standard average dust ingestion value in the literature. For example, for adults, the USEPA recommends 0.56 mg day^{-1} and 110 mg day^{-1} of low and high dust ingestion rates, respectively [59]. Similarly, the average and high daily dust ingestion rates recommended for adults and toddlers are 20 and 50 mg day^{-1} and 50 and 200 mg day^{-1}, respectively [60].

On the other hand, the estimations of the mean daily dust ingestion of 4.16 and 50 mg day^{-1} and high dust ingestion rate of 100 and 200 mg day^{-1} for adults and children (six months to two years), respectively, have been used [61]. Second, the metrics presentation of results for toxicants or congeners detected is surface loading (mass of congeners per square meter) or surface concentration (mass of congener per mass of dust used for analysis), which are equally important [62]. Third, irrespective of the type of indoor environment (office, house, or hotel), average indoor exposure rates are used by some researchers; for instance, a previous report [56] presented the exposure rates of the workplace and home together. This indicates that various researchers use different average exposure values, units, or total exposure rates. Therefore, this non-uniformity of data presentation may limit comparison with most published data. A summary of the daily average dust ingestion obtained in this study and a comparison with similar results from other studies are shown in Table 3.9. The calculation was done assuming 100% absorption of intake, and mean adult and toddler ingestion rates of 20 and 50 mg day^{-1} and high dust ingestion rates of 50 and 100 mg day^{-1}, respectively [50, 60]. The mean and high dust ingestion rates for adults and toddles were calculated using the median and mean concentrations of BDE-209 and ΣPBDEs in house dust. Accordingly, the median value exposure rates of BDE-209 and ΣPBDEs ranged from 0.05–0.18 and 0.61–2.44 ng day^{-1} for toddlers, but ranged from 0.02–0.05 and 0.24–0.61 ng day^{-1} for adults, respectively. Similarly, the mean values ranged from 1.75–6.98 and 0.81–3.24 ng day^{-1} for adults and 0.7–1.75 and 0.32–0.81ng day^{-1} for toddlers. In Table 10, the comparison of adult exposure rate to PBDEs in both microenvironments using mean and high dust ingestion rates revealed that the human exposure to PBDEs in office dust was approximately nine and two times that in house dust, irrespective of the median or mean used for calculation. Similarly, compared with other studies, the mean and high daily dust ingestion exposure rates estimated from this study were 1–2 and 2–3 orders of magnitude lower than in developed countries, respectively. Therefore, this study provides the first report on the exposure rates of PBDEs to South Africans living in Pretoria.

Table 3.9. Summary of PBDE concentrations (ng g^{-1}) in the home dust of this study and other selected studies

n	Country	PBDEs congeners measured	Mean	Median	Range	References
31	**South Afr.**	Σ(BDE-3, 15, 47, 66, 100, 99, 85, 154, and 153)	34.9	12.2	<dl–154.9	This study
		BDE-209	16.2	0.91	<dl–78.9	
31	**Singapore**	Σ(BDE-28, 47, 100, 99, 154, 153, and 183)	660	98	11–12,000	(Tan et al., 2007)
		BDE-209	2200	1000	68–13,000	
17	**Kuwait**	Σ(BDE-28, 47, 100, 99, 85, 154, 153, and 183)	20	9.6	0.2–124	(Gevao et al., 2006)
		BDE-209	129	83	0.8–338	
30	**UK**	Σ(BDE-28, 47, 49, 66, 100, 99, 154, and 153)	77	46	7.1–550	(Harrad et al., 2008a)
		BDE-209	260,000	8100	<dl–2,200,000	
5	**Sweden**	Σ(BDE-28, 47, 66, 100, 99, 154, 153, and 183)	173	-	-	(Karlsson et al., 2007)
		BDE-209	470	-	-	
31	**Pakistan**	Σ(BDE-28, 47, 100, 99, 154, 153, and 183)	4.7	-	<0.2–64.5	(Ali et al., 2012)
		BDE-209	41.5	19.7	<2–1465	
64	**Canada**	Σ(BDE-17, 28, 47, 66, 100, 99, 85, 154, 153, 138, 183, and 190)	4,500	900	64–170,000	(Wilford et al., 2005)
		BDE-209	1100	630	74–10,000	
10	**USA**	Σ(BDE-47, 66, 100, 99, 138, 154, and 153)	10,482	9015	1780–25,200	(Hwang et al., 2008a)
34	**Germany**	Σ(BDE-28, 47, 66, 100, 99, 154, and 153)	74.9	30	5.88–814	(Fromme et al., 2009)
		BDE-209	354	312	29.7–1460	
33	**Belgium**	ΣPBDEs*	695	355	3–6325	(Covaci et al., 2010)
		BDE2-209	590	313	<1–5295	
43	**Belgium**	Σ(BDE- 47, 100, 99, 154, 153, 183, 196, 197, and 203)	104	27	4–1214	(D'Hollander et al., 2010)
		BDE-209	590	313	<5–5295	
20	**New Zealand**	Σ(BDE-28, 47, 49, 66, 100, 99, 154, and 153)	160	96	13–680	(Harrad et al., 2008b)
10	**Canada**	Σ(BDE-28, 47, 49, 66, 100, 99, 154, and 153)	1100	620	160–3600	(Harrad et al., 2008b)
		BDE-209	670	560	290–1100	
10	**UK**	Σ(BDE-28, 47, 49, 66, 100, 99, 154, and 153)	98	59	5.7–610	(Harrad et al., 2008b)
		BDE-209	45,000	2,800	120–520,000	
76	**China**	Σ(BDE-28, 47, 66, 100, 99, 85,154, 153, and 183)	63.93	30.51	6.39–639	(Huang et al., 2010)
		BDE-209	2598	1792	175–9602	

[n] = number of samples analyzed, * PBDEs included are not mentioned

The low distribution of BFR concentrations detected in different houses ranging from none to low highlights the fact that South Africans, particularly those living in Pretoria, are exposed to low concentrations of PBDEs and PBBs from house dust compared persons from other countries. This study could be extended to all regions of South Africa for the determination of the overall exposure rates for the entire population. To our knowledge, there are no data and exposure rates regarding BFRS in most African countries. Therefore, studies should be conducted to investigate these contaminants in other African countries where data regarding the environmental levels of BFRs and human exposure rates are limited.

Table 3.10. Summary of the estimated exposure (ng day^{-1}) of adult and toddlers to PBDEs via home dust ingestion in this study and other selected studies.

Country	Exposure group	BFRs	Mean dust ingestion rate		High dust ingestion rate		References
			Median	Mean	Median	Mean	
South Africac	Toddlers	ΣPBDEs[a]	0.61	1.75	2.44	6.98	This study
		BDE-209	0.05	0.81	0.18	3.24	
	Adult	ΣPBDEs[a]	0.24	0.70	0.61	1.75	
		BDE-209	0.02	0.32	0.05	0.81	
South Africad	Adult	ΣPBDEs[b]	2.19	2.33	5.48	5.83	(Kefeni and Okonkwo, 2012)
		BDE-209	<dl	1.052	<dl	2.63	
Canada	Toddlers	Σtri- hexa-BDEs	31	55	120	220	(Harrad et al., 2008b)
		BDE-209	28	33	110	130	
	Adult	Σtri- hexa-BDEs	12	22	31	55	
		BDE-209	11	13	28	33	
New Zealand	Toddlers	Σtri- hexa-BDEs	4.8	8.1	19	32	(Harrad et al., 2008b)
	Adult	Σtri- hexa-BDEs	1.9	3.2	4.8	8.1	
UK	Toddlers	Σtri- hexa-BDEs	2.9	4.9	12	20	(Harrad et al., 2008b)
		BDE-209	140	2200	560	9000	
	Adult	Σtri- hexa-BDEs	1.2	2	2.9	4.9	
		BDE-209	56	900	140	2200	
US	Toddlers	Σtri- hexa-BDEs	82	150	330	590	(Harrad et al., 2008b)
		BDE-209	65	80	260	320	
	Adult	Σtri- hexa-BDEs	33	59	82	150	
		BDE-209	26	32	65	80	

[a] Σ(BDE-3, 15, 47, 66, 100, 99, 85, 154, & 153), [b] Σ(BDE-47, 66, 99, 66, 85, & 153), [c] = house, [d] = office

6. Conclusions

This is the first study of its kind to be conducted in South Africa and Nigeria. The study reports the concentrations and compositional profiles of PBBs and PBDEs in the office and home dust samples from both countries. The concentrations of PBDE congeners detected in the office dust samples from South Africa were substantially lower than those reported in office dust in some developed countries. The low distribution of BFR concentration detected in different houses highlights the fact that South Africans, particularly those living in Pretoria, are exposed to lower concentrations of PBDEs and PBBs in house dust than individuals from other countries. Consequently, this study should be extended to all regions in South Africa to determine the overall exposure rates for the entire population. However, additional research should be conducted to ascertain whether polyurethane foams in mattresses, furniture, and other materials are treated with BFRs in South Africa. This will provide an overview of the extent of exposure in South Africans to these emerging contaminants because the materials are used on a daily basis.

Several reports have highlighted that dust is a heterogeneous mixture of biologically derived materials including BFRs and has been recognized as an important pathway of human exposure to PBDEs. Therefore, the PBDE concentrations in the office dust samples collected from Makurdi, Benue State Nigeria, were measured. The results reveal a significant difference between the samples. Further, BDE-209 was significantly different from the seven target congeners. The concentrations in office dust samples were higher than those in previous studies in samples collected from Pretoria, South Africa, but lower than those in samples collected from some developed countries.

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Pesticide Exposure in Horticultural and Floricultural Periurban Production Units in Argentina

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Abstract

This study summarizes the results of the OPCW project titled "Persistent pesticide contamination in horticultural periurban production units" performed at Universidad Nacional de General Sarmiento, Argentina. This project highlighted the impact of pesticide use on three different nontargets: workers, soil, and horticultural plastics. Therefore, an exposure study among horticultural and floricultural workers was conducted, revealing the correlation between the pesticide formulation and the exposure level. Further, the exposure during the mixing and loading stage for manual applications was almost as important as that arising from the application step. The degradation of selected pesticides was faster in the horticultural soil than in the control soils, probably due to the modification of the autochthonous microbial community. Finally, the relative pesticide amounts that reached the agricultural plastics (mulching and greenhouse polyethylene films) after pesticide application were determined. The chemical and photochemical degradation of deltamethrin absorbed on the polyethylene film were studied.

Keywords: horticulture, floriculture, periurban agriculture, pesticide, potential dermal exposure, plastic film.

1. Introduction

The use of pesticides in modern agriculture has contributed to a consistent increase in crop yields in the past decades [1]. However, there are several negative impacts such as pesticide environmental persistence [2]. Periurban agricultural activities are primarily focused on small horticultural and floricultural production units located in green belts around large cities. The impact of pesticides on the nontarget systems in periurban production units can be investigated based on three different components: the workers, the soil, and the agricultural plastics (Figure 1).

Figure 1. Potential interactions of pesticides with crops, workers, soil, and plastics.

Safe pesticide handling is a major concern regarding worker exposure during the mix/load, application, and re-entry operations in agricultural practices [2], [3]. This issue is particularly important in small-scale production units, like those surrounding Buenos Aires, where all the aforementioned operations are usually performed by the same laborer [4]. Under typical working conditions in fields, dermal absorption is potentially the most important pathway for the uptake of pesticides [5]. Thus, measurement of the potential dermal exposure (PDE) provides relevant information on the quantity of a chemical substance that contaminates the uncovered body regions and clothing worn by pesticide handlers [6]. However, PDE data cannot be exclusively used as a risk indicator because they must be related to acceptable exposure limits. Consequently, the margin of safety (MOS) [7] has been proposed as a useful risk indicator linking the acceptable exposure to a product with the mass deposited on the worker's cloth and skin. This mass can be estimated from the PDE.

The quantitative estimation of pesticide exposure levels in soils is essential for investigating their fate in horticultural and nonlabored soils. Although there are detailed studies on different soils devoted to extensive agriculture [9] and pesticide drift outside the crop fields [8], to our best knowledge, there is no systematic study on pesticide distribution in soils during the application stage using manual knapsacks at small-scale horticultural production units. The pesticides that reach the soil during application not only have profound effects on its biological state, but the molecules can also migrate to water resources, thus spreading the contamination.

Another important matrix reached by pesticides in horticultural and floricultural production units is the plastic sheeting used for greenhouse construction or mulching purposes [10]. Hence, most research has focused on investigating the absorption of pesticides, primarily on low-density polyethylene (LDPE), and the recyclability of the LDPE used in mulching practices [10]. Conversely, the quantitative estimation of pesticides that reach the plastic surfaces and their chemical transformations have not been comprehensively investigated.

In brief, this project aims to assess the pesticide exposure of nontarget systems (workers, soil, and agricultural plastics) and the distribution in horticultural and floricultural periurban production units. Further, the findings of this study will be used for proposing possible measures to minimize the potentially negative effects of pesticides under the aforementioned production conditions.

2. Evaluation of pesticide exposure among horticultural workers

The PDE results of a set of horticultural and floricultural workers of small production units located in Moreno district (Provincia de Buenos Aires, Argentina) are shown in Figure 2A. This PDE data correspond to different crops at the application stage, and is expressed as the total mass of pesticide on the cotton sampler coverall (in mg), and as the percentage of PDE (%PDE: ratio of pesticide on the worker's coverall and the total applied mass). The PDE was obtained by analyzing the cotton sampler coverall (Figure 2B), which were cut in predetermined sections (Figure 2C), extracted with solvents, and quantified by gas chromatography-electron capture detector (GC-ECD) according to a previously described methodology [11], [12]. The absolute mass of pesticide detected on the work coveralls ranges from 0.03–3.2 mg, whereas the %PDE ranged from 0.06–0.58% of the total manipulated pesticide. The exposure values of the application stage were similar to those found in the European Community in equivalent application scenarios [13]. Notably, these values were obtained for a unique application of a 20 L knapsack and did not include the exposure of the mix and load stage.

A

Crop	Pesticide	Hort/ Flor.	Pesticide applied/mg	EDP/mg	% PDE
Maize	Deltamethrin	Hof	583.2	3.2	0.55
Maize	Deltamethrin	Hof	466.0	2.7	0.58
Maize	Deltamethrin	Hof	1463.3	1.8	0.12
Broccoli	Deltamethrin	Hof	1368.9	1.7	0.12
Broccoli	Deltamethrin	Hof	594.4	1.3	0.22
Broccoli	Deltamethrin	Hof	583.0	0.5	0.09
Broccoli	Deltamethrin	Hof	1601.1	7.3	0.46
Tomato	Deltamethrin	Hgh[2]	380.6	0.52	0.14
Tomato	Deltamethrin	Hgh	433.2	0.24	0.06
Tomato	Deltamethrin	Hgh	394.1	0.16	0.04
Tomato	Deltamethrin	Hgh	321.1	0.36	0.11
Tomato	Procymidone	Hgh	1941.1	0.03	0.002
Tomato	Procymidone	Hgh	2315.0	0.04	0.002
Tomato	Procymidone	Hgh	2945.5	0.38	0.013
Tomato	Procymidone	Hgh	2914	1.63	0.060
Flowers	Endosulfan	Fgh[3]	5920	1.3	0.022
Flowers	Endosulfan	Fgh	9972	3.3	0.033
Flowers	Endosulfan	Fgh	8262	0.56	0.0068
Flowers	Endosulfan	Fgh	1575	1.85	0.12
Flowers	Endosulfan	Fgh	961	4.1	0.43
Flowers	Endosulfan	Fgh	4225.5	1.05	0.025
Flowers	Procymidone	Fgh	2697.9	1.48	0.055

Hof: horticultural open field. [2]Hgh: horticultural greenhouse. [3]Fgh: Floricultural greenhouse.

B

C

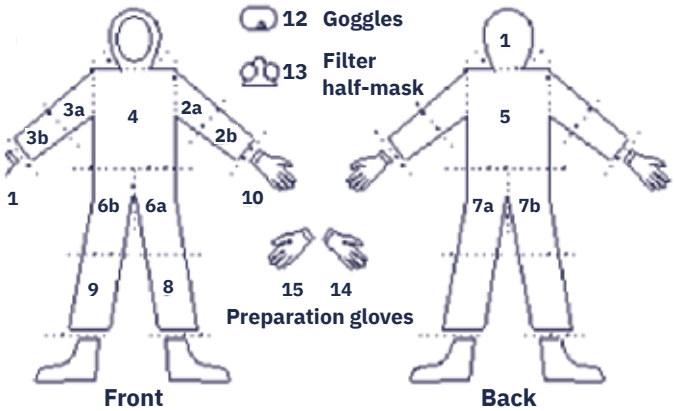


Figure 2. Potential Dermal Exposure (PDE) of horticultural and floricultural workers: B- cotton sampler overall; C- schematic of the sections of the sampler; A- PDE in mass (mg) and as a percentage of the applied pesticide (%PDE).

We previously determined that the mix and load stage could contribute to as much exposure as the application stage for manual pesticide applications in small horticultural production units [14], [15]. Therefore, we investigated the main factors that could modulate the exposure during the mix and load stage; these factors included the formulation type (solid or liquid, Figure 3A), the bottle size and seal (Figure 3B, C), the measuring devices (Figure 3D), and the formulation color (Figure 3E) [16]. Hence, we measured the potential manual exposure (PME), which is defined as the total amount of pesticide that reached the workers hands in a specific operation (measuring, transferring, rinsing, filling, Figure 3) [16]. To compare exposures when different pesticide amounts were used, the % PME was calculated as the ratio of the total amount of pesticides on the worker's hands during a specific operation and the total amount of pesticide used, expressed as a percentage.

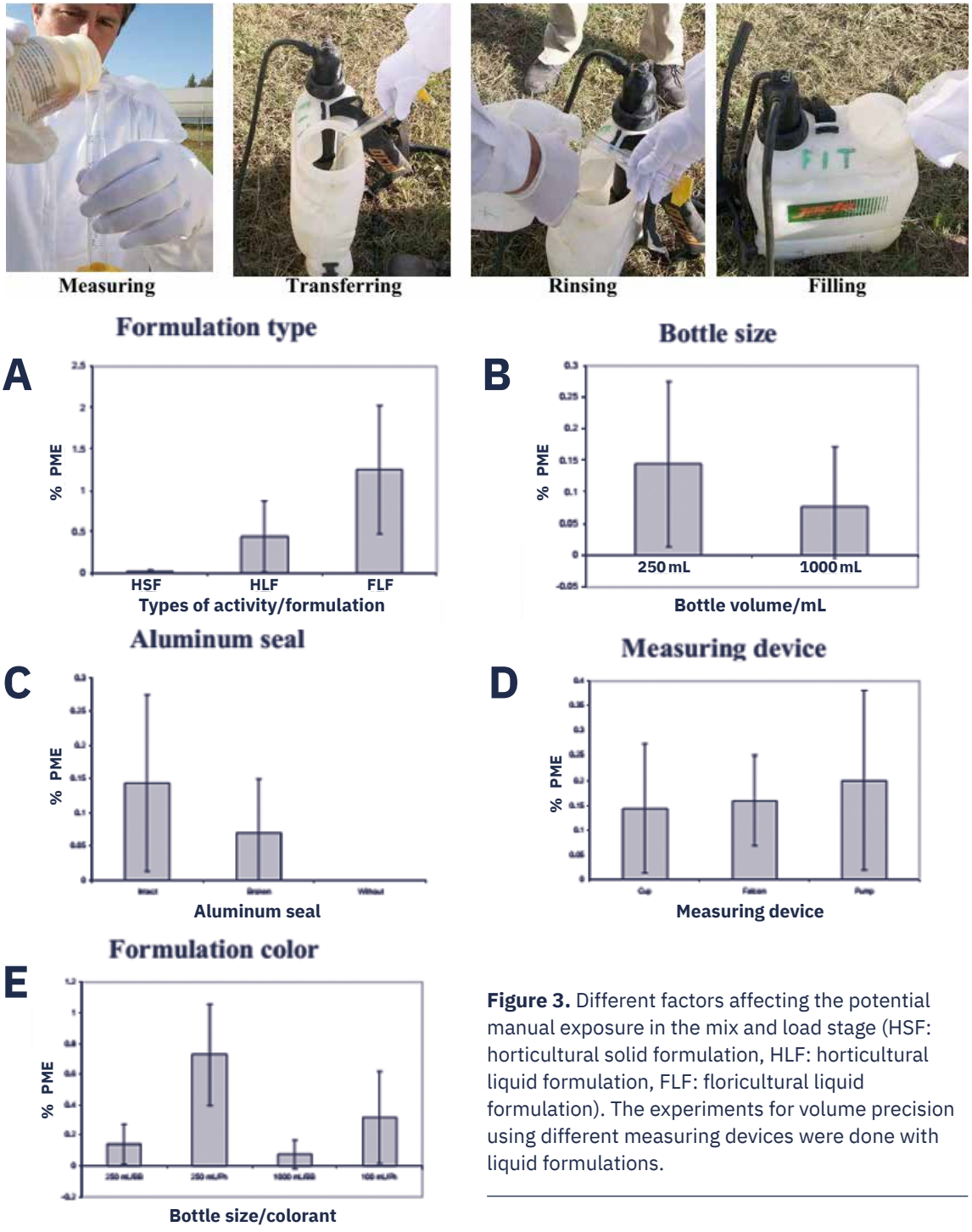


Figure 3. Different factors affecting the potential manual exposure in the mix and load stage (HSF: horticultural solid formulation, HLF: horticultural liquid formulation, FLF: floricultural liquid formulation). The experiments for volume precision using different measuring devices were done with liquid formulations.

The formulation type (solid or liquid) strongly influences the workers % PME (Figure 3A). The relative exposure was lower for solid formulations than for liquid formulations, both in the horticultural and floricultural scenarios. This behavior could be explained by the possibility of droplet splashing during the different steps of the mix and load stage (measuring, transferring, rinsing, filling). Based on the comparison of the % PME between powdered and granulated formulations, the granulated formulations were safer than the powdered products [16].

The size of the bottle containing the formulated liquid products was also studied, observing no difference in the PME when vessels of 250 mL or 1000 mL were used (Figure 3B) [16]. The presence or absence of an aluminum seal in the neck of the container was also assessed as another factor potentially contributing to PME. Breaking the seal or the presence of broken pieces of the seal in the bottle's neck, significantly increased the exposure compared to the case were no seal was present (Figure 3C, [16]).

The effect of the measuring device used to quantify the amount of formulated product () on the PME was analyzed (Figure 3D) [16], yielding no significant disparities between using a small cup, a Falcon tube, or a manual pump.

Surprisingly, when the variable was the formulation color (blue or uncolored) (Figure 3E) [16], an important difference in the PME was observed. The exposure levels were higher for the uncolored formulations, even when different bottle sizes were assayed (Figure 3E), suggesting that the addition of an inert dye to the formula could be a simple way to improve the exposure safety, at least when small bottle sizes (250–1000 mL) were handled.

3. Estimation of pesticide distribution between nontarget systems (soil, plastic, drift)

Having determined that 0.06–0.58% of the pesticide could reach the worker's cloths (section 1), we investigated the extent to which other nontarget subsystems, like soil (in the production unit or outside it by drift) or plastics could be exposed to pesticides. Therefore, we studied the pesticide distribution in small horticultural and floricultural production units between crop, soil, agricultural plastics (greenhouse and mulching sheeting), and drift. Figure 4 shows the percentage pesticide distribution referring to the total applied pesticide in horticultural open fields and horticultural and floricultural greenhouses. This parameter enabled comparison of the various situations in which different concentrations and volumes of pesticides were applied to various crops. The experiments were performed by applying different pesticides with manual knapsacks, in independent trials on different production units and under real working conditions with different workers [17].

We observed that besides the crop that is naturally the target, the relative amounts of pesticide found on soil or on soil plus plastic mulching were significant (Figure 4). In the case of broccoli and cauliflower, the amount of pesticide detected on the soil of open fields was higher than that found on the crop itself (Figure 4) [17]. In the case of strawberry open fields, the amount found on soil plus plastic mulching was similar to that found on the crop. Another interesting feature was that the pesticide distribution between the different nontarget systems differed between greenhouses (horticultural and floricultural) and open fields. In greenhouses (Figure 4) [17], a general pesticide distribution pattern was observed as fractions of the total amount applied, i.e., 2/3 crop, 1/4 soil, and 1/20 plastic. In all cases, when manual knapsack pesticide applications were performed, the pesticide drift into neighboring fields was < 5% of the total pesticide applied, and it declined to nondetectable values for distances longer than 7 m from the crop border.

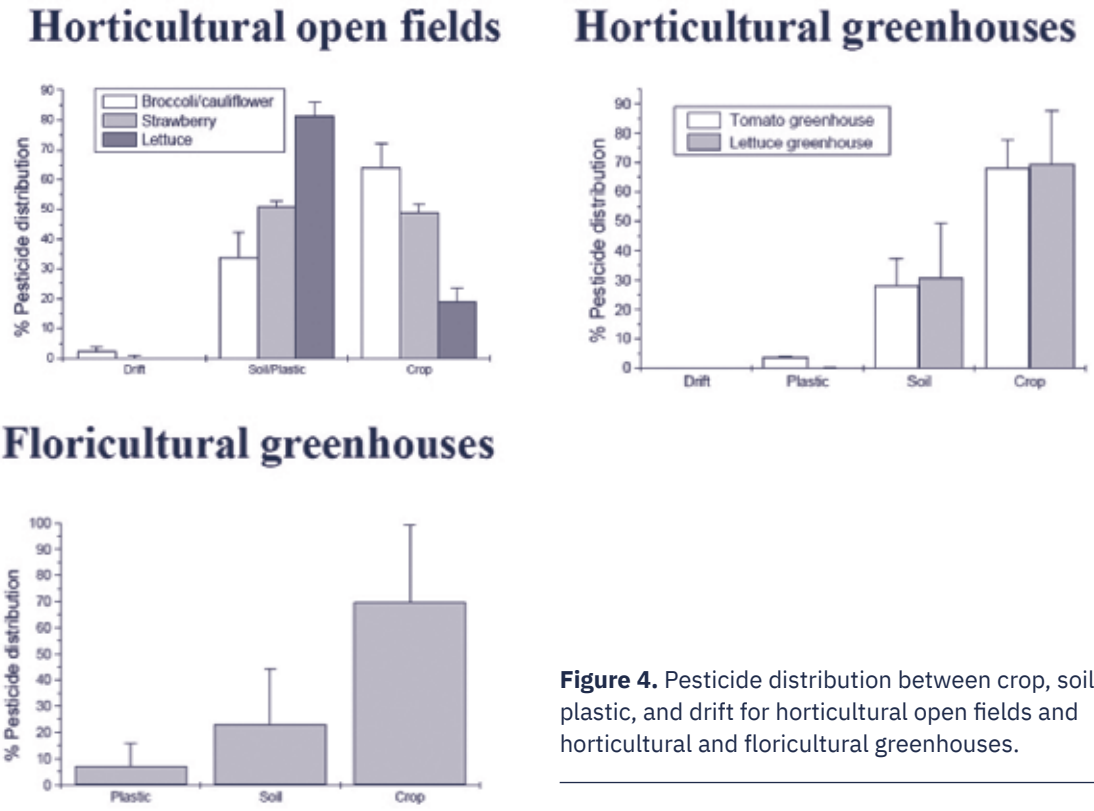


Figure 4. Pesticide distribution between crop, soil, plastic, and drift for horticultural open fields and horticultural and floricultural greenhouses.

An interesting conclusion of the previous measurements is that the amount of pesticide on the plastic surface of greenhouses was not negligible (Figure 4) [17]. Approximately 2% of the total pesticide applied was detected on the surface of horticultural greenhouses pesticide applied, whereas higher values were detected on the surface of floricultural greenhouses [17]. Considering this, we investigated the pesticide distribution in plastic greenhouses. To achieve this, we placed cotton sampling patches on the walls at three different heights and on the ceiling (Figure 5, center) [17]. Figure 5 shows the % relative pesticide distribution on the greenhouse plastics after application on four main sectors: lateral walls, front/back walls, crop roof, and aisle roof. In horticultural greenhouses, no specific distribution pattern was observed for two different crops: lettuce and tomato, whereas a higher exposure was detected on the lateral walls of floricultural greenhouses [17].

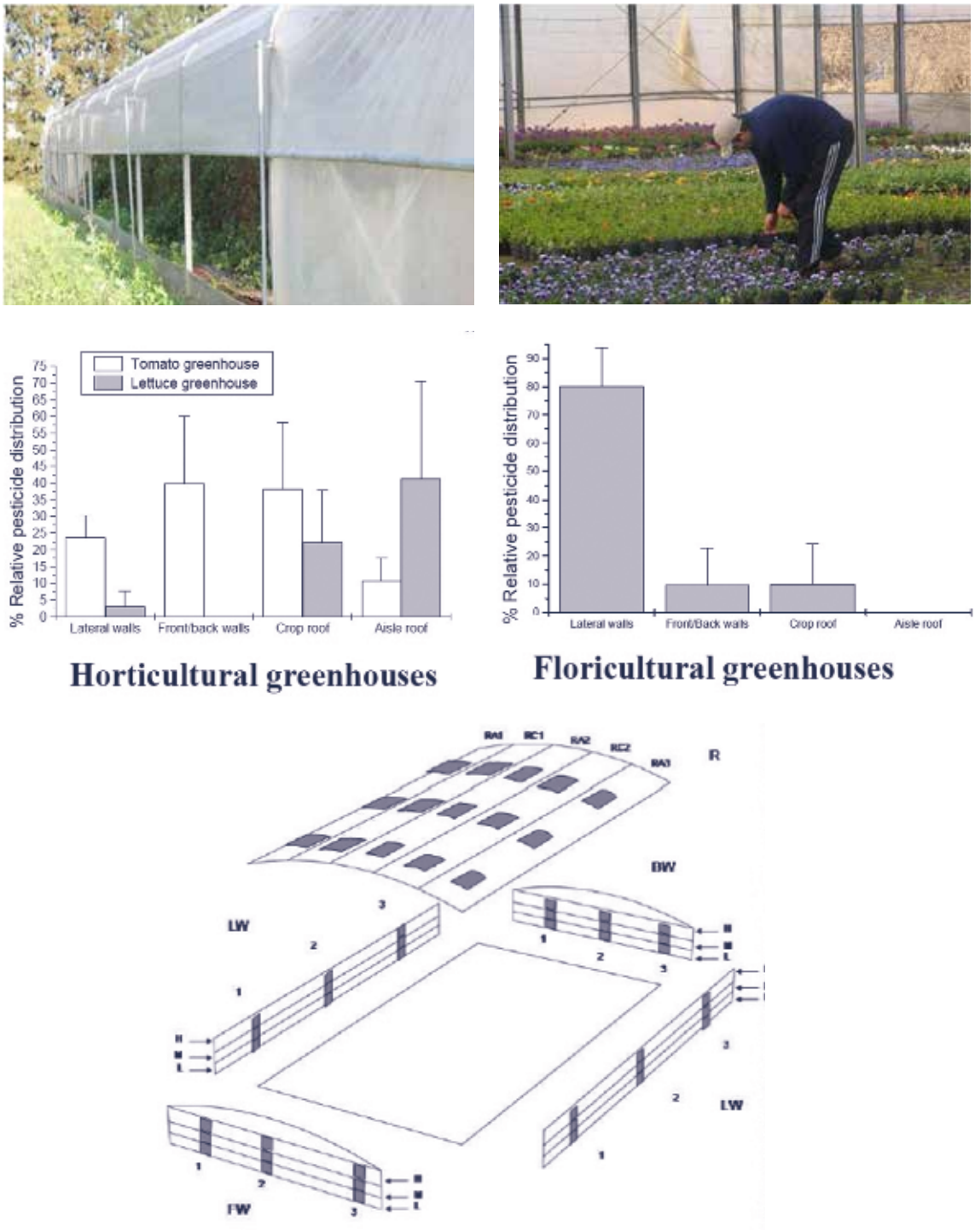


Figure 5. Pesticide relative distribution on greenhouse plastics.

The measurement of pesticides that could reach agricultural plastic films (mainly PE for greenhouses and mulching) is important because significant amounts of discarded plastic sheeting were usually found next to cultivated fields (Figure 6). Plastics could act as the source or sink for pesticides, impacting their environmental fate. Similarly, we recently reported that small pieces of the plastic film were found in horticultural soils, in up to 10% of the soil area. Evidently, plastic fragments have become a significant component in productive soils; hence, they must be considered to understand the pesticide fate in this environment. [18].

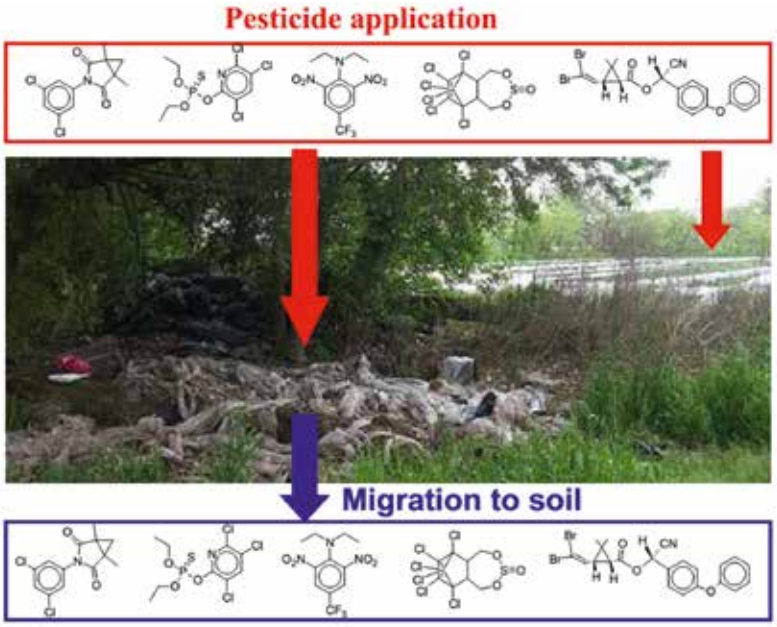


Figure 6. Used PE sheeting next to a horticultural production.

4. Degradation of different pesticides in horticultural soils

Since horticultural and floricultural soils are directly exposed to significant pesticide amounts, it is important to investigate the pesticide fate in this environment. However, this requires considering whether horticultural soils, in which different crops are cultivated and rotated in different sections of the same production unit, are homogenous (Figure 7).

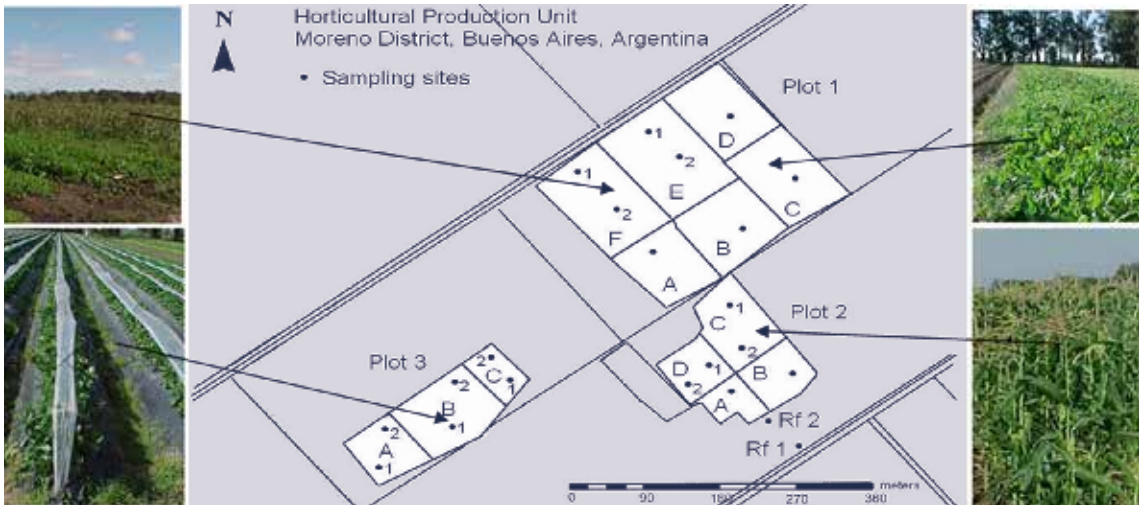


Figure 7. Different crops and sections of a small periurban horticultural production unit in Buenos Aires.

To achieve the aforementioned, we selected several physicochemical soil properties as indicators of soil conditions: microbial respiration, humidity, organic matter, conductivity, pH, and total phosphorous content. All measurements were done in selected sampling points (Figure 7) of three different subsections of a horticultural production unit located at the Moreno district in Buenos Aires, Argentina [19]. According to these selected properties, the mean values for each of the three subsections did not exhibit relevant heterogeneity within the production unit [19]

Table 5. Soil properties for the different sampling points of plots P1, P2, and P3.

Sample	Soil Properties							
	MR[1] (mg CO_2/g soil)	**Hum.**[2] (%)	**O.M.**[3] (%)	**Cond.**[4] (mS/cm)	**pH**	**R.V.**[5] (mL/g)	**Density** (g/mL)	**P**[6] (mg/g soil)
P1-A1	0.42	15.5	4.79	0.114	6.45	1.44	NM[7]	0.277
P1-B1	0.29	17.8	4.40	0.067	5.99	1.28	1.10	0.226
P1-C1	0.39	14.5	4.36	0.051	6.45	1.12	1.80	0.258
P1-D1	0.42	16.9	4.41	0.045	6.55	0.99	2.01	0.248
P1-E1	0.32	20.3	4.75	0.032	6.09	0.94	1.66	0.214
P1-E2	0.46	19.6	4.39	0.144	7.04	1.29	1.52	0.194
P1-F1	0.45	20.4	4.82	0.058	6.35	1.10	NM	0.185
P1-F2	2.41	21.3	4.61	0.133	6.15	1.34	0.29	0.202
P2-A	1.16	12.7	6.05	0.083	5.95	0.94	1.52	0.066
P2-B	0.60	22.3	6.42	0.031	5.39	0.76	1.91	0.088
P2-C1	0.68	22.4	4.01	0.143	5.17	1.02	1.82	0.104
P2-C2	0.46	14.1	6.31	0.050	6.09	0.94	1.63	0.204
P2-D1	0.84	17.5	4.27	0.027	5.19	0.81	1.90	0.149
P2-D2	0.38	13.6	4.28	0.046	6.03	0.70	1.62	0.290
P3-A1	0.25	16.3	4.35	0.268	7.05	1.20	1.62	0.160
P3-A2	0.55	21.6	4.42	0.061	5.65	0.73	1.70	0.164
P3-B1	0.57	19.6	5.71	0.056	5.75	1.05	1.57	0.155
P3-B2	0.51	19.4	4.70	0.063	6.21	0.77	2.50	0.147
P3-C1	0.44	14.9	2.78	0.027	5.21	1.12	1.52	0.186
P3-C2	0.37	17.1	6.23	0.054	5.65	0.83	NM	0.168

[1]MR: Microbial respiration (mg CO_2/g dry soil). [2]Hum: Humidity (% referred to dry soil). [3]O.M.: Organic matter content (% referred to dry soil). [4]Cond.: Conductivity. [5]R.V.: Retention volume (mL of water/g dry soil). [6]Total phosphorus (mg of P/g dry soil).[7]Not Measured.

When the homogeneity within the production unit was confirmed, we investigated the pertubation of the horticultural soil relative to a reference soil of the same edaphological kind, but not used for at least 20 years. This was achieved by determining the same set of physicochemical properties in the reference soil, which confirmed significant differences in the phosphorous and organic matter content. The phosphorus content in the horticultural system was twice that of the reference soil, whereas the organic matter in the horticultural soil was half that in the reference soil [19].

Considering these parameters, we investigated the possible differences in pesticide degradation rates between horticultural and reference soils. Consolidated samples were made with equal amounts of soil from each sampling point for both horticultural soil and the reference soil. The influence of soil characteristics on pesticide degradation was investigated by applying a single pulse of a mixture of pesticides (commercial formulations of chlorpyrifos, procymidone, and trifluralin) to the composite samples of both soil types. We also assessed the simultaneous degradation of a group of pesticides because simultaneous application of different active ingredients is a common practice among the horticultural workers. The pesticides were selected as representatives of the herbicide, insecticide, and fungicide groups. A single dose of 0.015–0.035 mg of each pesticide per gram of dried soil (twice the manufacturer's recommended dose) was applied. The soil pesticide content was determined at different exposure times by solvent extraction and quantification by GC-ECD. Figure 8 depicts the chlorpyrifos, procymidone, and trifluralin degradation profiles for both soils. All pesticides experienced faster degradation in the horticultural soil than in the reference soil, exhibiting first order exponential kinetics for procymidone and trifluralin in the first case. To evaluate whether the pesticide application impacted the microbiota, microbial respiration was measured, using composite samples with and without pesticides. The results (not shown) of microbial respiration versus time for both experiments indicated negligible differences between them [19].

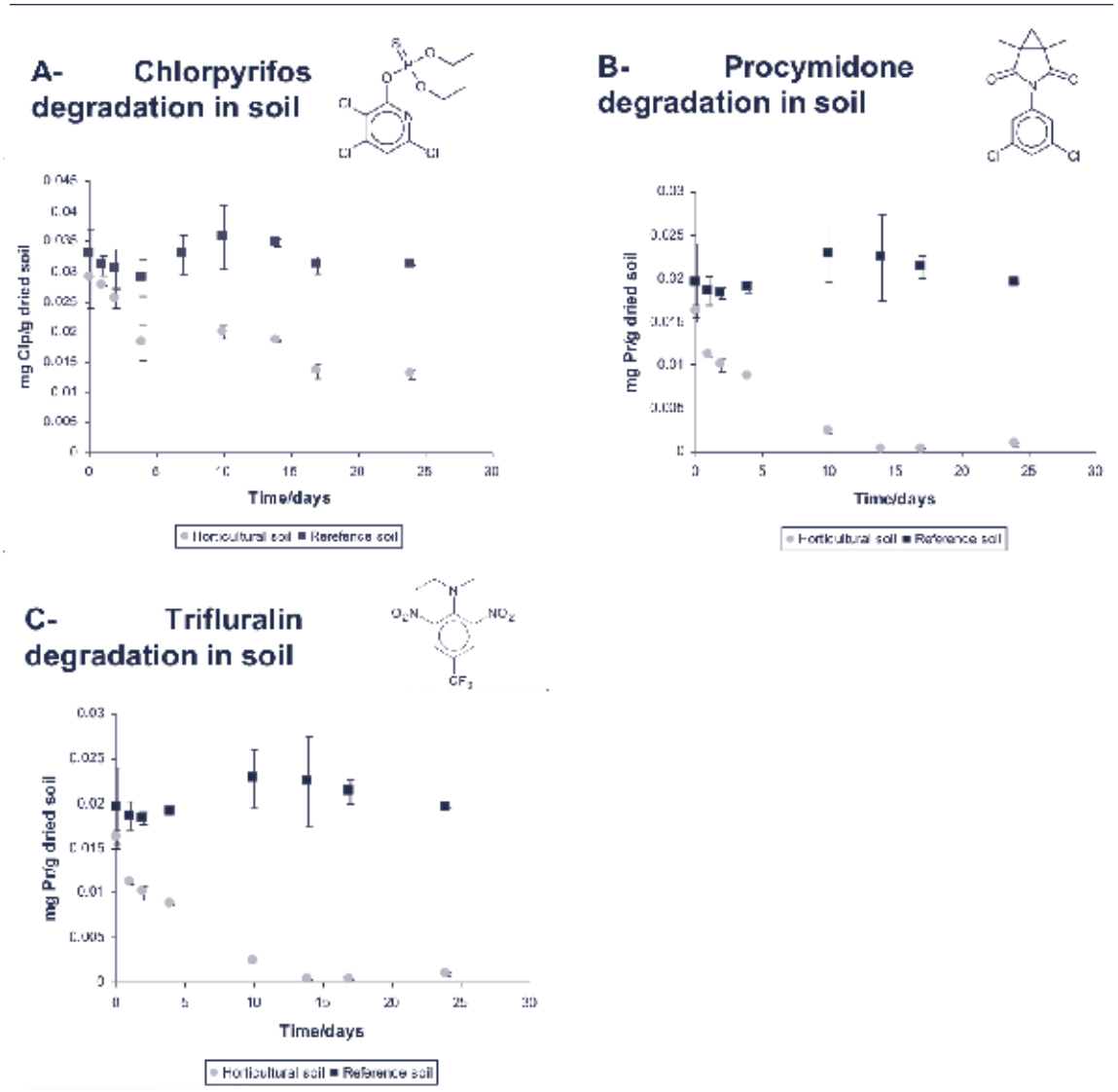
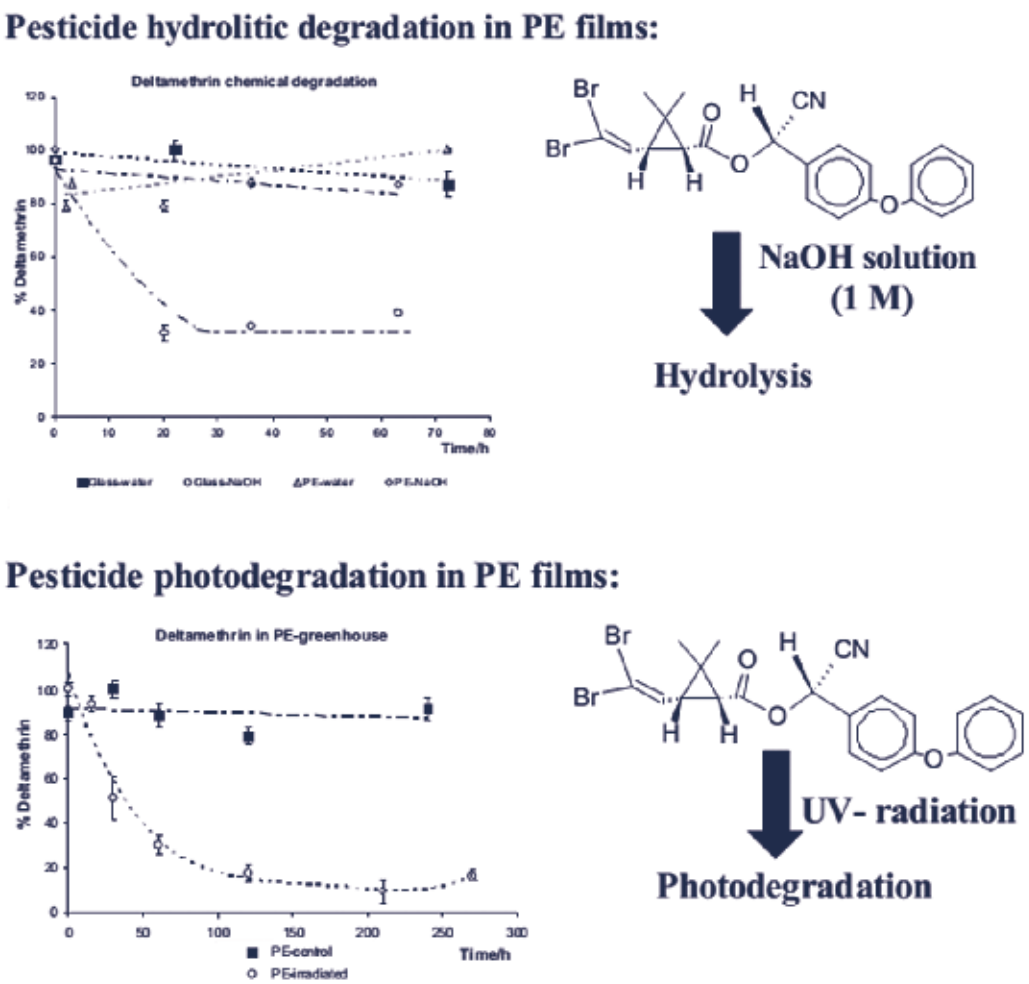


Figure 8. Chlorpyrifos, procymidone, and trifluralin degradation in microcosm assays of the horticultural and reference soils.

5. Stability and pesticide degradation processes on agricultural plastics

As previously discussed in section 2, horticultural plastic sheeting is significantly exposed to pesticides during the application stage. Therefore, significant amounts of these products are absorbed into the plastic film (Figure 4). Hence, it should be interesting to assess whether pesticides in the LDPE film could experience a protective effect against chemical or photochemical degradation [18]. To validate this hypothesis, we allowed deltamethrin to be absorbed in small LDPE sections (25 and 100 □m thick) and exposed them to a 1 M NaOH solution or to UV radiation (different experiments). In both cases, deltamethrin on a glass surface was also exposed as a positive control and deltamethrin absorbed on LDPE, but not exposed to NaOH or UV, was used as negative control. Figure 9 depicts the remaining deltamethrin content versus time. During the hydrolytic experiment, the deltamethrin that was absorbed into the LDPE and exposed to NaOH remained stable, whereas that on the glass surface (negative control) was significantly decomposed. These findings were attributed to a protective effect of the LDPE.

Conversely, when deltamethrin on both LDPE and glass was exposed to UV radiation, the photodegradation rate was higher on the LDPE than on the glass (Figure 9) [18]. These results could be explained by considering the amorphous polymer phase as a solvent with an infinite viscosity, where photodegradation can occur because of the mobility of the radical fragments, which is a phenomenon that is undesirable on glass [18].



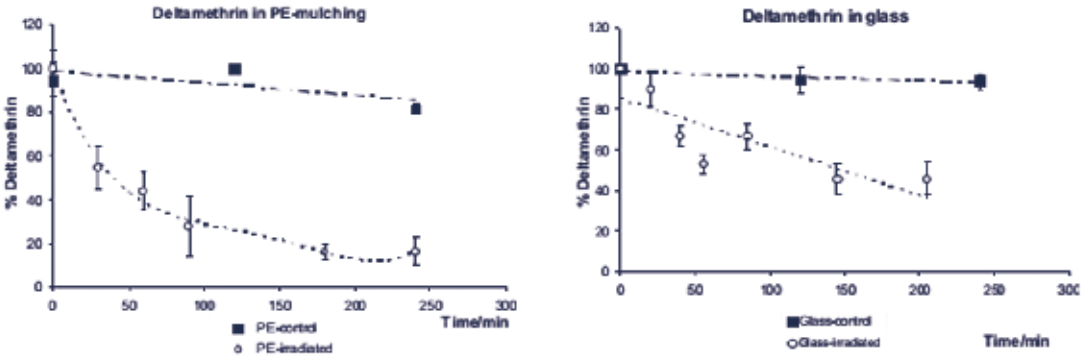


Figure 9. Hydrolytic and photolytic degradation of deltamethrin on PE films.

6. Educational training

Importantly, we conducted educational activities with the horticultural workers to raise awareness of the risk associated with pesticide manipulation. We observed that workers of small periurban horticultural production units are not typically cognizant of the risks associated with these substances. Hence, to contribute to their education in risk perception, conducted some awareness activities using Brilliant Blue—a harmless bromatological dye—as a pesticide surrogate. Workers were encouraged to perform their usual preparation and application activities using the pesticide surrogate and the cotton sampler overall described in section 1 (Figure 10). Once the preparation/application stages were complete, the blue dyes on the overall surface were used to show the workers the magnitude of the exposure.

Figure 10. Operator's educational training with a pesticide surrogate.

7. Conclusions

Pesticide exposure during horticultural and floricultural practices such as preparing and applying these products was evaluated by determining the PDE. The critical aspects that could impact the exposure during the mix and load step were also investigated. Simple factors like colored formulations could help to diminish workers exposure during the mix and load stage.

A relative mass distribution of pesticide between the crop and the nontarget systems (soil, plastic, drift) was done in open fields and in horticultural and floricultural greenhouses, determining that the soil and plastic exposure could be significant.

Horticultural soil heterogeneity was considered for a small production unit with different subsections. Pesticide degradation in horticultural and reference soils was investigated, revealing that degradation was enhanced in horticultural soil, possibly due to microbiota adaptation.

The hydrolytic and photolytic degradation of pesticides absorbed on LDPE was also studied, confirming that photolytic degradation was faster in the LDPE than in the control system. In the case of hydrolytic degradation, a protective effect was observed on the LDPE.

Finally, educational training activities regarding workers safety during pesticide manipulation were conducted with horticultural laborers.

Acknowledgments

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Insights into the Geochemistry of Serpentine Regolith in Sri Lanka

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Abstract

Serpentine soils are weathered products of ultramafic rocks composed of ferromagnesian silicates. These soils are considered as extreme environments in geoecology, owing to the high concentrations of heavy metals and low amount of calcium with abundant magnesium. The toxic metal release from serpentine soils into the surrounding areas and groundwater is an ecological, agricultural, and human health concern. We investigated the environmental conditions impacting the release of Ni, Mn, and total Cr. Further, we examined the releasing rates, mechanisms, and the possibility of forming highly toxic Cr(VI) from serpentinite soils found in different ultramafic localities in Sri Lanka by coupling interpretations garnered from chemical extractions and model experiments. Both Ni and Mn demonstrate rapid release rates in water from the Ussangoda soil (2.4 and 2.0 mol m^{-2} s^{-1}, respectively). Further, the release rates increase with increasing ionic strengths. Sequential extraction experiments, which were used to identify elemental pools, indicated that Mn is mainly associated with oxides/(oxy)hydroxides, whereas, Ni and Cr are bound in silicates and spinels. Despite multiple phases capable of releasing Ni and Mn, the reaction kinetics demonstrated that the antigorite mineral found in serpentine soil (i.e., the silicate fraction) is responsible for a majority of the Ni and Mn release. Overall, our results support that serpentinite soils provide a more highly labile and chemically modifiable source of Mn and Ni than Cr. Interestingly, no detectable Cr(VI) was released into soil solutions, potentially due to the abundance of HM. However, the dynamic interactions of Cr(III)-bearing silicates and birnessite provide a kinetically favorable route of Cr(VI) formation, which is ultimately tempered by humic matter.

Keywords: Serpentine soil, Dissolution and release, Toxic heavy metals, Ultramafic rocks, Antigorite, Fractionation

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1. Introduction

Heavy metal contamination of soil caused by the release of heavy metals from natural and anthropogenic activities is a widespread environmental problem with severe consequences for agricultural crop productivity and human health. Serpentine soil, which is derived from the weathering of ultramorphic

rocks, is found in many places around the world. Serpentine soil consists of extremely low levels of essential plant nutrients (e.g., N, P, Ca), extremely high levels of heavy metals (Ni, Cr, Co), and very poor water availability and retention [1-2]. Serpentinites are ultramafic rocks, and their soils are known as non-anthropogenic sources of metal contamination in the environment [1, 3-5]. Weathering and pedogenic processes of serpentinite rocks may release toxic elements, ions, and compounds into the surrounding environment [6-7]. The metal releasing process is regulated by a multitude of variables including solution pH, ionic strength, and the type and concentration of acid available [8-10].

Serpentine-associated soils are often subjected to agriculture in regions such as Northwestern Spain [11], Canada [12], Philippines [13], and Japan [14]. The crop plants in such areas can accumulate high concentrations of toxic metals such as Cr, Ni, and Mn in their edible parts [11, 13]. Hence, the cultivation of food plants in areas within and adjacent to serpentine outcrops and other heavy metal-enriched sites may be of particular concern, owing to phytotoxicity and metal accumulation [15]. The prolonged consumption of metal-accumulating plants may pose serious health risks when their consumption leads to concentrations above the toxicity threshold. Therefore, it is essential to understand the mechanisms, environmental factors, and rates of release before using such heavy metal-rich soils in agriculture.

1.1 Characteristics of serpentine soil

Relative to other soil types, serpentinite soils are characterized by higher concentrations of Cr, Ni, Co, and Fe, lower concentrations of plant nutrients such as Ca, K, N, and P, lower Ca/Mg ratios, and characteristic flora and physical properties [1]. The primary cause of serpentinite soil toxicity is low Ca concentrations combined with elevated soil concentrations of Mg and Ni. Whereas soil concentrations of Ni are typically range from 5–500 mg kg^{-1}, they may exceed 10,000 mg kg^{-1} in serpentinite soils [1, 16-17]. Thus, a larger Ni-available fraction is present in serpentinite soils. Numerous reports on serpentinite focus on the biogeochemical significance of the deposits [3, 18-19]. However, there are limited reports on the toxic metal release from serpentinite soil to the environment.

The pH of serpentinite soils ranges from ~4 to 9 [1, 20]. The Cr concentrations in the serpentinite soils typically range from 0.1–3.2 µmol L^{-1}, present as colloidal material [19]. The inhabitability of these soils for most plants has been attributed to the imbalance of Ca to Mg [21], the deficiency of plant nutrients [22], and the elevated concentration of heavy metals [23]. However, the correlation between the lack of plant productivity of these soils and the toxic levels of Cr has not been completely elucidated [1, 24]. The unique flora and fauna associated with these soils have been well-documented and the Cr concentrations in the plants can be as high as 600 mg kg^{-1} [1, 3].

Geochemical studies on serpentinites and serpentine soils have mostly focused on Cr [25-29]. Nickel has also attracted some attention [28, 30-32]. However, not many studies have focused on serpentines in the tropical regions where weathering rates are typically high, owing to the high annual rainfall and temperature. Several serpentine outcrops are present in Sri Lanka, which is a continental island in the equatorial belt [33]. The serpentine outcrops in Sri Lanka have received attention from botanists [18, 34]; further, the existing studies have revealed several potential nickel hyperaccumulators, i.e., species accumulating over 1000 µg Ni/g dry leaf tissue [35], from the Ussangoda site [36-38].

More importantly, the release of toxic heavy metals into surrounding areas is a human health concern related to the contamination of groundwater [39-40]. It is essential to investigate how solution chemistry (i.e., soil solutions) may assist or accelerate heavy metal release from the serpentine soil to inhibit the pathways of metal release into the surrounding environments. However, the mechanisms of heavy metal release into the environment remainin unresolved. The geochemistry of serpentine soils in Sri Lanka has not been comprehensively investigated in terms of the heavy metal partitioning and carbon fractions. Hence, this study was proposed to: (I) assess the geochemistry and metal partitioning and carbon in different serpentine soils in Sri Lanka, (II) critically assess the releasing behavior of toxic metal species such as Cr, Ni, and Mn from serpentinite soil based on different environmental conditions, (III) identify the Cr, Ni, and Mn release mechanisms and the potential routes of their environmental inputs, and (IV) examine the time-dependent oxidation/dissolution of Cr in serpentinite soil to understand the natural attenuation of Cr(VI) formation.

2. Serpentine soil environments in Sri Lanka

The geological boundary between Highland and Vijayan Complexes in Sri Lanka is identified as a mineralized belt with many serpentinite deposits in Ussangoda, Udawalawe, Indikolapelessa, Ginigalpelessa, Katupota, Yudaganawa, and Rupaha (Figure 1). Five of them, except the one at Rupaha, occur close to the geological boundary between two lithological complexes. However, the Rupaha deposit is more marble than serpentinite [41]. In southern Sri Lanka, three serpentinite bodies occur at Ussangoda (near Ambalantota), Ginigalpelessa (near Udawalawa), and Indikolapelassa (near Udawalawa). Although the erpentinite body at Ginigalpelssa is the largest, spanning an area of ~1 km^2, the one at Ussangoda is relatively small, covering an area of ~0.3 km^2, revealing contrasting features (less vegetation and rich in finer soil particles) compared to other deposits. The Indikolapelassa serpentinite body is situated close to the Ginigalpelesa body, covering an area of 0.3 km^2 [33]. However, there are limited reports on the serpentinite soil chemistry in Sri Lanka. Only two locations, Ginigalpelassa and Indikolapelassa, have been studied in detail regarding mineralogy and petrology [33, 41]. Accordingly, these serpentinite rocks comprised ~90% pyroxene and olivine minerals.

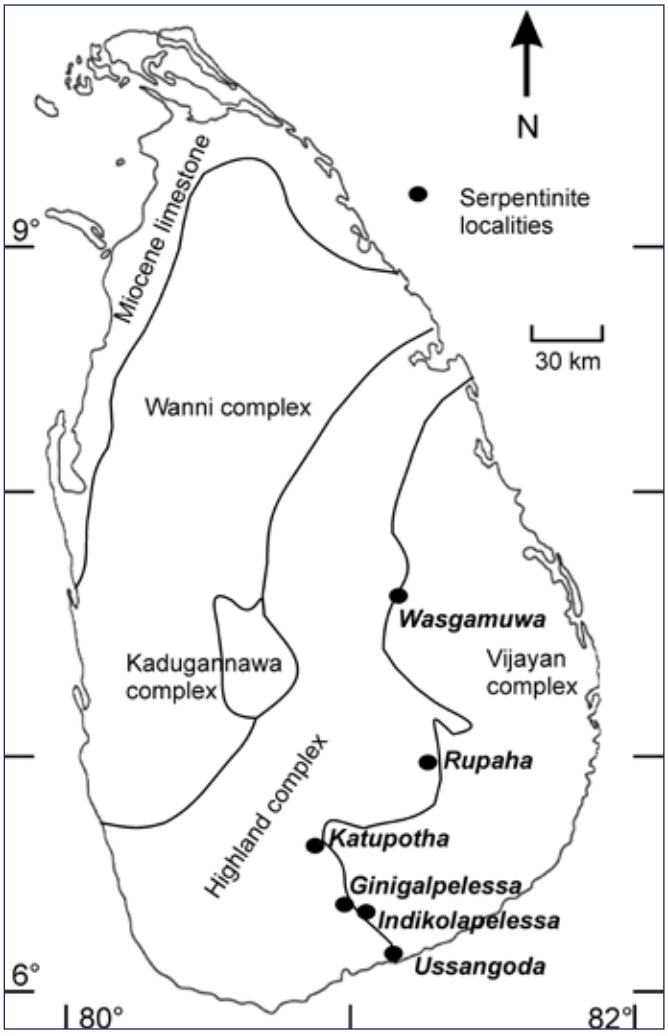

Figure 1. Serpentinite localities (black dots) in Sri Lanka along the lithological boundary between Highland and Vijayan Complexes. a) Ussangoda serpentinite rock, b) Ussangoda serpentine soil, c) Yudaganawa serpentine soil, d) Ginigalpelessa serpentine soil.

3. Geochemistry of serpentine soils in Sri Lanka

In a study conducted by Vithanage et al., general chemical properties such as pH, electrical conductivity, organic matter, and cation exchange capacity were discussed for four soil samples from Ussangoda, Yudhaganawa, Ginigalpelessa, and Indikolapelessa [42]. The pHs of the soils were near neutral (6.26–7.69). The electrical conductivities (EC) in the soil ranged from 33.50 to 129.90 μS cm^{-1}, indicating relatively low dissolved salts and major dissolved inorganic solutes. The highest reported EC was from the Ussangoda soil, which could be attributed to the deposition of salt spray from the sea. The highest organic carbon (TOC) percentage was from Yudhaganawa soil (3.54%), which is adjacent to a forested habitat. The TOC amount of the Ussangoda serpentine soil was 2.98%. Additionally, the microbial biomass carbon and the labile carbon of the Yudhaganawa serpentine soil were 0.31% and 327 mg kg^{-1}, respectively. The corresponding values for the Ussangoda serpentine soil were 0.29% and 150 mg kg^{-1}, respectively. The specific surface areas of the Yudhaganawa and Ussangoda serpentine soils were 70.99 and 67.32 m^2 g^{-1}, respectively. Further, more titrations performed at three ionic strengths yielded pH_{ZPC} values of 8.57, 8.90, 8.30, and 8.01 for Ussangoda, Yudhaganawa, Ginigalpelessa, and Indikolapelessa, respectively.

The XRD patterns were similar to the reported data in previous literature [27, 43]. Antigorite $(Mg,Fe)_3Si_2O_5(OH)_4$) is often the dominant mineral present, with minor amounts of chrysotile ($Mg_3(Si_2O_5)(OH)_4$), magnetite (Fe_3O_4), spinels, and clays [42]. The elemental composition of serpentine soils was obtained using X-ray fluorescence (XRF) and total digestion techniques. Both techniques were complimentary; major elements in metal oxides were determined by XRF spectrometry and in the elemental form via total digestion. Chemical composition data revealed that the soils consisted of Fe-Cr-Ni-rich aluminosilicates. Additionally, Mn was also high in the samples, particularly in Yudhaganawa and Ginigalpelessa soils. The Ni concentration was highest in Ussangoda soils, while Cr and Mn concentrations were higher in Yudhaganawa soil than in soils from the other localities [42]. EPMA analysis maps show the distribution of Ni, Mn, and Cr with Al, Fe, and Si phases. The areas of Cr and Fe enrichment are chromite, Cr-magnetite, or magnetite. The EPMA plots for Yudhaganawa soil better illustrate Cr distribution than that for serpentine soils from other locations [42].

3.1 Metal bound phases

Single and sequential extraction techniques have been widely applied to investigate the geochemical partitioning of trace metals in contaminated soils [44], riverine sediments [45], and estuarine sediments [46]. Chemical fractionation methods, based on sequential and extraction procedures, have been used to determine numerous contaminants in specific chemical pools [47] and to understand contaminant mobility and bioavailability [48]. This information facilitates the understanding of trace metal behavior in the environment system. Although the separation of various chemical forms of heavy metals is extremely difficult, the use of sequential extraction methods in this way provides an important approach. Metal concentrations for each chemical extraction step are shown in Figure 6. Manganese was found equally in the Fe-Mn oxide fraction (420.7 mg kg^{-1}, 37%) and in the residual fraction (351 mg kg^{-1}, 31%). The residual fraction was associated with silicates and other primary oxides such as spinels. Nickel dominated in the residual fraction (4,697 mg kg^{-1}, 72%), whereas Cr was mainly found in the residual and organic matter bound fractions (8,567 mg kg^{-1}, 83% and 508 mg kg^{-1}, 4.6%, respectively). The order of the geochemical fractions where Cr, Ni, and Mn are bound from highest to lowest are: 1) Cr: residual > organic matter bound > Fe and Mn bound > exchangeable > carbonate bound, 2) Ni: residual > Fe and Mn oxide bound > organic matter bound > exchangeable > carbonate bound, and 3) Mn: Fe and Mn oxide bound > residual > organic matter bound > exchangeable > carbonate bound.

Heavy metals existed as exchangeable or associated with organic matter, carbonates, Fe-Mn oxides, and sulfides in the soil matrix fractions. Chemical extractions were utilized to assess the geochemical partitioning of metals as well as to evaluate metal mobility and bioavailability in soils and sediments. However, changes in soil pH, ionic strength, and other environmental factors may affect metal

mobilization in soil environments, particularly with respect to time and land use. By coupling single and sequential extractions with chemical kinetic interpretations, it was possible to obtain better insight with respect to where and how Ni, Mn, and Cr were bound. More importantly, the fate and behavior of Ni, Mn, and Cr as they are mobilized or impacted by critical zone processes could also be interpreted for a wide variety of potential chemical changes, including those related to the addition of fertilizers and changes in rainwater chemistry [32].

Sequential extraction experiments provide information on the association of species, enabling the differentiation between elemental pools based on how they are attached or the minerals they are associated with, including carbonates, (oxy)hydroxides, or silicates. Even though these metals were dominantly bound in relatively unavailable forms, changes in the critical zone such as soil acidity, microbial activity, availability of chelating materials, and redox conditions could enhance the mobility, providing a continually changing flux into the environment. Notably, bioavailable, exchangeable, and carbonate bound fractions may have less overall Ni, Mn, and Cr; however, these fractions potentially provide a more labile source in soil environments.

Several differences were observed for serpentine soils from different localities. Nickel in Ussangoda and Yudhaganawa soil were primarily observed in the organic matter bound fraction. However, exchangeable Ni was higher for the other two soils. This could be ascribed to the ecosystem-level differences resulting from Ussangoda and Yudhaganawa being protected as national reserves, whereas, Indikolapelessa and Ginigalpelessa are not. In the case of Mn, all soils, except those from Ussangoda, exhibited the second highest Mn in the organic matter bound fraction and third in the residual fraction. This dissimilarity may be due to the differences in mineralogy. In the soils derived from serpentinite [26, 49-50], most of the Cr is bound in the structure of the primary minerals such as Cr-rich spinels (i.e., chromite) and Cr-substituted Fe oxides. The results support that antigorite (i.e., the dominant mineral identified in these serpentine soils via XRD) could be a contributor for Ni and Mn release, whereas, the Cr-spinels (chromite/Cr-muscovite) is a major potential source of Cr [32]. A substantial proportion of Cr was linked with organic matter; this high proportion was in good agreement with the high affinity of Cr for organic matter [51].

3.2 Single extractions

The DTPA treatment extracted 323 mg kg^{-1} (5.0%) of Ni and 76.3 mg kg^{-1} (6.8%) of Mn for the Ussangoda soil. The Ni (167.6 mg kg^{-1}, 2.6%) and Mn (45.51 mg kg^{-1}, 4.1%) extractions with 0.01 M $CaCl_2$ were lower than that of DTPA in the slightly acidic serpentine soils (Table 4). Similarly, extractable Ni and Mn using $NaNO_3$ and distilled water (pH 6.5) were comparatively higher in the Ussangoda soils (Table 5).

The DTPA and $CaCl_2$ extraction methods provide a proxy for evaluating the plant bioavailability of Ni and Mn in soils and soil solutions [52-53]. Since DTPA forms soluble complexes with metals, thereby reducing their activity in the soil solutions, Ni and Mn ions may be desorbed from the soil and enter into the solution. Extractions with $CaCl_2$ are commonly used to assess plant bioavailability, particularly in neutral or weakly alkaline soils. High concentrations of Ni and Mn release were observed from serpentine soils from all localities. The concentrations recorded from $CaCl_2$ extractions were ~50% or lower than the DTPA extractable concentrations (Table 4). Several studies have revealed that salt solutions do not accurately reflect the plant available metals, particularly in the case of non-calcareous soils, whereas DTPA or hydroxylamine methods are more predictive [54]. Similar to the other extraction results, Ussangoda and Yudhaganawa soils demonstrated a higher leaching capacity of Ni, Mn, and Cr than the other two soils (Table 4). This directly relates to the total metal concentration differences among soils from different localities.

Although this study did not reveal significant differences in soil pH among the four sites, a previous report [18] recorded low rhizospheric pH for soils at the Ussangoda site (4.3–4.9) followed by the Yudhaganawa site (5.05–5.65), suggesting that the lower pH at these sites may also contribute to the greater leaching capacity we observed. The solubility and mobility of Ni in soils increase as pH decreases, at least within the pH range of physiological significance [55-56]. Using ion-exchange kinetics, Echevarria et al. [57] documented pH as the main factor influencing Ni availability in a wide range of natural soils. In addition, Tye et al. [58] demonstrated that pH is also a major factor influencing Ni activity in a range of

contaminated soils. The more labile Ni is more prone to plant uptake [59] and may have contributed to the highest Ni content observed thus far, including levels of hyperaccumulation, for species growing at the Ussangoda site [18, 38]. No detectable Cr was reported from the $CaCl_2$ extractions from any serpentine soil, although DTPA exhibited bioavailable concentrations for the soils from Ussangoda, Indikolapelessa, and Yudhaganawa. The bioavailable Cr could be attributed to the higher affinity of Cr to adsorb to clay surfaces and humic matter [60].

The water and ionic strength extractable fraction of metals may be more representative of what is available for water pollution and plant uptake, including species that hyperaccumulate Ni at the Ussangoda site [18, 38]. Distilled water and $NaNO_3$ extractable data confirmed a higher release of Ni than Mn or Cr (Table 5). The highest distilled water extractable Ni and Mn was observed for Ussangoda soil and showed a reduction in the the sequence of Yudhaganawa, Indikolapelessa, and Ginigalpelessa. This sequence may be related to the total amount of Ni present in the soil, as observed from the XRF and total digestion data. However, the behavior of Mn release with distilled water and $NaNO_3$ differs from that of the total Mn present and is instead related to total Fe in the soil. This may be associated with the Fe and Mn bound fraction of soils. However, the Mn release sequence is similar to that of Ni. In the case of $NaNO_3$ extractable metal ions, with the decrease in ionic strength, the metal ion release also declined. Dissolution rates were enhanced by increasing the ionic strength due to surface protonation, which displaced ions from the surface sites [61]. Chromium did not exhibit any release in soils, except at Yudhaganawa with $NaNO_3$, although the total concentrations reported were higher than the Ni and Mn concentrations. Cr(III) is highly stable, due to which strong complex formation with humic matter may have been hindered, owing to dissolution [60]. Also, since chromite is highly resistant to weathering, it was not likely to be an immediate contributor to bioavailable Cr [50].

4. Heavy metal release

Several studies have been conducted to understand the different dissolution mechanisms of different minerals under various conditions [62-64]. A variety of methods such as leaching tests, single and sequential extractions, the effect of pH, and inorganic or organic acids have been used to assess toxic metal release from soils. Since the use of a single method is not capable of demonstrating the possible mechanisms (surface-controlled, ligand-promoted, and proton-promoted dissolution), a combination of several methods are integrated. In slightly acidic conditions, the mineral dissolution rate was dependent on the surface bound protons in the absence of complex-forming ligands [65]. However, in the presence of complexing and sorbing ligands, the mineral dissolution occurred as a process called ligand-promoted dissolution [66].

One of our recent studies investigated the dissolution of serpentinite soil in the simulated environments with the presence of organic and inorganic acids to observe the release of Ni and Mn to the environment [32].

4.1 Ni and Mn release

The rates of metal release from solid phases in water serve as a baseline to evaluate complex solutions (i.e., the effect of inorganic and organic acids). Both Ni and Mn are rapidly released at rates of 1.55×10^{-13} and 7.89×10^{-14} mol m^{-2} s^{-1}, respectively, within the first 24 h in Ussangoda soil (Figure 6.1). The rate of Ni release decreased after 24 h, while Mn reached a steady value after four days. In the case of Ni, however, the dissolution process did not reached its maximum value, as indicated by the increasing trend, even after 12 days. Based on these rates with water, Ni and Mn exhibited values exceeding the World Health Organization's limit for drinking water within a day (1 kg of soil to 1 L of water) under the same conditions.

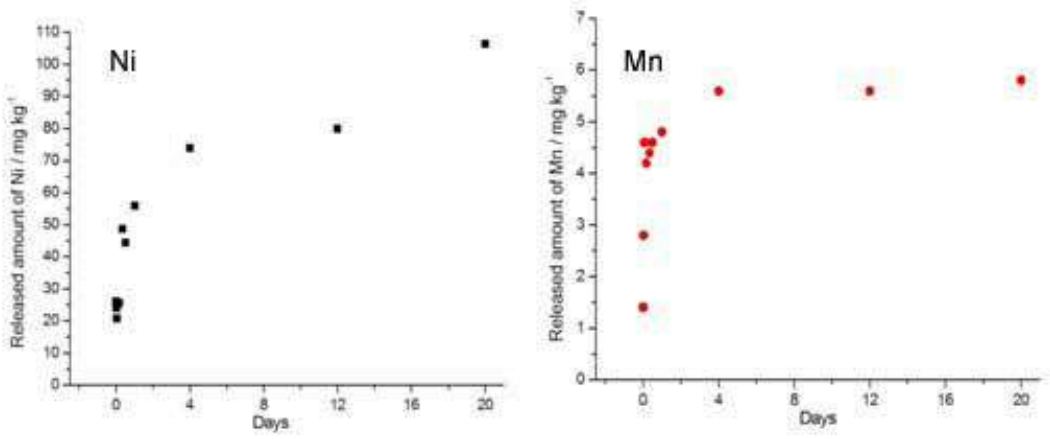

Figure 2. Release of Ni and Mn with distilled water as a function of time. The errors are smaller than the symbols used.

The Ussangoda serpentinite deposit lies along the coastal belt. Hence, it is possible to have high ionic strength conditions, leading to increased metal ion release. The addition of fertilizers and animal waste are additional concerns related to increasing the ionic strength in these soils and soil solutions. Hence, metal release changes were observed based on different ionic strengths. Experiments with varying ionic strengths demonstrated that an increase in ionic strength increased the release rate of Ni and Mn into solution (Figure 6.2). Dissolution rates were enhanced by increasing the ionic strength due to surface protonation, which displaced ions from surface sites [61]. However, there was no observed significant effect of ionic strength on Ni and Mn release at pH values between 8 and 9 (Figure 6.2), (i.e., the pH associated with pH_{ZPC} (pH 8.57) of this soil). When the solution pH approached the pH_{ZPC}, the surfaces of the serpentinite soil minerals were neutral (i.e., not charged); therefore, the effect of ionic strength was negligible [61]. Since these serpentinite soils (pH 6.7) as well as serpentinite soils worldwide were slightly acidic (pH ~6) [67], the ionic strength is an important consideration for Ni and Mn release. Importantly, the serpentinite soils that are capable of achieving high ionic strengths and acidic pHs will be an environmental concern.

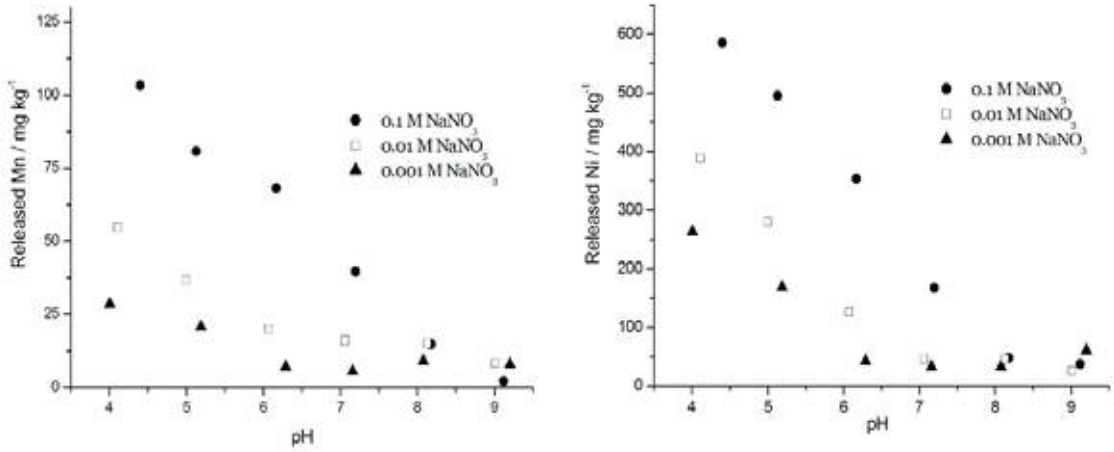

Figure 3. Release of Ni and Mn in the presence of three different ionic strengths in the soil solution. The errors are smaller than the symbols used.

Experiments conducted at an acid concentration range of 0.05–10 mM using three organic (citric, acetic, oxalic) and inorganic (H_2SO_4, HNO_3, HCl) acids revealed that Ni and Mn releasing rates increased in the following order: nitric/hydrochloric/acetic acid < sulfuric acid < citric acid < oxalic acid. The maximum release rates of Ni (5.84 mol m^{-2} s^{-1}) and Mn (2.56 mol m^{-2} s^{-1}) were observed in the presence of oxalic acid. The sequential extraction results indicated that Ni is released under acidic, reducing, and oxidizing conditions, whereas Mn is mainly associated with reducing fractions. The pH dependency of the dissolution revealed the linear relationships between the dissolution rate and pH. Different dissolution rates at the same pH values were ascribed to the accompanying anion of the acids. The effect of ligands (anion associated with the acid) greatly impacted the Ni and Mn releasing behavior. Therefore, pH, as well as complex-forming ligands, accelerate the serpentinite dissolution rate [32].

The acidity of the soil solution and the drainage controlled the dissolution and bioavailability of manganese [68]. Only Mn(II) was mobile in the environment (soil solution). However, Mn(III) was unstable and reduced or oxidized to form Mn(II) or Mn(IV), respectively. Therefore, the reduction conditions facilitate Mn leaching to groundwater and plant uptake [68].

4.2 Cr release

Hexavalent Cr is considered more toxic than trivalent Cr and hazardous by all exposure routes, owing to its mutagenic and carcinogenic properties [69]. Cr(III) is present in serpentinite soils around the world in high concentrations [67]. The redox potential of the Cr(VI)/Cr(III) conversion is extremely high; hence, a few oxidants present in natural systems have the capability to oxidize Cr(III) to Cr(VI). The dissolved oxygen and manganese oxides are promising oxidants that have demonstrated the potential to oxidize Cr(III) to Cr(VI) [6-7]. The oxidation of Cr(III) by manganese oxides is reportedly more rapid than that by dissolved oxygen [70]. After the oxidation process, Cr(VI) can readily leach into the deeper soil layer, causing groundwater pollution.

5. Conclusions

The weathering of ultramafic rocks and serpentinites produces serpentinite soils containing high concentrations of Cr as well as other potentially toxic elements including Ni, Co, and Mn, which can be released into the groundwater in the vicinity. Dissolution studies demonstrated that Ni and Mn in the serpentinite soils found in Sri Lanka were released primarily from the silicate fraction. In particular, the Ni and Mn were released from antigorite, which is the dominant mineral identified in serpentinite soils, whereas Cr was released from Cr(III)-oxides or Cr(III)-muscovite. Based on a variety of extractions, these serpentinite soils provided a labile source of Ni and Mn that could be released readily over a range of geochemical conditions. This study confirmed previous findings and provided additional evidence that Ni and Mn release from serpentinite soil is accelerated by complex-forming ligands, and that both ligands and protons corroborate the accelerated release of Ni and Mn from serpentine soils into surrounding environments. Considering toxic Cr(VI), there was no evidence confirming Cr(VI) formation by Cr(III) in serpentine soils in Sri Lanka, possibly because of the presence of humic matter in soil. Based on the findings, it can be concluded that the groundwater can be contaminated by the toxic metals in serpentine surrounding environments, depending on the environmental factors. Further, agriculture-related activities in the surroundings may provide an environment for toxic metal accumulation in plants.

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Part II.
Removal of Toxicants from the Environment

Remediation of Polycyclic Aromatic Hydrocarbon-polluted Soils Using Mushroom Cultivation Substrate

Remediation of Polycyclic Aromatic Hydrocarbon-polluted Soils Using Mushroom Cultivation Substrate

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Abstract

The potential of mushroom cultivation substrate (MCS) in the bioremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated soil was examined on a laboratory scale. The water extracts of four types of spent MCS, including *Pleurotus ostreatus*, *Pleurotus eryngii*, *Coprinus comatus*, and *Agaricus bisporus*, were capable of transforming PAH in aqueous reaction systems. The transformation profiles of PAH using spent MCS extracts were extremely similar to those obtained with fungal laccase. Soil microcosms were then established and the combinations of MCS, *Pleurotus ostreatus*, and alfalfa were compared in terms of the PAH dissipation and microbial communities. After a 60-day incubation in greenhouse, 32.9% of the 15 USEPA Priority PAHs was reduced in the MCS-amended microcosms, with anthracene, benzo[*a*]pyrene, and benz[*a*]anthracene being more degradable. Consequently, the toxic equivalent (TEQ) of PAH in these microcosms decreased by approximately 50%. MCS stimulated the growth of indigenous microorganisms and changed the community compositions of bacteria, fungi, and aromatic hydrocarbon degraders. Two species belonging to the phylum Ascomycota, class Sordariomycetes were enriched in all MCS-treated soil samples, coupled with unique changes in the PAH profile, implicating the involvement of laccase-like enzymes. Limited improvement was observed with the inoculation of a white-rot fungi *P. ostreatus*, possibly because of its poor colonization in soil. In addition, alfalfa appeared to antagonize the bioremediation effects of MCS. These laboratory-scale results, together with the ongoing field remediation experiment, suggest that MCS is a promising cost-effective and green biostimulation agent, assisting the development of MCS-based biostimulation of PAH-contaminated soil.

Keywords: Bioremediation; Biostimulation; Fungi; Laccase; Microbial community; Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds containing two or more fused benzene rings and have been identified as common persistent organic pollutants (Figure. 1). Both natural and anthropogenic sources, such as forest fires, volcanic eruptions, and fossil fuel combustions, contribute to the amount of PAHs present in the environment. It was estimated that the annual global emission of the 16 USEPA Priority PAHs was as high as 520 Gg [1]. PAHs are routinely found in soils throughout the world at various concentrations, posing potential risks to human and ecological health dueto their toxicity and carcinogenic properties [2]. According to a nationwide soil pollution survey, PAHs have become one of major organic pollutants in cropland soils in China [3].

Many techniques are available to reduce PAHs in soil [4]. Bioremediation is a promising strategy, which is primarily based on the biodegradation of PAHs. Previous studies have mainly focused on bacterial transformation; however, fungi are also attractive alternatives in this process [5]. Many ligninolytic fungi, mostly wood rot fungi, are potent PAH degraders [6]. The ability of fungi to degrade PAHs may be related either to their cytochrome P-450 enzymes, or the extracellular ligninolytic enzymes, including lignin peroxidase, manganese peroxidase, and laccase [7]. The potential of hazardous chemical transformation by fungi has been extensively evaluated in recent years [5], but there are still several limitations on fungi-based remediation. One of the most important considerations is the colonization of fungi in soil. Once introduced to the soil, allochthonous fungi are exposed to a hostile environment with less available nutrients than their natural habitats, as well as competition with native communities [6]. As such, biostimulation, which involves the addition of nutrients to promote the activity of indigenous microorganisms, has been identified as an alternative method to bioaugmentation, which is the introduction of allochthonous strains.

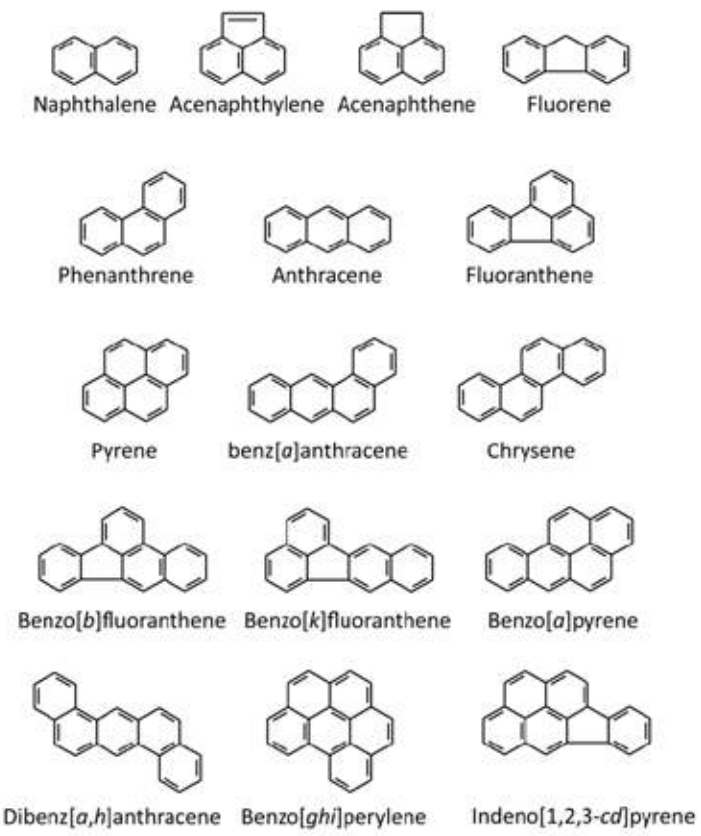

Figure 1. The 16 USEPA Priority PAHs.

Several substrates can stimulate the fungal degradation of organic pollutants in soil. One of the commonly used substrates is *mushroom cultivation substrate* (MCS), which provides bulk nutrients for indigenous soil microflora and contains considerable microbial or enzymatic activity [8], thus exhibiting the combined advantages of both biostimulation and bioaugmentation. In addition, plants are typically introduced to enhance PAH removal [9]. For example, alfalfa increased the abundance of PAH degraders as well as the dissipation of hydrocarbons in soil [10].

During the past decade, we have explored multiple aspects of the fungal transformation of PAHs and its use in soil remediation. However, in this study, we primarily focus on the bioremediation of PAH-polluted cropland soils. Laboratory- and field-scale experiments were conducted to develop practical bioremediation techniques using MCS, and to obtain insights into the possible mechanisms underlying the degradation of PAHs in soil.

2. PAH oxidation by spent mushroom compost extract

PAHs, particularly those with high molecular weight (HMW-PAHs), are known for their resistance to microbial degradation. Accumulating evidence indicates that fungal laccase can effectively oxidize benzo[*a*]pyrene and a few other PAHs, thereby making it potentially applicable in polluted soil remediation [11]. For example, we observed a 55.6% dissipation of benzo[*a*]pyrene by pure laccase in soil microcosms in the presence of ABTS, an artificial redox mediator [12]. However, the direct use of the enzyme at larger scales is hindered by cost and environmental limitations; thus, alternative strategies should be developed for the purpose of field application.

China produces an estimate of > 30 ´ 10^6 tons of edible mushrooms and more spent MCS each year. Spent MCS has been extensively utilized for soil conditioning and fertility improvement, but its use in soil remediation is still in its nascent stage. Spent MCS contains fungal mycelia and PAH-oxidizing enzymes, which may contribute to the transformation of PAHs. As such, spent MCS represents a sustainable and cheap resource for the clean-up of PAH-contaminated soil.

In the first experiment, we tested the transformation of PAHs using spent MCS extracts in aqueous reaction systems. We collected four types of spent MCS of commercial mushrooms: *Pleurotus ostreatus*, *Pleurotus eryngii*, *Coprinus comatus*, and *Agaricus bisporus*. The MCS were extracted with water by shaking for 1 h. The crude extracts were obtained by centrifugation. Laccase activity in these crude extracts ranged from 0.1 to > 8.0 units mL^{-1}. To explore its potential use in soil remediation, aqueous reaction systems consisting of crude extracts and selected PAHs were established. The reaction tubes were incubated (25 °C, 24 h) in dark, then the PAHs were analyzed to calculate the transformation. All the crude extracts of the four MCS resulted in a significant reduction of PAHs, particularly anthracene, benzo[*a*]pyrene, and benz[*a*]anthracene (Figure. 2) [13]. This result was extremely similar to that of fungal laccase oxidation [11], which suggests laccase-like activity; further, the result may correlate with the low ionization potential (IP) of these PAHs [14].

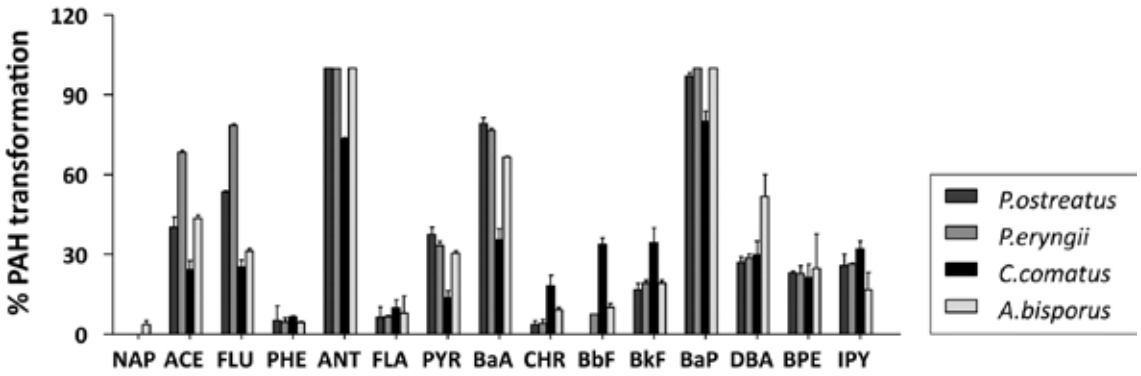

Figure 2. Transformation of 15 EPA PAHs by extracts of four types of spent mushroom composts [13]. The abbreviations for each PAH are: NAP, naphthalene; ACE, acenaphthylene; FLU, fluorene; PHE, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BaA, benz[*a*]anthracene; CHR, chrysene; BbF, benzo[*b*]fluoranthene; BkF, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene; DBA, dibenz[*a*,*h*]anthracene; BPE, benzo[*ghi*]perylene; IPY, indeno[1,2,3-*cd*]pyrene.

One additional advantage of spent MCS over pure laccase is the presence of redox mediators, which can enhance the enzymatic transformation of PAHs. This was clarified by the observation of increased PAH oxidation by pure laccase in the presence of boiled MCS extract [13]. Based on these findings, we believe that MCS is a candidate substrate for the remediation of PAH-contaminated soils.

3. Dissipation of PAH in soil microcosms amended with mushroom cultivation substrate

In the second experiment, we explored the potential of MCS in soil remediation with microcosm incubation [15]. The contaminated soil (14.6 mg kg^{-1}, 15 EPA Priority PAHs) used in the study was collected from a petroleum gas station in Wuxi, Jiangsu Province, China (30° 36’14’’N, 120°28’33’’E). Notably, the soil had an acidic pH. Five treatments, including the control, were established in triplicate, as shown in Table 1. The MCS used in this study consisted of crushed corncob (60%), wheat bran (30%), cattle manure (7%), sucrose (1%), superphosphate (1%), urea (0.5%), and gypsum (0.5%). The MCS with or without *P. ostreatus* was spiked into soil at a concentration of 5% (w/w). After incubating (60 d) in a green house, the soil samples were removed from each microcosm for molecular and chemical analysis.

Table 1. Experimental treatments [15]

Treatment	Abbreviation	MCS	P. ostreatus	Alfalfa
Control	CK	–	–	–
MCS amendment	S	+	–	–
MCS and P. ostreatus	SP	+	+	–
MCS, P. ostreatus and alfalfa	SPA	+	+	+
Alfalfa	A	–	–	+

3.1 Dissipation of PAH in soil microcosms

Following the incubation (60 d), the total amount of PAHs decreased from 14.6 to 10.4 mg kg^{-1} in the control treatment, suggesting natural attenuation caused by the activity of indigenous microbes. For the purpose of comparison, the dissipation of individual and total PAHs in all the treatments was calculated against the values of CK (Figure 3).

In general, the S and SP treatments resulted in high levels of PAH removal (Figure 3). Treatment with MCS resulted in 32.9±8.0% dissipation of total PAHs. The depletion of individual PAHs was extremely variable and appeared unrelated to the molecular weight. For example, the 5-ring compound benzo[*a*] pyrene was the second most degraded of the 13 detectable PAHs. In addition, anthracene and benz[*a*] anthracene were presumably susceptible to MCS treatment, with >60% removal observed. The addition of *P. ostreatus* with MCS (SP treatment) apparently increased the dissipation of benz[*a*]anthracene and benzo[*a*]pyrene; however, no significant difference was observed in the total PAH consumption between the S and SP treatments.

Interestingly, the removal of PAHs due to the addition of MCS was abolished by alfalfa; only 7.7% and 11.2% of total PAHs were degraded in the SPA and A alfalfa treatments, respectively. This trend was apparent for anthracene and benzo[*a*]pyrene, with the dissipation of these compounds decreasing from 100% and 98.4% with SP treatment to 8.0% and 5.0% with SPA treatment, respectively (Figure 3). As a result of the PAH dissipation, the soil toxic equivalent (TEQ) to benzo[*a*]pyrene in S and SP microcosms decreased by approximately 50% (Figure 4).

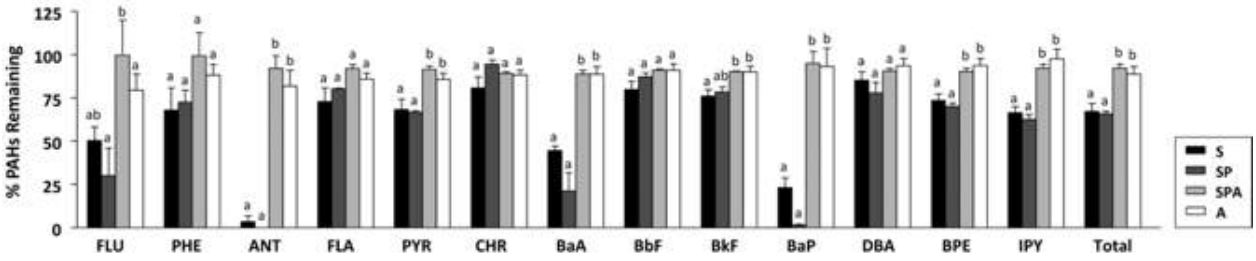

Figure 3. The percentages of PAH residual in soil after a 60 d incubation with mean values plotted with standard error bars [15]. The values were calculated against those of control treatments. Values with the same letter indicate that there are no differences. The abbreviations for each PAH are the same as those in Figure 2.

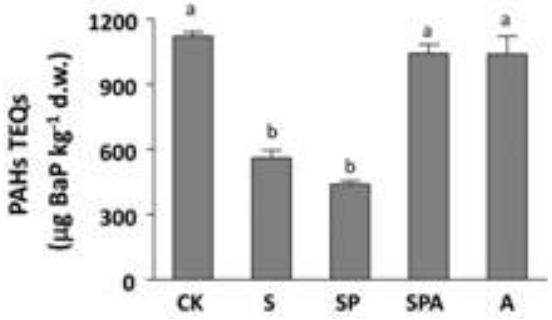

Figure 4. Soil TEQs in microcosms after a 60-d incubation. Values with the same letter indicate that there are no differences.

3.2 Effects of MCS on indigenous soil microbial communities

After the 60-d incubation, bacteria, fungi, and aromatic hydrocarbon degraders (AHDs) were enumerated by culture-based methods (Figure 5). In general, the control had the smallest bacterial and fungal populations, and the addition of MCS considerably stimulated the growth of all three groups (bacteria, fungi, and AHDs). However, the addition of *P. ostreatus* to the microcosms did not contribute to the growth of fungi and bacteria, as demonstrated by the almost equal or slightly decreased population sizes. Although alfalfa itself had marginal effect on the growth of bacteria and fungi, when combined with MCS and *P. ostreatus* (SPA treatment), it resulted in the same levels of growth observed with the treatment groups including MCS and *P. ostreatus* (treatment groups S and SP). The microbes that were potentially involved in the degradation of aromatics (AHDs) were enumerated using a most probable number (MPN) method. Treatment with MCS resulted in increasing numbers of AHDs (S, SP, and SPA treatments) (Figure 5C), whereas treatment with alfalfa alone did not significantly stimulate the growth of AHDs.

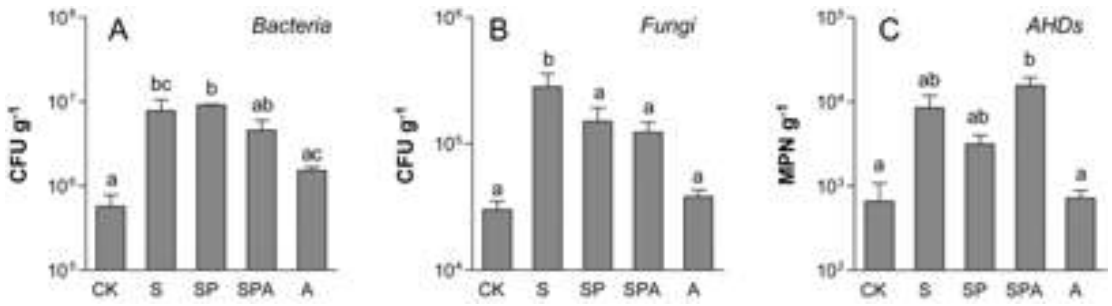

Figure 5. Population sizes of (A) bacteria, (B) fungi, and (C) AHDs in soils after a 60-d incubation [15]. Values with the same letter indicate that there are no differences.

The bacterial and fungal community compositions after the CK, S, SP, and SPA treatments were examined with a fingerprinting approach, DGGE (Figure 6). Despite the two outliers (S-1 and SP-1 in Figure 6A), the CK treatment group for bacteria differed from the remaining treatments, suggesting a shift in community composition. Dominant or clear bands were excised and subjected to sequence analysis; however, only eight sequences were retrieved because of the failure of reamplification.

The effects of treatment were more apparent in fungal than bacterial communities. The fungal community in the CK-treated microcosms was relatively diverse and consisted of more bands than those observed with other treatments (Figure 6B). Based on the sequence alignment, these bands were affiliated with the Chytridiomycota, Zygomycota, Basidiomycota, and Ascomycota phyla. Obviously, the addition of MCS reduced fungal diversity, as indicated by the disappearance of a few bands (such as F2, F3, F4, and F7) with S treatment. This reduction in diversity resulted in the enrichment of two bands (F6 and F8) that were closely related to the class Sordariomycetes of Ascomycota. Additional treatments with *P. ostreatus* inoculation and alfalfa did not significantly change the fungal community composition, as demonstrated by the high similarity among the S, SP, and SPA treatments (Figure 6B). Interestingly, there was no intense band corresponding to *P. ostreatus*, which belongs to the class Agaricomycetes of Basidiomycota.

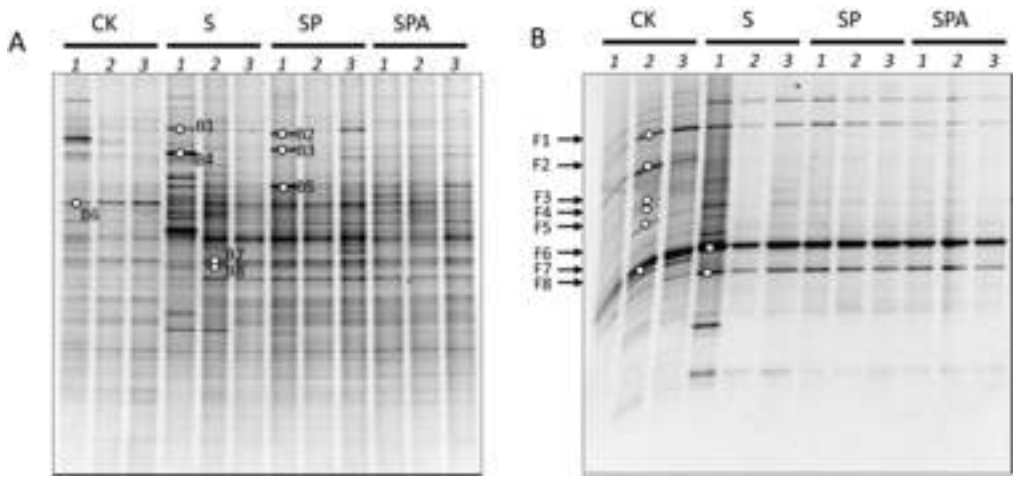

Figure 6. DGGE fingerprints of (A) bacterial 16S rRNA genes and (B) fungal 18S rRNA genes [15]. Three replicates of each treatment were included. The labeled bands were excised and sequenced.

4. Implications of MCS amendment on PAH-contaminated soil remediation

The addition of MCS to aged PAH-contaminated soil (S treatment) resulted in considerable dissipation of the total PAH concentration after the 60-d incubation compared to the control treatment (Figure 3). Since there was no sterile soil control with the MCS treatment, it was impossible to assess the effects of abiotic factors on the degradation of PAHs. For example, the organic matter of MCS may adsorb PAHs, thus reducing the bioavailability of the PAHs in soil [16]. Therefore, the depletion of PAHs with S treatment could be largely attributed to the enhanced degradation activity of indigenous microorganisms.

The addition of MCS to soil significantly changed the microbial communities. Although MCS is commonly used for edible mushroom cultivation, it also stimulated the growth of bacteria and AHDs simultaneously (Figure 5), possibly because of the enrichment of soil organic matter and nitrogen contents. The bacterial and fungal community compositions were considerably impacted by the addition of MCS (Figure 6), thus increasing the relative abundance of two Sordariomycete species increased. The reason for the selection of these species was unknown, and the unambiguous identification of active PAH-degrading species in soil was beyond the scope of this study.

Notably, the dissipation of individual PAHs was not dependent on the molecular weight, which is the major property influencing the degradability of PAHs by bacteria [17]. The concentrations of anthracene, benzo[*a*]pyrene, and benz[*a*]anthracene were reduced by >50% with S treatment (Figure 3). This provides insight regarding the underlying mechanism of PAH degradation because these three PAHs have been found to be susceptible to oxidation by fungal laccase [14, 18]. Similar degradation patterns can be also observed with crude extracts of spent MCS, as demonstrated above. Further, fungal laccase can also readily remove majority of the anthracene and benzo[*a*]pyrene in soil [11]. In contrast, bacteria have rarely been reported to degrade benzo[*a*]pyrene in soil [17]. Considering that some Sordariomycete species enriched in S treatment exhibit ligninolytic enzyme activity [19], fungal laccase-like enzymes could potentially play a role in the bioconversion of PAHs. However, possible explanations for the degradation of PAHs with S treatment exist. For example, fungi normally synthesize complex enzymes that degrade PAHs to an extent [20, 21]. Bacteria with laccase activity may also contribute to the degradation of benzo[*a*]pyrene and anthracene [22]. Notably, the interactions between fungi and bacteria may impact the degradation of PAHs in soil [23]. Hence, the mechanisms of PAH degradation by MCS biostimulation require further investigation.

Two additional bioremediation treatments appeared to be less efficient than biostimulation with MCS. Compared with the S treatment, the addition of *P. ostreatus* (SP treatment) to soil improved the degradation of several PAHs at nonsignificant levels, whereas the total PAH concentrations with S and SP treatments were approximately equal. The colonization of allochthonous microbial species in soil is often complicated by many factors and is a major concern in the application of bioaugmentation [6]. *P. ostreatus* has been employed in PAH depletion in some studies [24, 25]; however, in this study, *P. ostreatus* might not be a strong competitor in soil, which is demonstrated by the fact that no dominant band in the DGGE profiles could be identified as this white-rot fungus (Figure 6B).

The effect of MCS on the dissipation of PAHs was hindered by alfalfa (SPA Treatment, Figure 3), even though the presence of alfalfa has been reported to considerably decrease the levels of PAHs in soil [26]. Most interestingly, there was no significant difference in the microbial population sizes (Figure 5), and nearly identical fungal community compositions were observed between the SP and SPA treatments (Figure 6). Therefore, the competition for bulk nutrients between plants and microbes may be largely negligible, as further evidenced by the similar levels of carbon and nitrogen in both treatments. Interestingly, the addition of alfalfa to samples increased the soil pH from 4.58 with SP treatment to 4.98 with SPA treatment, as demonstrated in a previous study [27]. The optimal pH range for laccase activity is between 3.5 and 4.5, which might be an explanation for the decreased dissipation of benzo[*a*]pyrene, anthracene, and benz[*a*]anthracene in the soil microcosms of SPA treatment. Moreover, the effects of rhizodeposition (the release of organic compounds from plant roots into soil) on PAH degraders are complex [28], and the plant metabolites may hinder the biodegradation of PAH [29]. Further studies are required to understand the interaction between the roots of the alfalfa and soil microbial communities.

5. Conclusions

This study explored the potential of a mixed substrate used for mushroom cultivation (MCS) in the bioremediation of aged, PAH-contaminated soil. Three PAHs, i.e., anthracene, benzo[*a*]pyrene, and benz[*a*]anthracene, were most susceptible to degradation, which is consistent with the PAH degradation characteristics of fungal laccase-like enzymes. Other strategies were less effective than or inhibited biostimulation, thus suggesting the complexity of the regulation of PAH degrader activity. The results of this study, together with an ongoing field experiment of cropland remediation using MCS (Figure 7) demonstrate that MCS is a promising biostimulation agent for the bioremediation of PAH-contaminated soils [30].

Figure 7. An ongoing field remediation experiment of PAH-contaminated cropland using MCS. The polluted site is near a smelting plant in Nanjing, China.

Acknowledgements

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Part III.
Applications of Analytical Chemistry

Ionic Liquids and Nanomaterials: An Efficient Combination to Develop Novel Environmentally friendly Analytical Methods for Toxic Trace Element Determination

Ionic Liquids and Nanomaterials: An Efficient Combination to Develop Novel Environmentally friendly Analytical Methods for Toxic Trace Element Determination

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Abstract

Toxic elements can be largely introduced into the environment and food chain by industrial activities and natural sources such as effluent spilling and volcanic emissions, respectively. However, several toxic elements could cause severe damage to living organisms, even when these occur in food and water at extremely low concentrations. Therefore, it is important to utilize highly sensitive analytical methods for determining toxic elements at trace levels.

In recent years, the development of novel technologies based on the combination of nanomaterials and ionic liquids (ILs) has attracted considerable interest in analytical chemistry. Thus, by combining potentially greener solvents such as ILs with efficient and modern solid phases such as nanomaterials, the design and implementation of innovative and highly efficient analytical methods for the sensitive and selective determination of toxic elements (e.g., As, Hg, Cd, Pb, and Tl) is feasible. Likewise, when innovative hybrids materials, such as those composed by ILs and nanomaterials, are implemented in miniaturized solid-phase and liquid-phase microextraction procedures, high analytical recoveries are guaranteed and environmentally friendly analytical methods can be developed. Furthermore, the extraction and preconcentration methods implemented in flow injection and sequential injection analysis systems associated with elemental-specific detectors such as those based on atomic spectrometry are particularly useful for developing automated methods that increase the productivity of analytical laboratories. Here, the most recent progress on the application of ILs and nanomaterials to develop environmentally friendly analytical methods for monitoring toxic elements in environmental and food samples is discussed.

Keywords: Nanomaterials; Ionic liquids; Analytical Chemistry; Green Chemistry, Environmental safeguard, Chemical analysis; Trace elements; Ambient monitoring, Microextraction; Preconcentration.

1. Introduction

Green Analytical Chemistry (GAC) is an important part of the sustainable development concept. The public interest in protecting the environment has encouraged analytical chemists to explore novel sample-preparation techniques that could significantly reduce the adverse environmental impact of chemical approaches [1, 2]. Investigation on GAC methods includes numerous strategies to diminish the amounts of reagents consumed and wastes generated [1, 3]. For example, miniaturization of the sample preparation procedures in analytical chemistry, automation, and the search for alternative solvents and materials are important methods of diminishing the environmental side effects of analytical methods. These strategies have been the subject of a significant number of research efforts and recent progress in GAC [3, 4].

The miniaturization of analytical methods, primarily the sample preparation steps, is considered to be one of the main approaches complying with GAC principles. Microextraction is defined as a non-exhaustive miniaturized sample preparation method using an insignificant volume of extracting phase (≤ microliter range) relative to the sample volume [5]. Analytes can be extracted by small volumes of solid or semi-solid materials, e.g., in solid-phase microextraction (SPME), or alternatively by small volumes of a liquid phase, e.g., in liquid-liquid microextraction (LLME) [5, 6]. Therefore, microextraction techniques are an important contribution to the improvement of sample preparation and the development of environmentally friendly analytical procedures. The main analytical result indicates increased reliability, higher precision, increased time efficiency, and substantially reduced waste.

In the pursuit of substitute solvents and materials for microextaction, the goal is not just to replace the existing products, but to exploit the advantageous properties of the substitutes to enhance the selectivity, sensitivity, and reliability of the analysis, while reducing the time required for analyses [4]. Over the past decades, the unique properties of ionic liquids (ILs) and nanomaterials have enabled numerous applications in analytical chemistry. In particular, considerable efforts have been invested in replacing the volatile organic solvents in sample preparation procedures [7-10]. ILs have been applied to the development of various types of microextraction techniques, owing to their favorable chemical and physical characteristics. These characteristics include negligible vapor pressure, excellent extraction efficiency for several organic compounds and metal ions (as neutral or charged complexes), high thermal stability, and adjustable viscosity and miscibility with water and organic solvents [8, 11]. ILs are excellent alternatives to the volatile organic solvents typically utilized in microextraction methods, thus yielding high analytical recoveries and enrichment factors (EFs) [11, 12]. The development of novel sustainable analytical procedures is extremely important for GAC. Thus, the application of state-of-the-art solvents, like ILs, combined with microextraction techniques is potentially an excellent strategy for achieving greener sample preparation than classical techniques. In fact, several of the GAC objectives (e.g., minimal or zero waste generation, use of safer solvents, and development of miniaturized methods) are fulfilled by utilizing ILs in microextraction procedures [8, 13].

The introduction of nanotechnology in analytical chemistry, particularly, the application of various types of nanomaterials for developing novel methods of solid-phase microextraction (SPME) and liquid-liquid microextraction (LLME), is an extremely attractive strategy to achieve the separation and preconcentration of trace elements. This is largely ascribed to the high surface area and chemical stability of several nanomaterials, both in organic and inorganic media. Moreover, nanomaterials provide multiple active sites for adsorbing analytes, resulting in significantly higher surface-area-to-volume ratios that supply far greater extraction capacity and efficiency than conventional sorption materials. Thus, in recent years, several nanomaterials, including carbon nanotubes (CNTs), fullerenes, nanoporous silica, nanostructured metal oxides and Au nanoparticles (NPs) have been successfully used in the preconcentration and extraction of different analytes [14].

In addition to the individual application of ILs and nanomaterials, their combination can produce noticeable advantages for the development of modern analytical methods with highly efficient preconcentration of analytes. For example, coordinating NPs dispersed in ILs as extractant phases are promising alternatives for efficient microextraction. Therefore, the use of ILs, nanomaterials, and the combination of the two for developing batch and on-line microextraction procedures for the determination of trace elements can potentially induce strong innovation in analytical chemistry.

Methods involving the combination of ILs and nanomaterials are advantageous in flow-injection (FIA) and sequential-injection (SIA) analysis systems, thereby enabling further automation of the procedures and the overall analytical method. Additionally, the practical application, safety, and cost effectiveness of using ILs and nanomaterials along with microextraction techniques are practical advantages for developing environmentally friendly and efficient analytical methods with extensive applications in routine-analysis laboratories for trace element determination.

Herein, we discuss the abovementioned advantages of using ILs and nanomaterials in the development of state-of-the-art analytical methods for the preconcentration and determination of toxic trace elements. This study strongly correlates with the research project titled “Development of highly sensitive analytical methods based on ionic liquids-functionalized nanomaterials for toxic trace elements determination”, where the design and implementation of innovative and highly analytical methods for sensitive and selective determination of toxic elements, such as As, Hg, Cd, Pb, and Tl, were proposed.

2. Ionic liquids and nanomaterials: definitions and useful properties for green chemical analysis

2.1 Ionic Liquids

ILs are defined as salts with melting points close to or below room temperature. These are nonmolecular solvents that exhibit numerous unique properties such as negligible vapor pressure, thermal stability (even at high temperatures), as well as favorable viscosity and miscibility with water and organic solvents [15]. The other properties of ILs are shown in Table 1. These characteristics make them promising alternatives to environmentally unfriendly solvents which, contrary to ILs, generate volatile organic compounds. Moreover, several ILs can be synthesized based on the high number of anions and cations available. In fact, it is estimated that there could be up to 1018 ILs available for different applications [15].

Table 1. Selected physicochemical properties of ILs, nanomaterials, and IL-nanomaterial hybrids for extraction and preconcentration

ILs	Nanomaterials	IL-Nanomaterial hybrids
Non-combustibility	Large specific surface area	Easy regeneration
Negligible vapor pressure	Multiple active sites for adsorbing analytes	Fast extraction of analytes
High heat resistance	High resistance	High preconcentration of analytes
Low toxicity	Magnetic and optical properties	Less solvent consumption
High thermal stability	Possibility of physical or chemical functionalization	High chemical stability
High flame resistance Negligible volatility		Negligible vapor pressure
Good stabilizer of stable colloidal dispersions of nanomaterials		Tunable composition
Facilitates nanoparticles incorporation into the extraction phase		Polarity control
Increases the homogeneity of the active adsorbent sites		High chemical and thermal stabilities
		Novel magnetic and optical properties for more efficient extraction procedures

Typically, the ILs used in analytical chemistry can be composed of different organic cations including imidazolium, phosphonium, pyrrolidinium, pyridinium, or quaternary ammonium and several anions such us hexafluorophosphates, tetrafluoroborates, alkylsulfates, alkylsulfonates, nitrate, acetate, hydroxide, chloride, and bromide [16]. Less common anions include trifluoromethanesulfonate and bis(trifluoromethylsulfonyl)imide [17]. Figure 1 depicts some of the most common ILs and ion combinations.

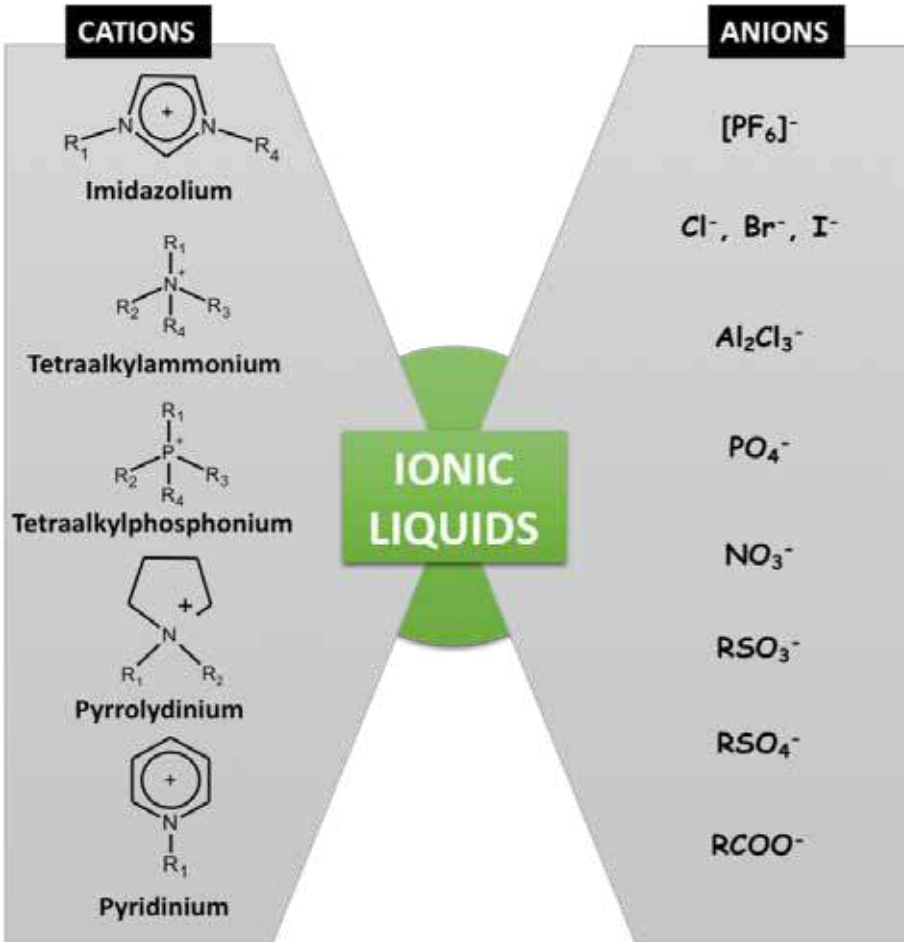

Figure 1. Cations and anions that are typically combined to obtain different ILs. R1, R2, R3, and R4 are alkyl chains such as ethyl, propyl, butyl, pentyl, and hexyl.

Imidazolium-type ILs have been extensively used in analytical chemistry [13, 18, 19]. This could be attributed to their relatively low cost and straightforward synthesis. Furthermore, the alkyl-chain length of the imidazolium ring and the counter anion can induce a variety of properties, such as low melting points, reusability, tunable viscosity, and solubility. Based on previous reports, viscosity increases proportionally with the alkyl-chain length, while the solubility in water decreases [16]. Therefore, both parameters are normally considered when selecting an appropriate extracting phase because low solubility enables minimal IL consumption, whereas high viscosity could lead to practical drawbacks during microextraction procedures. Notably, an increase in the alkyl-chain length is often followed by the formation of aggregates of IL in water above a certain concentration (IL-based surfactants) [16].

3. Nanomaterials

The concept of nanomaterials includes natural or synthetic particles that are ≤ 100 nm in size [20]. The general properties of nanomaterials are shown in Table 1. The synthesized nanomaterials can be modified to improve the efficiency of technological processes; however, their potential applications depend on the material composition [20]. CNTs and fullerenes (nC60), as well as inorganic particles such as metal oxides, are among the most interesting nanomaterials due their remarkable properties and extensive applications for developing analytical methods (Figure 2).

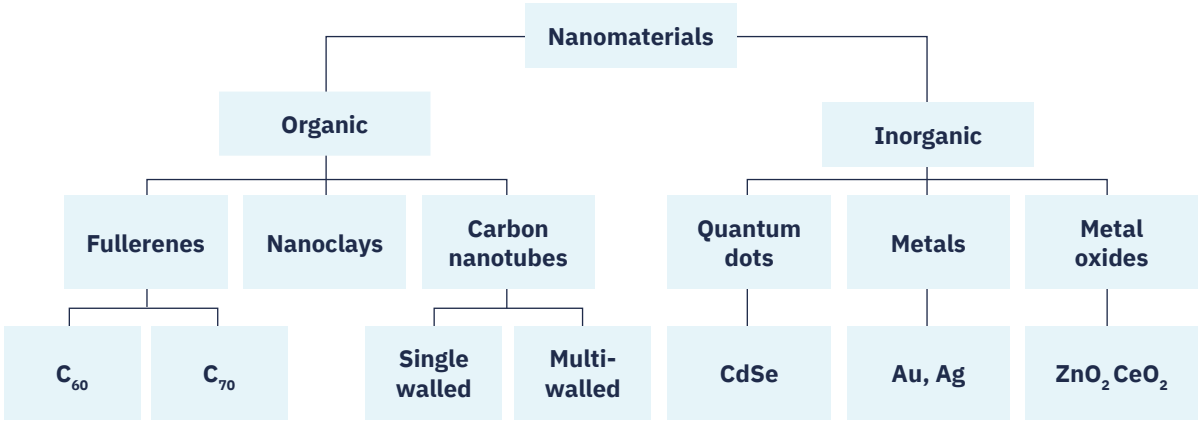

Figure 2. General classification of most common nanomaterials.

CNTs are allotropes of carbon, such as diamond and graphite, comprising curved or closed carbon hexagonal networks that form nanometer-sized tubes. Thus, the structure is essentially a self-rolled sheet of graphite. CNTs are light, hollowed-out, porous systems with high mechanical resistance. Hence, CNTs have been used for structural strengthening of light materials. In general, CNTs have diameters between fractions of nanometers and tens of nanometers and lengths of up to several micrometers. Their ends are normally capped by fullerene-like structures. CNTs are considered as hollow graphitic nanomaterials comprising one (single-walled carbon nanotubes, SWNT) or multiple (multi-walled CNTs, MWNTs) layers of graphene sheets [21, 22].

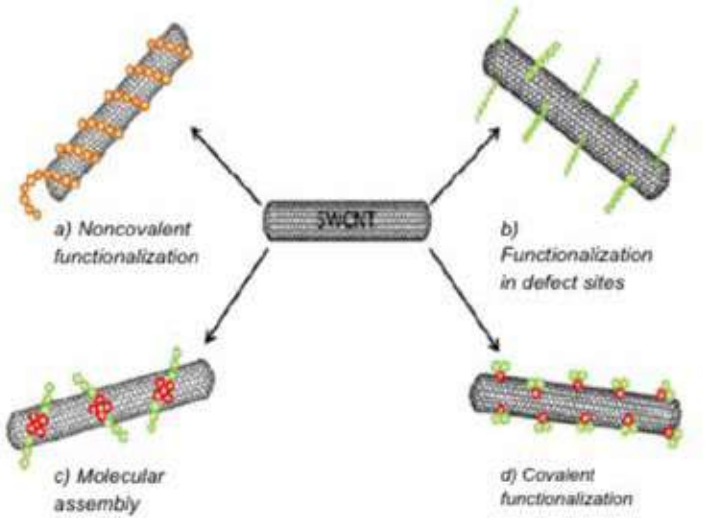

Figure 3. Different approaches for functionalization of single wall CNTs (SWCNT).

CNTs are characterized by their high surface areas and favorable electrical, chemical, mechanical and conducting properties. Owing to these characteristics, CNTs have garnered considerable attention. CNTs may exhibit metallic and semiconducting electron transport properties. Further, they possess hollow cores, which can store guest molecules. Solid-phase extraction is a suitable technique for preconcentrating organic and inorganic analytes, and the use of CNTs in SPE has attracted increased interest to analytical chemistry [23, 24]. Majority of the applications involve organic analytes, but CNTs can be also applied to inorganic analytes. Consequently, several reports have suggested the functionalization of CNTs by treating with oxidizing agents, whereas others suggest functionalization using a combination of organic reagents and other sorbents [24]. Figure 3 depicts some of the common functionalized forms.

The functionalization of CNTs facilitates modification of the physical and chemical characteristics of the CNTS [25]. The presence of covalently attached functional groups influence the retention or affinity of the CNT surface and important properties such as polarity, hydrophilicity, and other specific interactions. The functional groups may also alter the diffusional resistance, reducing the accessibility and affinity of the CNT surfaces for specific analytes.

NPs synthesized as the oxides of various metals (e.g., Zn, Ce, Ti and Fe) and non-metal NPs, such as those comprising Si, are other classes of nanomaterials. These types of nanomaterials have a broad application spectrum ranging from sunscreen production to the fabrication of electronic devices. In particular, Si-based NPs have garnered the most attention in analytical chemistry; their high surface areas and high reactivity favor surface chemical modification and functionalization [26]. Therefore, the functionalization of nanomaterials is becoming increasingly essential for analytical developments involving procedures, thus leading to higher selectivity and efficiency when these materials are incorporated into the analytical methods.

4. Analytical extraction and preconcentration methods for trace element determination based on ILs and nanomaterials

4.1 Liquid-liquid microextraction methods based on ILs

There is an increasing demand for novel sample preparation techniques in analytical chemistry to achieve efficient extraction and preconcentration of several analytes, including trace elements [5]. In recent years, considerable efforts have been made to reduce the scale of liquid-liquid extraction techniques and explore new alternatives to traditional volatile organic solvents. Thus, the synergy obtained by implementing ILs in LLME techniques is increasingly becoming mainstream in sample preparation [13, 18, 19]. Different microextraction techniques such as dispersive liquid-liquid microextraction (DLLME), In situ solvent formation microextraction (ISFME), temperature-assisted tandem dispersive liquid-liquid microextraction (TA-DLLME), and ionic liquid-assisted ion-pairing LLME, have emerged as effective sample preparation approaches, owing to their simplicity, rapidity, and adaptability to a wide variety of samples and analytes [4]. An example of the typical experimental steps performed by employing the DLLME technique is detailed in Figure 4.

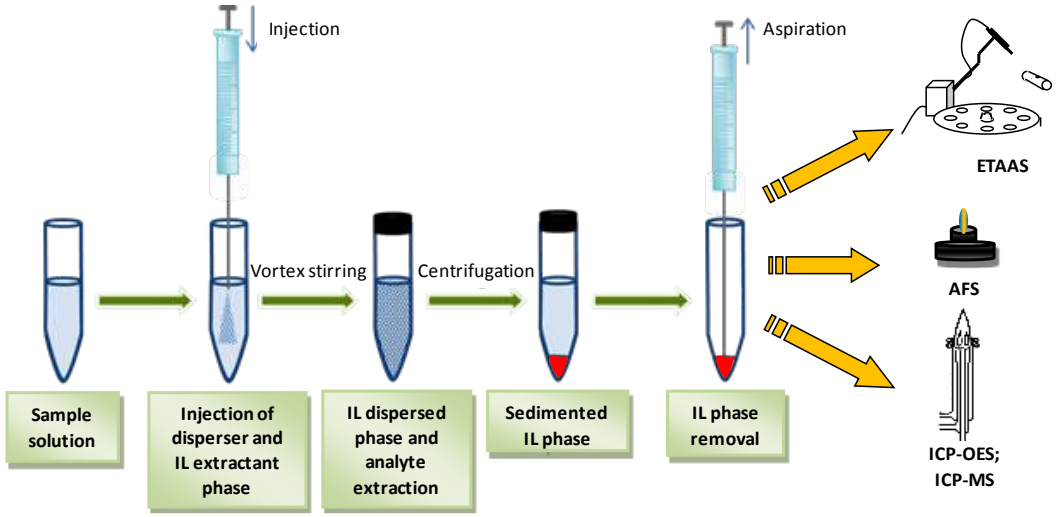

Figure. 4. Schematic diagram of an IL-DLLME procedure for preconcentration and determination of trace elements with different detectors.

The use of ILs as solvents for LLME has enhanced the sensitivity and selectivity of methods for the extraction and preconcentration of metal species and the analysis of different matrix samples. ILs are attractive and efficient tools for improving the limits of detection, selectivity, and sensitivity in the total and speciation analysis of several metals. Hence, ILs are undoubtedly valid alternatives to the organic solvents that are conventionally utilized in analytical chemistry, owing to the high recoveries and sensitivity-enhancement factors obtained after their application [7-10]. Furthermore, the practicality, safety, and cost-effectiveness of implementing ILs along with microextraction techniques, are practical advantages for developing environmentally friendly analytical methods, which can be extensively used in routine-analysis laboratories for efficient trace metal determination [7-10].

As mentioned earlier, ILs are advantageous as extractant solvents for trace metal preconcentration and determination. However, although there is increasing information regarding the different properties of ILs, the total potential of these unique solvents for separation, preconcentration, and speciation has not been elucidated. To date, ILs have been primarily used as conventional solvents; however, these ILs possess numerous properties that require further investigation. Hence, it is necessary to determine the benefits of different organic solvents to achieve more advantageous methods. There are limited reports on the mechanisms involved during metal extraction [13, 18, 19].

Furthermore, although progress has been made regarding the automation of IL-LLME techniques, additional research is required to exploit its capabilities in chemical analysis. Also, the attachment of functional groups to the chemical structures of ILs can facilitate the highly selective and direct extraction of trace elements, but still requires further development.

Although ILs are superior to volatile organic solvents because of their negligible vapor pressure, the ability to modify their chemical structures and versatilities compared to conventional organic solvents must be considered [11, 12]. Therefore, the future application of ILs in sample preparation strongly depends on overcoming limitations such as toxicity and reduced biodegradability of several ILs [27]. Additional efforts are still required to address these limitations. Finally, sample preparation in GAC would achieve considerable progress if the future developments of LLME progressively incorporate recycling and the reutilization of IL waste.

5. Development of solid-phase extraction methods with nanomaterials

Solid-phase extraction is a preconcentration technique with several advantages such as simplicity of operation, versatility, low cost of equipment, short extraction time, elimination of organic solvents, and possible automatization. The sorbent material is critical to this technique because the sorbent determines the retention of analytes in the preconcentration methods. There are two SPE techniques, i.e., on-line SPE with columns implemented in flow analysis systems and dispersive SPE performed in the off-line or batch mode. Several methods using nanomaterials have been developed with the former; however, the latter has been applied together with magnetic nanomaterials because it is easier to collect the dispersed solid-phase material [28, 29]. For example, several studies based on the implementation of magnetic NPs for microextraction procedures have been reported in recent years [30-35]. Magnetic nanomaterials have an additional advantage in that their separation from the sample after analyte preconcentration is achieved by a magnetic field, thus eliminating tedious centrifugation steps [30]. Magnetite (Fe_3O_4) NPs have received more attention than other magnetic nanomaterials, owing to the feasibility of preparation and excellent magnetic properties. However, a silica protection layer is normally required to ensure its chemical stability and improve the dispersibility in microextraction systems [36, 37]. Additionally, magnetic NPs have been used in extraction methods, primarily for organic analytes, rather than inorganic ones; this could be attributed to the possible interferences of Fe on trace metal determination. The adsorbents are readily regenerated, analyte extraction is rapid with high extraction efficiency, and solvent consumption is minimal, thus enabling the development of greener methods that have considerable applicability in analytical laboratories [38].

Another group of nanomaterials is based on substances containing carbon structures. Among them, CNTs are strong nanomaterials with excellent physical properties such as thermal conductivity, excellent electrical properties, and remarkable field emission properties [39]. CNTs also have excellent chemical properties that are essential for analyte preconcentration, such as a highly hydrophobic surface that facilitates strong adsorption of specified compounds by noncovalent forces (e.g., hydrogen bonding, π-π stacking, electrostatic forces, van der Waals forces, and hydrophobic interactions). Therefore, CNTs have been widely employed as efficient sorbent materials for analyte preconcentration [39, 40]. Another important carbon-based nanomaterial is graphene, which is a crystalline allotrope of carbon with a 2D structure formed by carbon atoms. Its favorable physico-chemical properties, including large surface area, high dispersibility, and hydrophobicity render it suited for the preconcentration of analytes [41].

On the other hand, metal oxide NPs like Mn_3O_4 are a class of nanosized materials with significantly increased surface areas, high sorption, and strong acid sites [42]. They are appropriate sorbents for removing metal ions from various samples and may be applicable for developing novel preconcentration methods for trace element determination [42]. However, the disadvantage of these nanomaterials is that they are prone to the formation of flocks or gels. Hence, their surfaces require a passive coating made of inert materials such as silica to prevent aggregation of the NPs [42].

Notably, despite the great enthusiasm for the introduction of nanomaterials in daily life because of their remarkable properties and applications, there are still significant gaps in the understanding of the real nature of their behavior in our environment. Thus, many more studies are required to comprehensively explore the potential risks of human exposure to nanoscale components of the currently commercially available products, as well as future products [43]. On the other hand, the possibility of using minute quantities of nanomaterials when analytical preconcentration is performed based on microextraction techniques, like those reported earlier in this study, is a profound advantage because it reduces the discharge of nanomaterials into the environment. Furthermore, the recycling and reutilization of nanomaterials should also be considered when analytical methods are applied in the laboratory.

6. Possible combination of ILs with nanomaterials for analytical preconcentration

As mentioned previously, nanomaterials and ILs both have both very interesting properties (Table 1) that may enable significant improvements in the analytical performance of the preconcentration methods (e.g. high sensitivity, selectivity, and interference elimination. The development of novel technologies based on the combination of nanomaterials and ILs for application in analytical chemistry is a matter of remarkable interest. Among various possibilities, ILs have also been proposed as excellent tools to functionalize nanomaterials, thus conferring specific characteristics for the efficient extraction of analytes. Modern approaches for the functionalization of NPs with ILs are being undertaken and applied in extraction and preconcentration methods. Likewise, the physical adsorption of ILs onto the surfaces of nanomaterials is another convenient strategy for the efficient separation and preconcentration of the analytes. Moreover, the incorporation of nanomaterials in miniaturized SPE and LLE procedures might guarantee a high recovery of analytes while developing environmentally friendly analytical methods.

ILs are proposed as excellent tools to functionalize nanomaterials. Further, they have extensive applications in fluid engineering using NPs and several reports have already described the functionalization or modification of these materials [26]. However, the functionalization of NPs with ILs in analytical chemistry has not been widely exploited because the main applications have been centered around the development of novel sensors to detect organic compounds using ILs as a disperser, but without much for preconcentration of trace elements. In fact, metal determination with IL-functionalized NPs have scarcely been used [44]. For example, Mahmoud et al. reported the synthesis of new sorbents based on the surface modification of nanosilica with a hydrophobic IL such as 1-methyl-3-ethylimidazolium bis(trifluoromethylsulfonyl)imide [EMIM+Tf2N–] [45]. The material obtained by this procedure was successfully used for the SPE of Pb in water samples. The first approach proposed by Mahmoud et al. for the synthesis of [NSi-OH-EMIM+Tf2N–] functionalized NPs was the derivatization of the silica surface by the physical adsorption of [EMIM+Tf2N–] on activated nanosilica (10–20 nm). A second approach consisting of the chemical derivatization of nanosilica with [Emim+Tf2N–] was proposed. Also, Farahani et al. prepared a sorbent for the simultaneous separation and preconcentration of Pb and Cd from milk and water samples [44]. An IL was deposited on the surface of magnetic NPs (IL-MNPs) and used for the SPE of these metals. The IL-MNPs carrying the target metals were then separated from the sample solution by applying an external magnetic field. Under optimal extraction conditions, a preconcentration factor of 200 was obtained. Despite the advantages presented in this study, the method was completely developed under batch conditions, whereas the implementation in flow systems remains limited.

Our research project titled “Development of highly sensitive analytical methods based on ionic liquids-functionalized nanomaterials for toxic trace elements determination”, which received funding from the Organisation for the Prohibition of Chemical Weapons (OPCW), comprises the design and implementation of innovative and highly efficient analytical methods for the sensitive and selective determination of toxic elements (e.g., As, Hg, Cd, Pb, and Tl) based on the application of ILs and different nanomaterials. Thus, the ability of various types of phosphonium ILs to form ion pairs with a complex obtained by the reaction between molybdate anion and As(V) species was evaluated [46]. It is important to determine different As species because the toxicity of this element in living organisms depends on the amount absorbed, the nature of the species, and the exposure routes. In fact, inorganic As species are more toxic than their organic counterparts, with methyl derivatives being thousand-fold less toxic than the inorganic species. In our study, As(V) species were initially complexed by molybdate anions, followed by an ion-pairing reaction with a phosphonium IL (tetradecylhexylphosphonium dicyanamide), and then extracted by a LLME procedure using a few microliters (80 µL) of tetrachlorethylene. The organic phase was then injected directly into the graphite furnace of the electrothermal atomic absorption spectrometry (ETAAS) instrument for As determination. Using only 5 mL of sample, an analyte extraction efficiency of 100% and 130-fold preconcentration factor were obtained. This method was applied for As species determination in different wines and waters of the Mendoza province (Argentina) at trace levels. Notably, the Mendoza province is recognized worldwide by the intense production of high quality wines. Therefore, novel analytical methods are extremely important to monitor the presence of toxic elements and their

species in the produced wine. Likewise, since water is scarce in the Mendoza province because of the characteristics of its climate, the evaluation of water quality is exceedingly important for determining its availability for human consumption and other uses.

In a different analytical method using a combination of ILs and nanomaterials for trace element preconcentration and determination, As speciation analysis was performed in garlic samples. Garlic (*Allium sativum* L.) is a vegetable widely used for cooking and is highly consumed in several countries. Hence, the determination of toxic trace elements is a fundamental issue. A highly sensitive analytical methodology for the determination and speciation of inorganic As species by ETAAS detection using d-SPME technique combining CNTs with ILs was developed. The phosphonium-type IL, trihexyltetradecylphosphonium chloride, was used to form an ion pair with the complex formed between the molybdate and As(V). CNTs (1.0 mg) were dispersed to remove As(V), then separated from the supernatant by centrifugation. Carbon nanotubes were redispersed with a surfactant, tetradecyltrimethylammonium bromide, and ultrasound. The dispersed particles containing the analyte were injected directly into the ETAAS. Under optimal conditions an extraction efficiency of 100% and a preconcentration factor of only 5 mL of sample extract were achieved. Carbon nanotubes exhibited remarkable performance as adsorbents with high adsorption capacity (25 mg As/g CNTs). The methodology was successfully applied for the determination of As species in garlic samples collected from the Mendoza province. It is important to highlight the significance of the garlic analysis because this food was intensively produced in the Mendoza province and exported to several places worldwide. Thus, the quality control of garlic and the evaluation of the levels at which toxic elements might occur are mandatory to preserve consumer health and open the doors to international markets.

7. Conclusions

The current trends in modern analytical chemistry are aimed at the development of green, simple, and highly sensitive methods for trace analyte determination. Sample preparation methods based on extraction and preconcentration prior to analyte determination have undergone intensive research. This can be attributed to the increasing demand of accurate and precise measurements at extremely low levels of inorganic and toxic analytes in diverse matrices. The GAC concept was introduced in preconcentration methods through the miniaturization and application of new extractant phases based on environmentally friendly and state-of-the-art materials. Within this framework, nanomaterials, ILs, and a combination of the two can be utilized for developing miniaturized liquid-phase and solid-phase extraction techniques that can be efficiently coupled to advanced instrumentation. As a result, efficient analytical tools that can be utilized in toxic trace element determination and speciation analysis can be developed. Further, these methods enable easy implementation in routine analytical laboratories and are aligned with the concept of green chemistry, thus preserving our environment. In our aforementioned research project, we developed state-of-the-art methods for determining the toxic elements in various types of samples combining IL and nanomaterials. We believe our proposed methods will have significant implications for studies of toxic trace elements in water, watersheds, sediment samples, and many other complex environmental and significant health samples. Further, the successful application of the developed analytical methods demonstrates their great potential for toxic element determination in food, thus generating powerful analytical tools for routine laboratories dedicated to food quality control.

8. References

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