Phytoremediation of Polychlorobiphenyls (PCB's) in Landfill E-Waste Leachate with Water Hyacinth (E.Crassipes)

E.A Omondi, P.K Ndiba and P.G Njuru

Abstract: The presence of e-waste in a landfill can release persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), into the environment. PCBs are a family of more than 200 chemical compounds (congeners), each of which consists of two benzene rings and one to ten chlorine atoms. This study investigated use of water hyacinth (Eichhornia crassipes) for phytoremediation of landfill leachate waste containing PCB. Landfill leachate was simulated in the laboratory by spiking water samples with PCB to obtain concentrations of 5, 10 and 15 µg/L, which were in one to two orders of magnitude above the US Environmental Protection Agency (EPA) limit of 0.5 µg/L or 0.5 ppb. Water hyacinth plants were grown in 2 L samples of the PCB spiked water for 15 days and evaluated for tolerance and bioaccumulation of PCB. Phytoremediation of PCB spiked water by the plants was evaluated by measuring the change in concentration of PCB. The plants tolerated PCB concentrations in the range of 5 to 15 µg/L without depicting any serious adverse effect except for change in root color and an initial wilting of peripheral leaves. Water hyacinth reduced the concentration of PCBs in the leachate over 15 days from 15 to 0.42 µg/L for the 15 µg/L initial concentration sample and to below the GC/MS detection limit of 0.142 µg/L for the 10 and 5 ug/L initial concentration samples. Bioaccumulation of PCB in the plant tissue was evaluated through solid phase extraction and testing of samples for PCB with GC/MS. Bioaccumulation of PCBs at a concentration of 0.179 µg/g was observed in the water hyacinth roots for the 15 µg/L sample but none was detected for the lower initial PCB concentration and shoots. The study demonstrated potential of water hyacinth plants in phytoremediation of PCBs in e-waste leachate.

Key words: Phytoremediation, Polychlorobiphenyls, Water Hyacinth, Bioaccumulation, e-waste

1.0 INTRODUCTION

1.1 Background Information

Excessive generation of municipal solid wastes (MSW) has been identified as one of the most serious environmental problems in the world. Up to 95% of the MSW collected worldwide are disposed off in landfills (Kurniawan et al., 2006), where they undergo a series of physicochemical and biological changes. Degradation of the wastes to various levels combines with percolated rainwater to generate a highly contaminated liquid known as "leachate." Persistent organic pollutants (POPs) are chemical substances that are extremely stable in the environment and are known to accumulate in biological tissue thereby posing risk to both human health and the environment. POPs originate from a variety of human activities especially agricultural and industrial activities, and electronic waste or e-waste. POPs can be conveyed for thousands of miles through air or water currents and may be found in remote ecosystems far from their source, including where they have never been used (EUROPA, 2007). Polychlorinated biphenyls (PCBs) are POP's comprising a family of more than 200 chemical compounds (congeners), each consisting of two benzene rings and one to ten chlorine atoms. PCBs are used extensively in industry as coolants and lubricants in transformers, capacitors, and other electrical appliances, because of their chemical inertness, resistance to heat, non-flammability, low vapor pressure, and high dielectric constant. Products containing PCBs include old fluorescent lighting fixtures, electrical appliances containing PCB capacitors, old microscope oil, and hydraulic fluids.

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Polychlorinated biphenyls (PCBs) in landfill leachate are of environmental concern because they are toxic, persistent and bio-accumulative. The use and disposal of such substances in landfill is not a sustainable practice. However, for different social and economic reasons such substances are still in use and are regularly released and disposed of to the environment through landfills. The characteristics of a landfill leachate depend on a number of factors such as the nature and origin of the waste. In cases where e-waste is disposed of in a landfill, the leachate will consist of heavy metals and persistent organic pollutants. The presence of organic pollutants in leachate is one of the reasons why regulatory bodies do not favor treatment of leachate with municipal sewage (Zenon Environmental Systems, 1989) and, therefore, the need to pretreat it. Many advanced soil remediation techniques exist in both industrialized and developed countries. They include gas phase chemical reduction (GPCR), mechano chemical dehalogentation (MCD) and thermal desorption. Some promising techniques such as base catalyzed decomposition (BCD) and sonic technology are still under development either at the laboratory stage or at the pilot study. However, due to the financial constraints, many advanced technologies are unlikely to be adopted by the developing countries. Consequently, finding suitable and affordable technologies for remediation of e-waste landfill leachate in developing countries is critical. Constructed wetlands are commonly used to treat municipal wastewater, acid drainage, agricultural runoff, animal wastes, and industrial wastewater. Water hyacinth plants in constructed wetlands are efficient in removal of a vast range of pollutants including suspended materials, biochemical oxygen demand (BOD), nutrients, organic matter, heavy metals and pathogens in a process known as phytoremediation. Because water hyacinth is an invasive plant of environmental concern, its use in phytoremediation of PCB's will offer a dual solution to environmental concerns. This study evaluated phytoremediation of e-waste in landfill waste using water hyacinth plants, as a simple and economic method of treatment of e-waste landfill leachate.

2.0 METHODOLOGY

2.1 Introduction

To achieve the objectives of this study, growth of water hyacinth plants in PCB contaminated water was simulated under laboratory conditions. Tolerance of the plants to PCB contamination was evaluated. Removal of PCB from the water by water hyacinth plants for three initial concentrations of PCB and an exposure period of 15 days was measured and compared with control in PCB free water. Bioaccumulation of PCB in the plant tissue was evaluated by measuring PCB concentration in roots, stems and leaves.

2.2. Water Hyacinth Planting, Growth and Maintenance

Young water hyacinth plants were obtained from Nairobi Dam, which is heavily invaded by the plants. Plants of similar size, health and foliage color and an average height 15 ± 2 cm were selected. The plants were transported in large open containers in the water of origin, to maintain growth. The plants were placed into the containers to form a canopy for control of splashing during transportation. Experimental plants that were not in use during the study were kept floated in the nutrient boosted water and enclosed in a lockable welded wire mesh cage with the shoots exposed to environment.

2.3 Supplement Nutrient Level Determination.

Essential nutrients for plant growth from NPK fertilizer were added to the water medium to support vibrant growth of plants. The N:P ratio is important for the growth of plant because phosphorus uptake is directly proportional to nitrogen availability (Reddy et al., 1990). Reddy and Tuker (1983) found N:P ratio of 2.3 to 5 in a water medium yielded optimum growth of water hyacinth biomass. Confirmatory tests for the nutrient dosage were carried out by addition of 17:17:17 NPK fertilizer at 0.6 g/L below and above the 3.2 g/L dosage recommended by Reddy et al. (1990). The plants were left to grow for a period of 15 days. The dosage yielding fastest growth was adopted for this study. The dosage of 3.2 g/L NPK fertilizer yielded the best overall growth of 375% for the roots and 13.8% for the shoots (Table 2.1). The dosage also resulted in healthier foliage.

Table 2.1 Nutrient Requirement for Water Hyacinth

Sample	NPK Dose (g/L)	Roots			Shoot		
		Initial Height (mm)	Final Height (mm)	Percent Growth (%)	Initial Length (mm)	Final Length (mm)	Percent Growth (%)
1	2.8	2	57	275	155	172	11.0
2	3.2	2	78	375	159	181	13.8
3	3.8	2	44	220	157	178	13.4

2.4 Preparation of Model Leachate Solution

PCB contaminated solution was prepared PCB congener, Penta CB, commonly referred to as CB101, was used. 20 ml of 2, 2', 4, 5, 5'-Pentachlorobiphenyl (PCB No.101 solution, analytical standard solution for environmental analysis, 10 ng/µL in isooctane) procured from Kobian Scientific. The chemical remained under custody of the supplier until the experimental set up was ready for its use. PCB solution with concentrations of 5, 10, and 15 µg/L, which were one to two orders of magnitude greater than the US EPA guideline limit of 0.5 µg/L for PCB (EPA, 2007), were prepared and placed in containers 1, 2 and 3, respectively. PCB disperses poorly in water and, therefore, requires the aid of a surfactant to improve solubility. Methanol was used as the surfactant in this study. The three PCB contaminated solutions were prepared by spiking 5 g of soil powder with computed volumes of PCB and analytical grade methanol. The matrix was thoroughly mixed in a Pyrex glass tube by shaking for three minutes and brought to two liters with tap water (e.g. Chu et al., 2003).

2.5 Experiment Set-Up

2.5.1 Tolerance to PCB Tests

The tolerance of water hyacinth to PCB was evaluated by comparing the extent of growth in a polluted environment to that of non-polluted free - nutrient enriched water. Three similar plants were grown in 5, 10, and 15 □ g/L concentrations of PCB 101 and their growth evaluated by measuring the length of a single marked shoot (petiole) and the fiber roots in every container and comparing it to that of control (PCB free environment). Measurements of the lengths of roots and shoot were taken every three days over a period of twelve days. Additionally, plants were observed for occurrence of dented growth, wilt and rust coloration. During the exposure period, the plants growth, health and vigor were noted. On the fifteenth day and after sampling for PCB tests, the plants were lifted off the solution, rinsed in distilled water and allowed to drip for five minutes (Yongchul et al., 1999). They were then weighed using Denver XL-+810 electronic balance. The difference in weight was used to establish the overall growth by change in mass. Growth parameters of the plants were used to determine the tolerance of the plants in different concentrations of PCB. The stems and rinsed roots were stored for bioaccumulation tests.

2.5.2 Phytoremediation Tests

A set of three, two liter containers, were thoroughly washed in tap water and rinsed with distilled water. The containers were filled with 5, 10 and 15 µg/L, PCB leachate solutions prepared previously and enriched with essential nutrients by adding NPK 17.17.17 at 3.2 g/L for vibrant growth of the hyacinth. Selected young and healthy water hyacinth plants were weighed on LIBROR AEG-220 electronic balance to obtain their initial weight before exposure to the PCB matrix solution. The lengths of shoot and roots were then measured and the plants placed in the PCB matrix solutions in a protected area, open to the natural environment and monitored. The test plants were left to grow in the solution for a retention period of 15days, which is close to the 15 days recommended for wetlands (Leir et al., 1996). On the twelfth day, 10 ml of the matrix solution was sampled and stored at 4^{o}C for extraction and PCBs analysis. Analyses for PCB were carried out at the Chemistry Department of Jomo Kenyatta University of

Agriculture and Technology (JKUAT) using Konik 400B GC/MS instrument equipped with a capillary column and automatic injector equipment.

2.5.3 PCB Bioaccumulation Tests

The plants removed from the solution at the end of the twelfth day were prepared for bioaccumulation tests following USEPA SW-846 Method 3540C. The plant materials were cut into smaller pieces of approximately 15 mm sizes and oven dried for 48 hours at room temperature to control possible volatility. To ensure uniform distribution of the bioaccumulated pollutants in the various samples, the dry materials were ground into powder using a mortar and pestle. About 5 g of the ground samples were weighed and extracted with methylene chloride-acetone (1:1) using automated soxhlet extractor. The extract was then subjected to sulfuric acid for clean up. Subsequently, two millimeters of the aliquot of the extract was injected into a GC/MS (Model) for PCB analysis (Alma et al., 2010).

2.6 PCB Extraction Process

Water samples were extracted and analyzed using EPA Method 608 while plant material were extracted and analyzed using USEPA SW-846 Method 3540C. Solid samples were weighed before extraction using a bath type soxhlet extractor unit No.3456A from IKEDA Scientific Company Limited. The extract was then filtered overnight in a filtration column comprised of filter paper; anhydrous sodium sulphate and activated charcoal to remove color. The extract was then concentrated to smaller volumes of about 1 mL in a rotary vacuum evaporator - RE100 and finally stored for analysis. Extraction of liquid samples was carried out by measuring 500 mL of the sample in a graduated measuring cylinder and transferring to a reparatory funnel for liquid-liquid extraction using a 1:1 ratio hexane: acetone mixture and organic layer separated from water layer. The organic layer was then transferred to a filtration column and left overnight for color removed. After extraction, the liquid extracts were stored at 4oC until analysis.

2.7 Mass Spectrometer Calibration

Three concentrations of calibration standards were prepared by adding volumes of stock standard 10 ng/uL PentaCB 101 (2, 2', 4, 5, 5'-Pentachlorobiphenyl) in isooctane to a volumetric flask and diluting to volume with isooctane. One of the external standards was prepared at a concentration above the minimum detection limit (MDL) and the other concentrations corresponded to the expected range of concentrations in the test samples in order to define the working range of the detector. Using injections of 2-5 µL, each calibration standard was analyzed according to Section 12 of EPA Method 608, and peak height and area responses against the mass injected read from the chromatograms (Figures 2.1 and 2.2) and tabulated in standard curve data (Table 2.2). This information formed the calibration for measurements of compounds present in the analytes of PCB spiked and plant waste matrix material. The results were interpreted by making reference to the national institute of standards and technology (NIST) PCB library chromatograms (Fig 2.3).

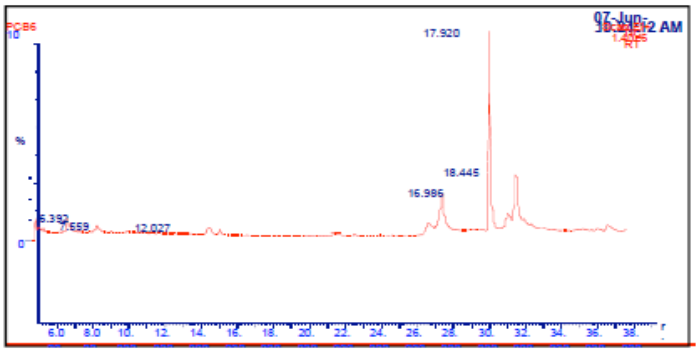

Figure 2.1 Chromatogram Standard - PCB Eluting at Retention Time 17.98 min

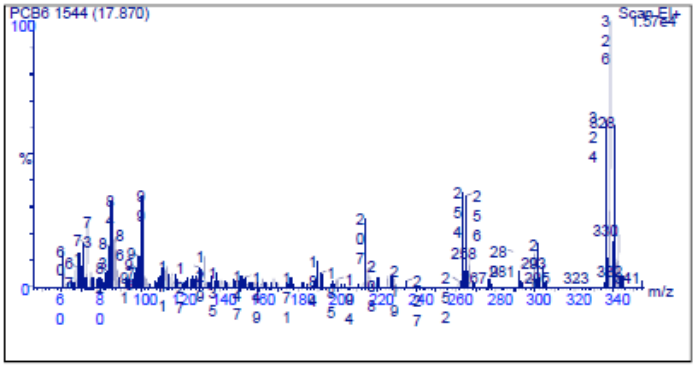

Figure 2.2 Mass spectrum of the PCB

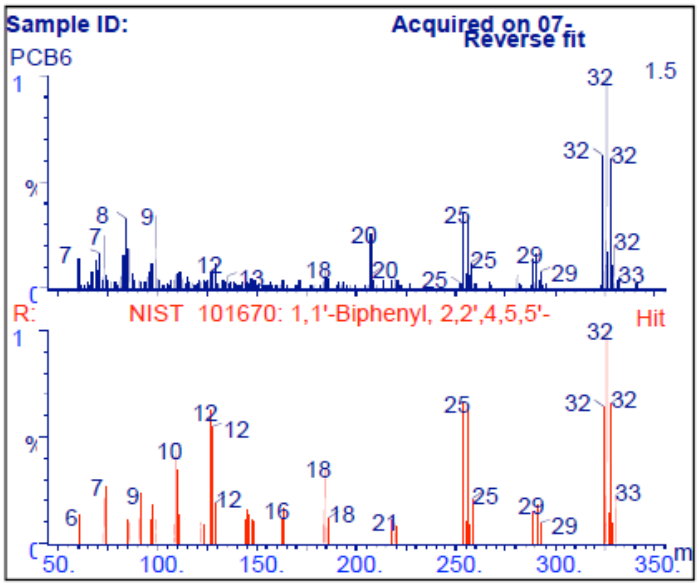

Figure 2.3 Library confirmation of the PCB

Table 2.2 Standard calibration curve Data

Conc. (µg/L)	0	0.5	1	2	3	4
Peak Area (mm^2)	0	110684	206987	443011	701985	893222

The standard calibration data were used to prepare a standard calibration curve for the PCBs shown in Figure 2.4 (Wager et al., 2011). Six number peaks areas corresponding to injection of pure PCB substance were recorded from the GC chromatograms generated by varying the quantity in µL, injected and the attenuation setting for each chromatogram peaks. A calibration line for the substance (Figure 2.4) was plotted using Vernier's Graphical Analysis program. A linear regression was performed on the line to determine the slope and y-intercept of the line. The concentrations for the unknown mixture were determined by reading the area under the chromatogram peak and the corresponding concentrations.

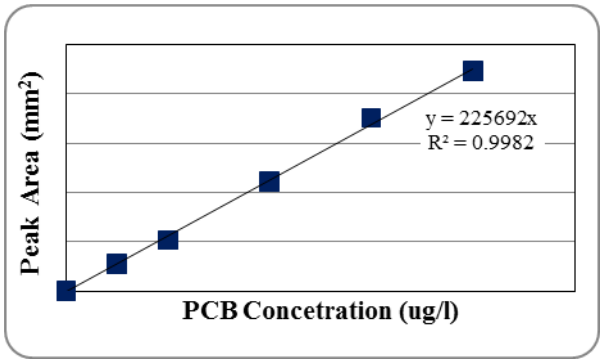

Figure 2.4 PCB Standard Curve.

2.8 Measurement of PCB Levels

PCB levels in the extracted samples were initially analyzed using Konik 400B GC/MS at the Kenya Coffee Research Foundation in Ruiru but the detection level of the equipment was found to be in adequate. Consequently, analyses were carried out using Finnigan GC 8000 series with Voyager EI-MS Detector, CE Instrument at JKUAT which resulted in more distinct peaks.

2.8.1 PCB Chromatogram Analysis

The reference standard of PCB 101 of 0.01 g/L concentration was injected into the Finnigan GC 8000 series with Voyager EI-MS Detector, CE Instrument and the peak area units read. Samples were concentrated to dryness and later reconstituted with 250 mL of HPLC grade of hexane before injection to the GC. An aliquot of the extract was injected into the gas chromatograph (GC) where the analytes were separated by the GC and detected by a mass spectrometer (MS). Since the sensitivity of the MS was low, a double injection was performed by increased volume from one micro liter to two micro liters.

2.8.2 PCB Quantification from Chromatograms

Quantification was based on peak areas from mass chromatograms. To convert the peak areas to mass of analyte, the peak areas were calibrated. The two main strategies were available based on external and internal standards. With external standards, the area of one or more mass chromatogram is calibrated with a known amount of the analyte injected into the GC-MS in a different experiment. Detection limits of a few nanograms can be achieved with this technique. In this study, internal standards which give most accurate quantitative results were used. Ten millimeters of compounds were added to the sample before isolation of the analytes began. After sample extraction and cleanup, only the ratio of response between the analyte and the internal standard was measured. This ratio multiplied by the amount of the internal standard gave the amount of the analyte injected into the GC-MS system. The amount was converted to concentration using appropriate dilution factors.

3.0 RESULTS AND DISCUSIONS

3.1 Water Hyacinth Tolerance to PCB

The tolerance of water hyacinth to PCB in water was evaluated by comparing the growth of roots and shoots in unpolluted and polluted environments. For both environments, the plants were grown in water enriched with 3.2 g/L of 17:17:17 N:P:K fertilizer to provide nutrients for stimulating growth. The results are presented in the following sections.

3.1.1 Plant Growth by Length

The growth of shoots and roots are presented in Figures 3.1 and 3.2, for PCB free environment and Figures 3.3 and 3.4, for three concentrations of PCB spiked water.

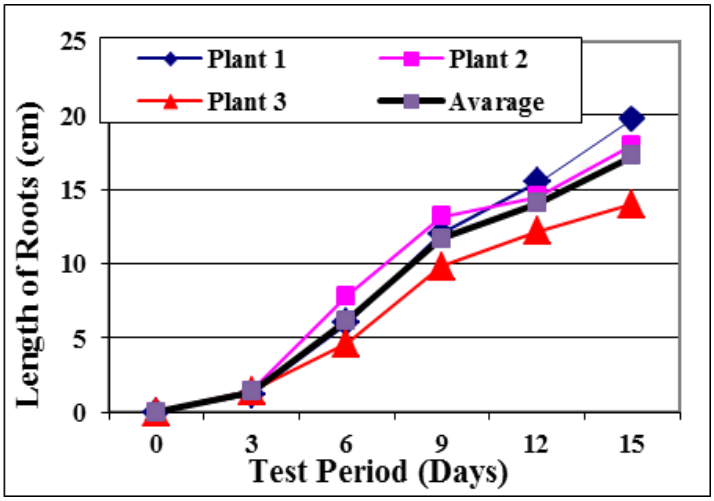

Fig 3.1 Growth of Roots in PCB Free Water

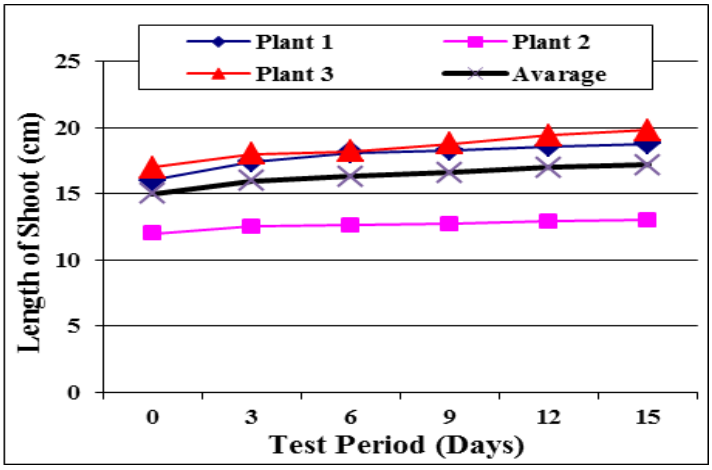

Fig 3.2 Growth of Shoots in PCB Free Environment

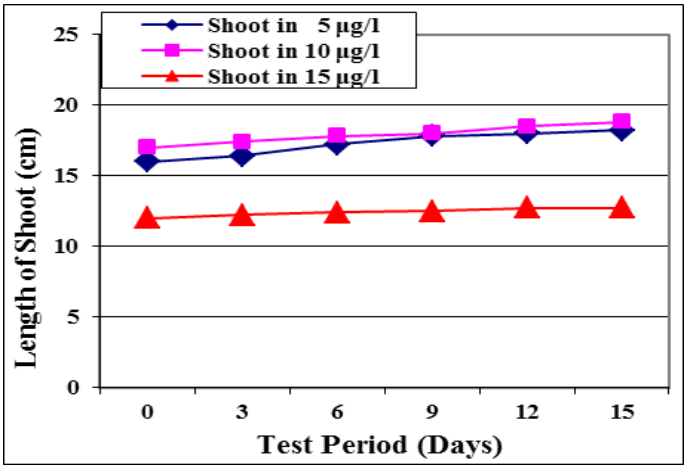

Fig 3.4 Growth of Shoots in PCB Spiked Water

Both the roots and shoots showed significant growth the roots being particularly vibrant. The roots grew gradually for the first three days and at an increased rate for the next twelve days achieving 12 to 16 mm lengths, an increase of 12 to 18 times. The shoots grew uniformly elongating by 1.0 to 2.8 mm or 10 to 25% growth over 15 days. The growth of roots and shoots in nutrient enriched water spiked with 5, 10 and 15 µg/L PCB was observed by measuring the roots and shoots after every three days over a period of fifteen days. The roots grew from almost nil to between 12 and 14 cm; they grew gradually for the first three days and rapidly for the remaining twelve days. The shoots showed nearly uniform growth of between 0.8 and 2.2 cm or 9 to 12%.The growth of the plants in both PCB free water and in PCB spiked was similar in pattern and percentage increment. Consequently, it was concluded that PCB concentrations in the 5 to 15 µg/L range did not have a significant effect on the growth of the plants.

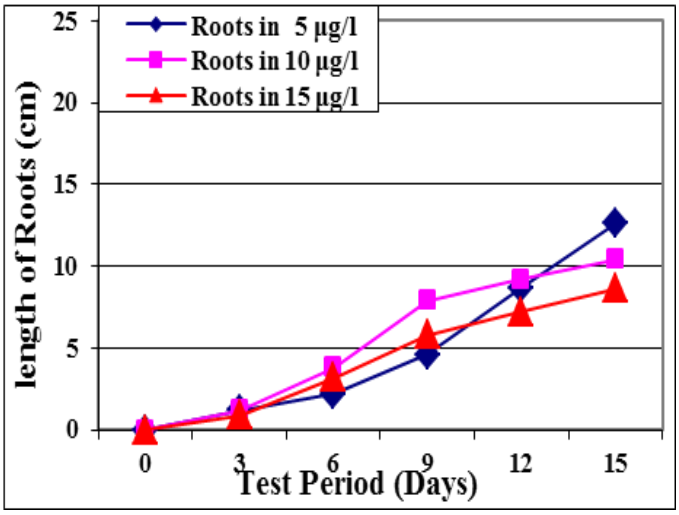

Fig 3.3 Growth of Roots in PCB Spiked Water

3.1.2. Growth by Weight

The change in weight of water hyacinth plants was measured by weighing plants before and after growing them in nutrient enriched water spiked with 5, 10 and 15 µg/L concentrations of PCB (Table 3.1).The plants achieved 11.2, 10.2 and 9.10 % increase in weight for the 5, 10 and 15 µg/L PCB concentrations, respectively. The percentage weight gain appeared to decrease with increase in PCB; however, the small range of weight increase, difference in initial weights and use of only three samples made the results inconclusive.

Table 3.1 Growth by Weight for Plants in PCB Spiked Water

Initial PCB concentration (µg/L)	Initial Plant Weight (g)	Final Weight (g)	Growth by Weight (g)	Percent Growth by Weight (%)
5	68.15	75.8	7.65	11.2
10	83.32	91.8	7.48	10.2
15	97.36	106.2	8.84	9.10

3.2 Observations of Plant Growth in PCB Spiked Water

The growth of water hyacinth in PCB spiked water was monitored visually. In the first three days, the roots changed color from white to purple (Plate 1) with those in 15 µg/L PCB solution showing more intensity than those for 5 and 10 µg/L. Additionally, older peripheral leaves in 15 µg/L PCB spiked water showed signs of wilting. However, the effect did not seem to extend to other leaves as they remained vibrant throughout the fifteen days test period. These results indicate occurrence of initial stress in the plants.

3.3 Phytoremediation of PCB

The results of phytoremediation of PCB by water hyacinth are summarized in Table 3.5. The concentrations of PCB after 12 days for three different initial concentrations are derived from the results of PCB analysis presented in chromatograms in Figure 3.5. Water hyacinth reduced PCB concentrations from 5 and 10 µg/L to below detection limit of 0.142µg/L and from concentrations of 15 µg/L to 0.42 µg/L.

The GC-MS was used to measure the concentration of the analytes in their complex mixtures. The chromatograms for products derived from the extracted PCB spiked water (Figures 3.5 and 3.6) depict peaks obtained for two samples from the 15 µg/L PCB concentration test. The peaks at 17.903 minutes (Figure 3.5) and 17.92 minutes (Figure 3.6) were for PCB101 initially spiked in water; the identities of the other peaks were not clear.

3.4 Bioaccumulation

PCB concentration in the roots and shoot extracts for 5 and 10 µg/L initial concentrations were below detection limit. Figure 3.7 shows the GC-MS chromatogram for products derived from water hyacinth roots for the 15 µg/L initial concentration solution. The peak at 17.922 minutes was for the PCB101 spiked in water. The identities of the other peaks were not clear. A summary of PCB concentration in the extracts for the roots is presented in Table 3.6. PCB concentration in the extract averaged 0.54 µg/L translating to 0.179 µg/g of roots. The results indicate a detectible bioaccumulation when the plants were exposed to higher concentrations of PCB of 15 µg/L. Detection of PCB in the roots only may be associated with the more vibrant growth of roots as compared to shoots. However, it may also indicate slow translocation to the shoots, biodegradation or phytovolatilatization of the PCB; however, these mechanisms were not investigated in this study.

(a)

(b)

Plate 1: Root color for (a) PCB free water and (b) 15µg/L PCB spiked water

Table 3.5 Results of Phytoremediation Tests

Initial concentration (µg/L)	Sample No.	PCB Solution
5	1	BDL
	2	BDL
	Average	BDL
10	1	BDL
	2	BDL
	Average	BDL
15	1	0.44
	2	0.40
	Average	0.42

* BDL – Below Detection Limit

Table 3.6 Bioaccumulation Test Results

Initial concentration (µg/L)	Sample No.	PCB Concentration	
		Leaves*	Roots*
5	1	BDL	BDL
	2	BDL	BDL
	Average	BDL	BDL
10	1	BDL	BDL
	2	BDL	BDL
	Average	BDL	BDL
15	1	BDL	0.53
	2	BDL	0.55
	Average	BDL	0.54

* BDL- Below Detection Limit

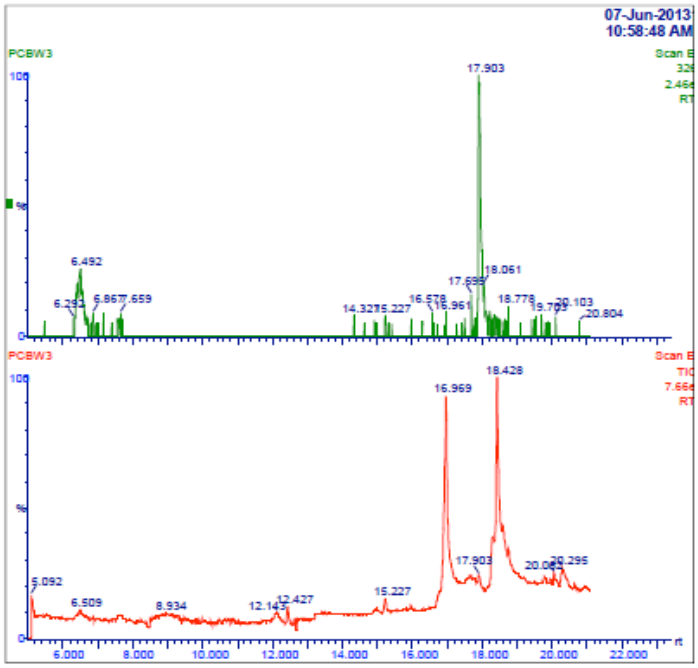

Fig. 3.5 PCB Spiked Water Sample I Chromatogram (15µg/L)

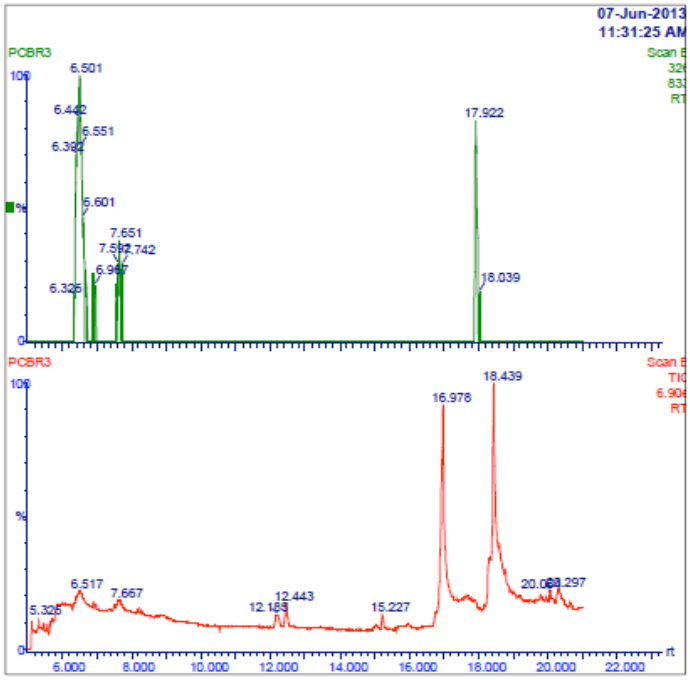

Figure 3.6 PCB Spiked Water Sample II Chromatogram (15µg/L)

4.5 General Discussion

The results of this study demonstrated the ability of water hyacinth to tolerate PCB concentration below 15 µg/L consistent with the finding of others (e.g. Cunningham et al., 1995). Presence of PCB appeared to affect root color and cause initial wilting of the shoots. The effects reveal stress in the plants and suggests a defense mechanism of the plants to pollution. Additionally, there was notable adverse effect on the plants health within the first three days of acclimatization but were later overcome by the plant as vibrant fiber roots developed. Water hyacinth was shown to phytoremediate PCB concentrations in the range 0 to 15 µg/L. There is need to investigate the ability of water hyacinth to phytoremediate higher PCB concentrations. The plants under 15 ug/L PCB, bio accumulated PCB's within its tissues as from below detection limit to an average of 0.179 µg/g in the roots of the plants. While this result signifies the ability of the plant to bioaccumulate, it does not establish whether the plants also biodegraded the PCB's or whether phytovolatilatization of the PCB's might have also played a role.

4.0. CONCLUSIONS AND RECOMMENDATIONS

This study investigated the ability of water hyacinth to tolerate, phytoremediate and bioaccumulate PCB. The study concluded that water hyacinth can tolerate a range of PCB concentrations of one to two orders of magnitude above the EPA limit of 0.5 µg/L. The hyacinth can phytoremediate PCB in a simulated PCB contaminated solution from an initial concentration of 15 µg/L to 0.42 µg/L and from 10 and 5 µg/L to below the detection limit of 0.142 µg/L. Bioaccumulation of up to 0.179 µg/g PCB in roots was established as one of the phytoremediation mechanisms. Consequently, the study found that Therefore, water hyacinth phytoremediation of PCB contamination water has potential for inexpensive and environmental friendly managemet of PCB contaminated waters such as landfill leachates.

The study recommended:

Evaluation of the tolerance of water hyacinth plants to PCB concentrations above 15 µg/L.

Evaluation of water hyacinth phytoremediation of PCB concentrations greater than 15 µg/L.

Characterization of leachates from landfills such as dandora in Nairobi Kenya to establish potential for release of POP from disposed e-waste.

Establishment of water hyacinth remediation of PCB in actual landfill leachate.

Investigation of biodegradation and phytovolatilization as mechanisms for phytoremediation PCB contaimation.

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