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The endangered African Great Ape: Pesticide residues in soil and plants consumed by Mountain Gorillas (Gorilla beringei) in Bwindi Impenetrable National Park, East Africa

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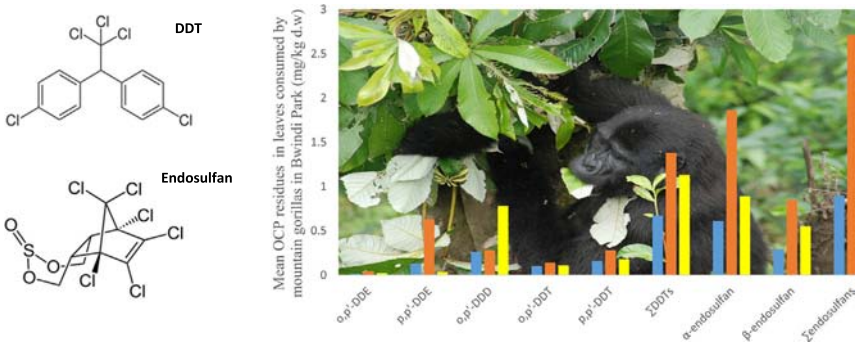
HIGHLIGHTS

- Pesticides in and around Bwindi Park have been investigated for the first time.
- Interviews revealed glyphosate as the most widely used pesticide near the park.
- GC analyses of plant leaves showed exposure of the apes to trace levels of OCPs.
- Hazard indices suggest possible health risks to the mountain gorillas.

GRAPHICAL ABSTRACT



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ABSTRACT

Bwindi Impenetrable National Park situated southwest of Uganda is a biodiversity hotspot that is home to about half of the world's endangered mountain gorilla (*Gorilla beringei*). Given its ecological significance and mounting pressures from agricultural activities such as tea growing, continuous monitoring of the levels of chemical toxins like pesticides in the park and surrounding areas is needed for effective conservation strategies. Furthermore, persistent organochlorine pesticides (OCPs) like DDT were used in agricultural gardens and indoor spraying in Kanungu district between the 1950s and 80s. The focus of this study was to explore the possible exposure of mountain gorillas to OCPs and cypermethrin used by the farmers in the areas near the park. Data from our interviews revealed that glyphosate is the most widely used pesticide by the farmers in areas surrounding the park, followed by cypermethrin, and mancozeb. Samples of leaves from plants consumed by mountain gorillas along the forest edges of the park and surficial soils (15–20 cm depths) were collected from three sites (Ruhija, Nkuringo and Buhoma) and analysed for the presence of cypermethrin and OCPs residues. Concentrations of total (∑) DDTs and ∑endosulfans were up to 0.34 and 9.89 mg/kg dry weight (d.w), respectively in soil samples. Concentrations of ∑DDTs and ∑endosulfans in samples of leaves ranged from 0.67 to 1.38 mg/kg d.w (mean = 1.07 mg/kg d.w) and 0.9 to 2.71 mg/kg d.w (mean = 1.68 mg/kg d.w), respectively. Mean concentration of ∑DDTs in leaves exceeded the European pharmacopeia and United States pharmacopeia recommended

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maximum residue limit values for DDTs in medicinal plants (1.0 mg/kg). In addition, calculated hazard indices for silverbacks (36.35), females (57.54) and juveniles (77.04) suggested potential health risks to the mountain gorillas. *o,p′*-DDT/*p,p′*-DDT ratios (0.5–0.63) in samples of leaves confirmed recent input of dicofol-DDT type in Bwindi rainforest.

1. Introduction

Pesticides are a wide spectrum of synthetic compounds with biocide activity used to remove weeds, fungi and insects to increase agricultural productivity (de Souza Pinheiro et al., 2011). Approximately 2 million tonnes of pesticides are used annually worldwide to increase crop productivity, out of which, 47.5% are herbicides, 29.5% are insecticides, 17.5% are fungicides, and others accounting for the remaining 5.5% (Sharma et al., 2019). In public health programmes, pesticides have been extensively used to control disease vectors notably the worldwide use of dichloro-diphenyl-trichloroethane (DDT) in malaria vector control (van den Berg et al., 2020; Van Den Berg et al., 2017; WHO, 2011). Unfortunately, some of these pesticides can adversely affect non-target species by inducing carcinogenic, teratogenic, mutagenic, neuro-toxic effects, as well as alterations of the reproductive processes in experimental animals and man (Akan et al., 2014). For instance, between 1986 and 1998, pesticide-linked poisoning accounted for 6.5% of the identified causes of death in wildlife in Europe (as cited in Krief et al., 2017), while excess levels of Dichloro-diphenyl-dichloroethylene (DDE), DDT, chlorpyrifos and imidacloprid in the vicinity of Kibale National Park have been associated with facial dysplasia in chimpanzees and baboons (Krief et al., 2017).

Organochlorine pesticides (OCPs) (such as DDT, endosulfan, lindane and dieldrin) have a longer history of application in Uganda compared to other alternative pesticides (Kasozi et al., 2006; Ssebugere et al., 2010). These OCPs are characterised by their high chemical stability, poor water solubility and low vapour pressures, which makes them persistent in different environmental matrices/media (Arinaitwe et al., 2016; Ssebugere et al., 2010; Wasswa et al., 2011). The persistent nature of these pollutants raises very serious environmental concerns as they are known to pose problems of chronic toxicity to both animals and humans which are exposed to them via air, water and food intake (Olisah et al., 2020; Taiwo, 2019). For instance, in animal studies, reproductive risks associated with the exposure to high levels of DDTs and endosulfans such as implantation failures (Al-Hussaini et al., 2018; Milesi et al., 2015), spontaneous abortion (Rupam et al., 2018) and disruption of the sex steroid hormone synthesis (Ozmen and Mor, 2012; Yan et al., 2019) leading to infertility and hence species extinction in the long run have already been documented.

Another class of synthetic organic insecticides is *pyrethroids*. These are derived from pyrethrin and have been widely used in households and agriculture across different parts of the world since the 1980s because of their low toxicity compared to other insecticides, such as organophosphorus and carbamic ester compounds (Tang et al., 2018; Yoo et al., 2016). However, uncontrolled use coupled with diffusion of these pesticides have facilitated their entry in the food chain through contaminated water and soil (Bhattacharjee et al., 2012; Debbab et al., 2014; Tang et al., 2018). By far, cypermethrin, a type II pyrethroid insecticide according to the World Health Organization (WHO) classification (WHO, 2020) is the most widely used insecticide (Jin et al., 2011; Nantia et al., 2017). The harmful effects of cypermethrin in animals including skin destruction (Hameed et al., 2020), decreased female reproduction rate (Zhou et al., 2018), gene alteration in embryonic stem cells (Rebuzzini et al., 2020), immune cell death and DNA damage (F. Huang et al., 2016) among others have already been documented. In Uganda, cypermethrin is cheap and readily available making it the most widely used insecticide by the farmers (Kagezi et al., 2019; Oesterlund et al., 2014; Okonya and Kroschel, 2015).

Bwindi Impenetrable National Park, the focus of the present study represents one of the oldest, most complex and biologically rich systems on earth (Blomley, 2003; Hamilton et al., 2000; Makombo, 2018). The park is situated in the Kigezi region in southwest of Uganda. With an area of 330.8 km^2, Bwindi represents an important epicenter within the Albertine Branch of East Africa's Great Rift Valley. Globally, the park has been listed in the top 20 of the 200 priority areas for biodiversity and ranked as the highest priority for the conservation of restricted-range animals in Africa (Hatfield, 2004; Laudati, 2010). More than 160 species of trees and 100 species of ferns grow in Bwindi in addition to being home to chimpanzees, leopards, forest elephants and half of the world's endangered mountain gorilla (*Gorilla beringei*) (Gray et al., 2013; Makombo, 2018). Putting aside its phylogenetic position as one of the closest living relatives of humans, mountain gorilla ecotourism represents a valuable economic resource for the country. This ecotourism provides over 50% of all revenue earned by the Uganda Wildlife Authority (UWA) enabling resources for conservation activities in other parks as well (UWA, 2018).

Unfortunately, human pressures have for decades surrounded and impacted the Bwindi ecosystem (Hamilton et al., 2000; Hickey et al., 2019) with some parts of the forest and woodlot being replaced by tea plantations and small scale farming (Twongyirwe et al., 2011). The increased use of pesticides (such as OCPs and cypermethrin) in the areas surrounding the Park is likely to increase exposure of these gorillas to pesticides as they feed on the vegetation in farmlands around the edges of the park. It has been noted that a large portion of the mountain gorilla diet is herbaceous vegetation (Rothman et al., 2014; Rothman et al., 2007) and the gorillas often favour to forage around the forest edges where there is secondary vegetation (Korbee, 2007). They also occasionally crop raid, moving through agricultural areas to feed on cultivars as well as the herbaceous vegetation that grows in abandoned farmlands (Seiler and Robbins, 2016). Motivated by the fact that mountain gorillas are endangered, this paper explored the possible exposure of mountain gorillas to the pesticides related to agricultural activities around the park.

The objectives of the present study were to; (i) document the types of pesticides used by the farmers in the vicinity of Bwindi Impenetrable National Park, (ii) investigate the levels of the selected pesticides with more emphasis on OCPs like DDT and endosulfan and Pyrethroid like cypermethrin in soils and leaves of plant species consumed by mountain gorillas along the forest edges of the park and (iii) assess the health risks associated with the consumption of leaves by mountain gorillas.

2. Materials and methods

2.1. Study area

The area of study was Bwindi Impenetrable National Park (Fig. 1), which is situated south-west of Uganda, bordering the Democratic Republic of Congo (0°53′ to 1°8′ South; 29°35′ to 29°53′ East). The altitudinal range of the park is 1160–2607 m with characteristic topography which is extremely rugged and composed of many steep-sided hills and narrow valleys (Babaasa et al., 2004). The climate is tropical with two rainfall peaks from March–May and September–November. The annual precipitation lies in the range of 1130–2390 mm with minimum and maximum annual mean temperatures in the ranges of 7–15 °C and to 20–28 °C, respectively. Together with some remnant lowland forest outside the boundary, the park is an important water catchment area

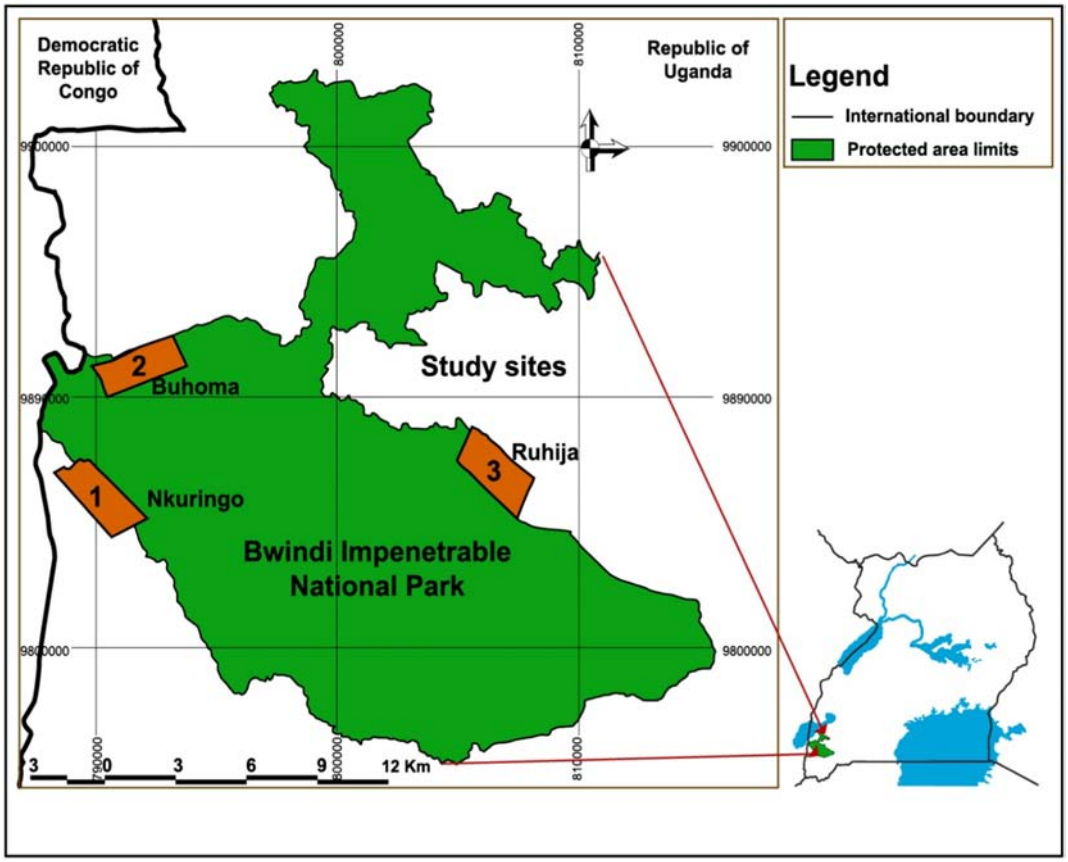

Fig. 1. Map of Bwindi Impenetrable National Park showing the three sites where samples of soil and leaves were collected.

serving surrounding agricultural lands. The soils are mainly humic red loams, moderately to highly acidic and deficient in bases (Nkurunungi et al., 2004). The park covers an area of 330.8 km^2 on the edge of the western arm of the Great East African Rift Valley and is a habitat to mountain gorillas.

The study area was stratified into three strata (Nkuringo, Buhoma and Ruhija). Nkuringo lies along the southern boundary of the park while Buhoma and Ruhija are located in the northern and eastern parts of the park, respectively. The three areas were selected because they are located in the vicinity of the park and majority of the people in these areas are involved in both subsistence and commercial agriculture. The use of pesticides in and around the park continues to pose a threat to the life of these primates (Rothman and Bryer, 2019).

2.2. Interviews-led questionnaires with the farmers near the park

To determine the types of pesticides commonly used by the farmers in the vicinity of the park, we randomly recruited 90 farmers aged between 25 and 75 years (30 from each site) who volunteered to participate in our interviews. A structured questionnaire containing open and close-ended questions was adapted for this study as described by Jallow et al. (2017) but with slight modifications. All the questions were translated from English into the local language (Lukiga and Lufumbira) during the interview. The questionnaire contained two sections; the first section collected information about the farmers' demographic characteristics such as age, gender, place of residence, education level, and type of agricultural field they had while the second section consisted of questions related to pesticide use such as identity of the commonly used pesticides, perceptions and practices on the use of pesticides, distance of the farm from the park, number of years of pesticide usage among others. All the data collection was carried between June and July 2016. The results from the questionnaires were analysed in terms of the different types of pesticides commonly used by farmers.

2.3. Sample collection and storage

Soil samples were collected following the method described by Moges et al. (2013). Two main land use types were considered (protected forest and farmlands). Soil samples were taken at a depth of <15 cm using an auger. Sample plots had dimensions of 20 m × 20 m with an "X" design and the samples were taken from the four corners and center of each sample plot. Approximately a 50 g sample was taken. A total of 60 soil samples (2 land use types × 2 replicates of sample plots in each land use × 5 sampling points × 3 sides (Nkuringo, Ruhija and Buhoma) were randomly collected for soil analysis.

Leaf samples were taken from the sample plots of 10 m × 10 m dimension as described by Rissell et al. (1990), with each site having 12 plots all inside the forest and separated by a 1000 m distance. The sampling was done along the "X" design and samples of the three most preferred gorilla leaf plants that were near the line were plugged using a clean pair of scissors. Choices of plant foods were randomly selected from the list generated by Ganas et al. (2008), from which three preferred leaves; *Basella alba*, *Laportea aestuans* and *Triumfetta tomentosa* were selected for the study. A total of 36 leaf samples were collected. All the samples were wrapped in aluminum foil and placed in labeled airtight bags, kept in ice-coolers at approximately −4 °C and transported to the Pesticide laboratory at Makerere University where they were kept in a freezer at −18 °C to avoid microbial degradation before extraction and analysis. The water content of the leaves was determined by constant weight drying in an oven at 60 °C and their levels were reported on a dry weight basis.

2.4. Pesticide reference standards, solvents, reagents and glassware

Eight pesticide reference standards (α-endosulfan, β-endosulfan, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDD, *p,p′*-DDE, *o,p′*-DDE and cypermethrin) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All the standards were above 99% purity. The analytical stock solutions of each pesticide were prepared using *n*-hexane and stored in amber flasks maintained at −18 °C. Dichloromethane (DCM), acetone, cyclohexane and *n*-hexane used were all of pesticide residue grade. Florisil (PR grade 60–100 mesh), ammonium chloride and anhydrous sodium sulfate used were of analytical grade (BDH, England). All the glasswares were cleaned by first soaking them for 2 h in tap water mixed with a detergent and then rinsed with hot water followed by acetone. The glasswares were then dried in an oven for 4 h at 105 °C.

2.5. Analytical procedure

2.5.1. Extraction and clean-up of soil samples

The soil was extracted following the method described by Åkerblom (1995). Soil samples were sieved to remove the gravel and then thoroughly homogenized. Two portions of the homogenized samples (20 g each) were weighed. The first portion was placed in a pre-weighed petri-dish and dried overnight in an oven at 105 °C. After drying, the petri-dish and its contents were weighed to obtain the moisture content. The second portion was transferred to a clean 150 mL conical flask, and 14 mL of ammonium chloride solution (0.2 M) was added to it. The flask was then swirled and left to stand for 15 min. 100 mL of a mixture of acetone/cyclohexane (1:1 v/v) was added to the flask, which was tightly stoppered thereafter. This mixture was shaken vigorously for 1 min, then less vigorously (for every 10 min) for 1 h. The flask and its contents were left to stand over-night and then shaken intermittently for another 2 h and the contents were then left to settle. Water was cautiously added to the mixture until the organic phase filled the neck of the stoppered flask. The organic phase was transferred into an Erlenmeyer flask (E-flask) and then dried using anhydrous sodium sulfate. The resultant extract was concentrated to 1 mL on a rotary evaporator at 30 °C and dissolved in cyclohexane (2 mL) for clean-up.

Clean-up of the soil extracts was done using the method described by Ssebugere et al. (2010). Briefly, a glass column (1 cm i.d. × 15 cm) was plugged with glass wool previously washed with cyclohexane. The column was then packed with florisil (2 g) followed by anhydrous sodium sulfate (10 g). The column was conditioned with 2 mL of an acetone/*n*-hexane mixture (1:9 v/v) and then *n*-hexane (5 mL). The concentrated soil extract was then added and eluted with a mixture of acetone/*n*-hexane (10 mL) followed by *n*-hexane (10 mL). The resulting eluate was then concentrated to 2 mL on a rotary evaporator and to near-dryness using a stream of nitrogen gas, and reconstituted in *n*-hexane for gas chromatographic analysis.

2.5.2. Extraction and clean-up of leaf samples

The leaf samples were extracted following the method described by Alam (2013). Briefly, the leaves were ground to a free-floating powder using a mortar and pestle. The sample (50 g) was mixed with *n*-hexane (135 mL) followed by DCM (15 mL). The mixture was shaken for 16 h (using a shaker) and left to stand for 15 min. The combined *n*-hexane and DCM extract was treated with anhydrous sodium sulfate (10 g) to remove traces of water and then passed through the glass wool conditioned with *n*-hexane. The collected extract was then concentrated under reduced pressure using a rotary vacuum evaporator at 30 °C and redissolved in *n*-hexane (3 mL) for clean-up.

The clean-up glass column was plugged with glass wool previously washed with *n*-hexane. It was then packed with florisil (2 g) followed by anhydrous sodium sulfate (10 g). The column was then conditioned with *n*-hexane. The concentrated leaf extract was added to the column and finally eluted with 100 mL of a mixture of DCM/*n*-hexane (1:9 v/v). The resulting eluate was further concentrated to 2 mL on a rotary evaporator and to near-dryness using a stream of nitrogen gas, and then reconstituted in *n*-hexane for gas chromatographic analysis.

2.5.3. Gas chromatographic analysis

A Varian (CP-3800, Palo Alto, CA, USA) gas chromatograph (GC) coupled to an electron capture detector (ECD) was used for analysis. The GC was equipped with both a semi-polar (CP-Sil 19 CB, J & W Scientific, Folsom, CA, USA) and a non-polar (CP-Sil 5 CB, J &W Scientific, Folsom, CA, USA) fused-silica capillary columns (each 30 m × 0.25 mm i.d. × 0.25 μm film thickness). The column temperature was initially set at 90 °C for 1 min and then raised to 180 °C at a rate of 30 °C min^{-1}. It was then further increased at a rate of 4 °C min^{-1} to 260 °C and held at this temperature for 16 min. Hydrogen (99.99% purity) was used as the carrier gas with a flow rate of 1.2 mL min^{-1}. The injector and detector temperatures were kept at 230 °C and 300 °C, respectively, together with a 30 mL min^{-1} make-up nitrogen gas flow. A Turbochrom (Perkin–Elmer Corporation, 1989–1995, Norwak, CT, USA) 4.0 chromatography station was used for chromatographic data processing. The GC was operated in a splitless mode with the injection volume of 1 μL per injection. Reference standards of individual pesticides were used to identify and quantify the analytes.

The results were later confirmed using an Agilent 6890N GC coupled to a mass spectrometer (MS) (Agilent 5975 inert XL Quadrupole, Palo Alto, CA, USA). The GC was equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The GC oven temperature was initially set at 90 °C, held for 1 min and then increased to 180 °C at a rate of 30 °C min^{-1}. It was further raised to 260 °C at a rate of 4 °C min^{-1} and then maintained at that temperature for 10 min. The injector and detector temperatures were both kept at 250 °C. Helium (99.999% purity) was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The GC–MS was operated in a splitless mode with a purge-off of 1 min and injection volume of 1 μL per injection. The MS solvent delay time was 3.57 min and the scanned mass range was 50–550 *m/z*. The full scan ion-monitoring mode was used and the analytes were identified using the internal standards method. GC–MSD Chemstation Software (G1701dad.02.0sp1, JAS CWA, USA) was used for data acquisition and processing.

2.6. Quality assurance/quality control

The Limits of detection (LODs) were calculated by a signal to noise ratio of three. The LODs for *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *o,p′*-DDD, cypermethrin, *α*- and *β*-endosulfan ranged from 0.01 to 0.3 mg/kg. Samples were considered positive when their residue levels were above LODs. Analytes below the LOD were taken to be 1/2 the LODs. Recoveries were obtained by spiking blank samples with 0.1 and 0.5 mg/kg of the individual pesticide standards. The recovery assays were replicated three times. Generally, good recoveries (84.1–104%) were obtained for all the target analytes at the 0.5 mg/kg than 0.1 mg/kg spiking level (81.4–100%). Consequently, no adjustments were made on the residue data since the majority of the recoveries were ≥70%.

2.7. Statistical data analysis

Descriptive statistics in form of mean levels and ranges were calculated for each of the analytes. Non-detects were treated as zero. Shapiro-Wilk test was used to confirm the normality of the data. Spearman's rank-order correlation coefficient was used to establish the relationship in the pesticide residue levels between the samples of leaves and soil at $p \leq 0.01$ level of significance. Correlation coefficients were calculated for only positive quantifiable samples. All statistical analyses were done using SPSS 21.0 (Chicago, IL, USA).

2.8. Health risk assessment in mountain gorillas

Given the phylogenetic position of mountain gorillas as one of the closest living relatives of humans, estimated daily intake (EDI) for pesticide residues, characterization of health hazard quotient (HQ) and hazard index (HI) based on humans was adopted for this paper (Bhandari et al., 2019).

2.8.1. Estimated daily intake (EDI) of pesticides residues

EDI was calculated according to the Eq. (1)

$$EDI = ([PS] \times Q)/BW \quad (1)$$

where [PS] is the average concentration of pesticide residues in leaves expressed in mg/kg, Q is the amount of food/plants/leaves consumed by a mountain gorilla, and BW is the body weight of mountain gorilla. The average daily food/plant consumption of 18.8 kg/silverback/day, 14.9 kg/female/day, and 14.9 kg/juvenile/day were considered in this paper while the body weights were considered to be 200 kg, 100 kg and 75 kg for the silverbacks, females and juveniles respectively (Rothman et al., 2008).

2.8.2. Characterization of health hazard quotient (HQ) and hazard index (HI)

Chronic/long-term HQ assessment (cHQ) was calculated based on the EDI, and the acceptable daily intake (ADI) according to the Eq. (2)

$$cHQ = EDI/ADI \quad (2)$$

ADI values expressed in mg/kg bw/day for DDTs (0.01) and endosulfans (0.006) were obtained from the EU Pesticides database (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=1521). ADI value of cypermethrin (0.05 mg/kg bw/day) was taken from the Australian Government Acceptable Daily Intakes for Agricultural and Veterinary Chemicals (2005) (Reviewed in Syed et al., 2014). The HI for the eight pesticides analysed in our study was calculated according to the Eq. (3).

$$HI = cHQ1 + cHQ2 + cHQ3 + \ldots + cHQ8 \quad (3)$$

3. Results and discussion

3.1. Types of pesticides used by the farmers in the vicinity of the park

All the 90 farmers interviewed from the three study sites (demographic characteristics in **Table S1**) revealed the use of five pesticides (glyphosate, cypermethrin, mancozeb, 2,4-D amine and dimethoate). It was noted that glyphosate was the most commonly used pesticide by the farmers (52% in Buhoma, 50% in Ruhija and 44% in Nkuringo) followed by cypermethrin (44% in Nkuringo, 37% in Ruhija and 36% in Buhoma) and mancozeb (16% in Nkuringo, 12% in Buhoma and 8% in Ruhija) while 2,4-D amine and dimethoate were only used in Ruhija (**Fig. S1**). In a related study, Kagezi et al. (2019) reported the use of pesticides by 325 household farmers growing Arabica coffee in Uganda. According to their study, equal proportion of farmers (15.4%) used glyphosate and cypermethrin followed by mancozeb (7.7%). Their study indicated that the highest percentage of pesticides users (40%) was recorded in southern Uganda. This trend was also similar to the cross-sectional study carried out in Palisa and Wakiso districts in Uganda where cypermethrin (used by 28 farmers), mancozeb (used by 17 farmers) and glyphosate (used by 13 farmers) were the commonly used pesticides by small scale farmers in Wakiso district (Oesterlund et al., 2014). Another study by Okonya and Kroschel (2015) on pesticide use and knowledge of smallholder potato farmers in Uganda also confirmed the use of cypermethrin as the preferred insecticide (20.1%) while mancozeb was the frequently used fungicide (71.6%) and glyphosate as the preferred herbicide (5%). Their study indicated that out of 68 farmers interviewed in southwestern highlands, 100% used insecticides and fungicides on their potatoes.

These frequently used pesticides are generally cheap and readily available to the Ugandan farmers (Kagezi et al., 2019). All the farmers interviewed in our study used knapsack sprayers to apply the pesticides in their gardens, which were within the radius of 3 km from the Park. However, only 24% of the farmers interviewed had received specialized training regarding pesticides usage from the agricultural extension officers (**Table S2**). 47% of the farmers interviewed could easily distinguish the different trade names of the pesticides and their uses while the rest did so with many difficulties. This could possibly be attributed to the fact that they relied on other farmers who grow the same crops. The farmers near the Bwindi Park are also persuaded by the pesticides' sellers operating in the nearby trading centers who are profit minded. There was evidence of small quantities of pesticides being kept in unlabeled containers in addition to poor pesticides disposal.

Majority of the farmers (97%) wore gumboots during pesticides application but almost none of them wore facemasks or pair of goggles or gloves and this exposes the farmers to these chemicals either by inhalation or by direct dermal contacts. Nonetheless, mancozeb is classified as a class U (unlikely to present acute hazard in normal use) pesticide, glyphosate is a slightly hazardous (class III) pesticide while cypermethrin, dimethoate and 2,4-D are moderately hazardous (class II) pesticides according to the World Health Organization classification (WHO, 2020).

Overall, our preliminary findings suggested that most wildlife, including mountain gorillas, living in this park were not exposed to the more toxic and persistent OCPs (like DDT) as well as extremely hazardous (class IA) and highly hazardous (class IB) pesticides, since there was no mention of these in the interviews. However, combined effects of the different pesticides, other deposition methods (e.g., atmospheric deposition of OCPs like DDT and endosulfan) and adulteration by sellers cannot be underestimated.

3.2. Levels of cypermethrin in samples of soil and leaves

Tables 1 and 2 show the levels of pesticide residues in samples of soil and leaves respectively. Cypermethrin residues were not detected in any of the samples. This could be attributed to the pesticide formulation and application dosage, its short half-life and low mobility in soils. In the present study, both soils and leaves were sampled about 3–4 weeks after the most recent application of cypermethrin by the farmers and therefore, the pesticide could have degraded to its metabolites. A study by Mantzos et al. (2016) showed that the dissipation of cypermethrin in soils followed first-order kinetics and its half-life depended on the pesticides formulation. The authors reported that the half-life of microgranular (MG) and emulsifiable concentrate (EC) formulations ranged between 22.01 and 24.24 days and the insecticide's residual concentrations reached levels below limit of quantitation (LOQ) 14 days after application with the EC formulation alone. Another study by Gupta et al. (2011) showed that no cypermethrin residues were detected in the soil even on zero (0) day following the application of Roket 44EC (profenofos 40% + cypermethrin 5%) and Action-505EC (chlorpyrifos 50% + cypermethrin 5%) formulations on tomato crop at the recommended dose. At double dose, cypermethrin residues were not detected in soils when Roket 44EC was used. However, on application of Action-505EC, 0.032 μg/g of cypermethrin residues were detected in the soil on day zero, which changed to non-detectable levels on the 7th day.

Another study by Jyot et al. (2013) reported that cypermethrin residues were not detected in soil samples collected 15 days after application of Nurelle-D 505 (chlorpyrifos 50% + cypermethrin 5%) formulation on chili (*Capsicum annuum* L.) at both single and double dosages. A related study by Sulaiman et al. (2020) indicated that

Table 1
Levels (mg/kg d.w) of OCP residues and cypermethrin in soil samples collected from Ruhija, Nkuringo and Buhoma sites.

Pesticide residues	Ruhija (N = 8)			Nkuringo (N = 8)			Buhoma (N = 8)			All samples (N = 24)		
	Mean	Range	n > LOD	Mean	Range	n > LOD	Mean	Range	n > LOD	Mean	Range	n > LOD (%)
o,p′-DDE	0.013	n.d-0.08	2	n.d	–	0	n.d	–	0	0.0044	n.d-0.08	2(8.3)
p,p′-DDE	n.d	–	0	n.d	–	0	n.d	–	0	n.d	–	0(0)
o,p′-DDD	0.028	n.d-0.34	1	n.d	–	0	n.d	–	0	0.0094	n.d-0.34	1(4.2)
o,p′-DDT	n.d	–	0	n.d	–	0	n.d	–	0	n.d	–	0(0)
p,p′-DDT	n.d	–	0	n.d	–	0	n.d	–	0	n.d	–	0(0)
∑DDTs	**0.041**	–		–	–		–	–		–	–	
α-Endosulfan	n.d	–	0	0.18	n.d-2.18	1	0.098	n.d-1.18	1	0.093	n.d-2.18	2(8.3)
β-Endosulfan	n.d	–	0	0.82	n.d-9.89	1	n.d	–	0	0.27	n.d-9.89	1(4.2)
∑endosulfans	–			1	–	–	–			–	–	
Cypermethrin	n.d	–	0	n.d	–	0	n.d	–	0	n.d	–	0(0)
α-/β-endosulfan	–			0.22	–	–				–	–	

N-number of samples; LOD-limit of detection; n.d-non-detectable; n-number of samples with levels above limit of detection; %-percentage.

cypermethrin residues were not detected in all the soil samples at all depths (<50 cm) collected from the Malaysian oil palm plantation at intervals of 0 to 36 days after cypermethrin treatment. Laboratory studies have also indicated that the persistence of cypermethrin in soil strongly relates to the soil microbial activity and with other factors that affect the soil microbial activity (Gu et al., 2008; Gu et al., 2010; Xie and Zhou, 2008).

These studies have shown that the decrease in the half-lives of cypermethrin was due to the increase in soil organic matter (Gu et al., 2008; Jun et al., 2013; Xie and Zhou, 2008) and the increase in soil temperature and moisture (Gu et al., 2010). Microbial metabolism of cypermethrin as a carbon and nitrogen source by several cypermethrin-degrading bacterial strains has extensively been investigated by other studies (Bhatt et al., 2019; Bhatt et al., 2020; Chen et al., 2014; Xiao et al., 2015).

A study by Sulaiman et al. (2020) indicated that cypermethrin is unstable under sunlight and is easily washed off by rain. The authors reported that plant metabolism or photolysis may rapidly breakdown cypermethrin into its potential metabolites including α-cyano-3-phenoxy benzyl alcohol and 3-phenoxybenzaldehyde. No data from our literature review were available pertaining to cypermethrin residues within leaves consumed by mountain gorillas. However, a considerable number of studies have documented cypermethrin residue levels in plants especially on fruits and less on leaves (Gupta et al., 2011; Jyot et al., 2013; Mohapatra, 2014; Mukherjee et al., 2012; Nahar et al., 2012; Priyadarshini et al., 2017; Singh et al., 2015; Uddin et al., 2016). Nevertheless, in all the aforementioned studies, cypermethrin was less persistent and reached non-detectable levels shortly after its application (Table 3).

3.3. Levels of OCPs in samples of soil and leaves

3.3.1. Soil samples

Table 1 presents the concentrations of OCPs in soil samples. DDTs were detected in soils from Ruhija only while endosulfans were detected in soils from Nkuringo and Buhoma only. The concentrations of α-endosulfan and β-endosulfan ranged from non-detectable (n.d) to 2.18 mg/kg dry weight (d.w) and n.d to 9.89 mg/kg d.w with mean of 0.093 and 0.27 mg/kg d.w respectively. The mean concentration of total (∑DDTs) was 0.041 mg/kg d.w. Concentrations of o,p′-DDE and o,p′-DDD ranged from n.d to 0.08 mg/kg d.w (mean = 0.013 mg/kg d.w) and n.d to 0.34 mg/kg d.w (mean = 0.028 mg/kg d.w) respectively. In all the soil samples analysed, only 2.5%, 8.3% and 4.2% of the soil samples showed detectable levels of ∑DDTs, α-endosulfan and β-endosulfan, respectively.

Elsewhere in the world, the concentrations of o,pv-DDE and o,p′-DDD in our study were higher than those reported in the horticultural soils from Switzerland (o,p′-DDE = n.d to 0.01 mg/kg d.w and o,p′-DDD = n.d to 0.17 mg/kg d.w) (Hilber et al., 2008) but lower than those reported in soils from India (o,p′-DDE = n.d to 0.265 mg/kg d.w) (Mishra et al., 2012). In general, the mean concentration of ∑DDTs in our study was higher than that reported in soils from the Corn Belt of northeast China (0.0243 mg/kg) (Wang et al., 2020) and agricultural soils along the southwest coast of India (0.013 mg/kg d.w) (Khuman et al., 2020) but lower than that reported in soils from Southern United States (0.211 mg/kg) (Bidleman and Leone, 2004), Mexicali valley in Mexico (0.047 mg/kg) (Sánchez-Osorio et al., 2017) and Nigeria (7.67 mg/kg) (Egbe et al., 2020). Furthermore, the mean concentrations of α-endosulfan and β-endosulfan in our study were higher

Table 2
Levels (mg/kg d.w) of OCP residues and cypermethrin in samples of leaves collected from Ruhija, Nkuringo and Buhoma sites.

Pesticide residues	Ruhija (N = 12)			Nkuringo (N = 12)			Buhoma (N = 12)			All samples (N = 36)		
	Mean	Range	n > LOD	Mean	Range	n > LOD	Mean	Range	n > LOD	Mean	Range	n > LOD (%)
o,p′-DDE	0.015	0.07–0.11	2	0.046	0.12–0.23	3	0.024	0.06–0.09	4	0.028	0.06–0.23	9(25)
p,p′-DDE	0.13	0.09–0.19	8	0.63	0.27–1.32	8	0.034	0.08–0.09	5	0.27	0.08–1.32	21(58.3)
o,p′-DDD	0.26	n.d-3.15	1	0.28	0.97–1.2	3	0.78	0.98–1.92	6	0.44	n.d-3.15	10(27.8)
o,p′-DDT	0.1	0.31–0.32	4	0.14	0.31–0.38	5	0.11	0.31–0.34	4	0.12	0.31–0.38	13(36.1)
p,p′-DDT	0.16	n.d-0.97	2	0.28	0.32–0.78	6	0.18	0.45–0.64	4	0.21	n.d-0.97	12(33.3)
∑DDTs	**0.67**	–		**1.38**	–		**1.13**	–		**1.07**	–	
α-Endosulfan	0.61	1.1–1.36	6	1.86	4.11–5.61	5	0.89	1.93–2.42	5	1.12	1.1–5.61	16(44.4)
β-Endosulfan	0.29	1.26–2.26	2	0.85	n.d-10.2	1	0.55	3.24–3.34	2	0.56	n.d-10.2	5(13.9)
∑endosulfan	**0.9**			**2.71**	–	–	**1.44**			**1.68**	–	
Cypermethrin	n.d	–	0	n.d	–	0	n.d	–	0	n.d	–	0(0)
α/β endosulfan	**2.10**			**2.18**								
o,p′-DDT/p,p′-DDT	**0.63**			**0.5**	–	–	**0.61**			**0.57**	–	

N-number of samples; LOD-limit of detection; n.d-non-detectable; n-number of samples with levels above limit of detection; %-percentage.

Table. 3
Persistence of cypermethrin residues in field soil and plant leaves/fruits samples after treatment elsewhere in the world.

Country	Sampling year	Matrices	Initial levels	Day to BDL	References
Malaysia	2013	Soil	n.d	–	Sulaiman et al. (2020)
		Oil palm leaves	0.37 μg/g	2nd	
India	2014/2015	Curry leaf	13.09 mg/kg	10th	Priyadarshini et al. (2017)
Bangladesh	2014	Soil	1.992 mg/kg	7th	Uddin et al. (2016)
		Okra fruit	2.775 mg/kg	10th	
Bangladesh	2009	Tomatoes	0.29 mg/kg	15th	Nahar et al. (2012)
India	–	Soil	–	15th	Jyot et al. (2013)
		Green chili	0.32 mg/kg	10th	
India	2013	Chili	1.46 mg/kg	25th	Singh et al. (2015)
Greece	2011	Soil			Mantzos et al. (2016)
		(Cultivated plot)	0.009–0.015 mg/kg	14th	
		(uncultivated plot)	0.020–0.025 mg/kg	14th	

BDL-Below detection limit; n.d-not detected.

than that reported in soils (n.d to 0.0 17 mg/kg d.w and 0.006 mg/kg d.w respectively) from India (Chakraborty et al., 2015), Pakistan (Syed and Malik, 2011), Canada (Becker et al., 2011) and South Korea (Kim et al., 2020) but lower than that reported in soils from Ethiopia (2.073 mg/kg d.w and 1.891 mg/kg d.w respectively) (Westbom et al., 2008). Spatial distribution of OCPs in soils are influenced by factors such as, on-going pesticide use, point sources, land use, soil pH and total organic carbon (Wang et al., 2009) thus partly explaining the variation in OCPs distribution in our study and other studies elsewhere.

Our data from the farmers' interviews did not explicitly reveal if or not DDT is still being applied in agricultural land in this area. However, detectable levels of DDT metabolites DDE and DDD in 12.5% of the soil samples from Ruhija site suggests historical use of DDT in the area. Once in the environment, DDT undergoes slow aerobic or anaerobic degradation to the more stable and persistent metabolites DDE and DDD (Ma et al., 2016; Ssebugere et al., 2010; Umulisa et al., 2020; Yang et al., 2013). Surprisingly, detectable levels of DDE and DDD were present only in soil samples from Ruhija site. These findings implicate the possibility of local variation in the distribution of DDT and its metabolites around the Bwindi park given its big area of 330.8 km^2 (Wang et al., 2019). In a related study, Bhandari et al. (2020) investigated pesticides residues in 147 soil samples from agricultural fields in Nepal and their findings showed that only 8% of the soils contained quantifiable residues of DDT and its metabolites. In Uzbekistan, a study on the arable sierozem-meadow soils by Haytbay et al. (2018) revealed that DDT metabolites (*o*,*p*′-DDT, *o*,*p*′-DDE and *o*,*p*′-DDD) were not detected in the top soil samples (<30 cm depths) except two DDT metabolites (*p*,*p*′-DDE and *p*,*p*′-DDD) which were detectable in the soil samples with total mean concentrations of 0.504 and 0.668 μg/kg d.w, respectively. These studies support local variations in levels of DDTs in soils, as observed in our study.

Regarding endosulfans, α- and β-endosulfans were detected only in soils from Nkuringo site in the ranges of n.d to 2.18 and n.d to 9.89 mg/kg d.w, respectively. Commercial technical endosulfan is mixture of α- (70%) and β- (30%) stereo-isomers of endosulfan (Sun et al., 2014; Yadav et al., 2016). Therefore, the observed lower concentrations of α-endosulfan in the soils compared to β-isomer in the present study could be attributed to the rapid decomposition of α-endosulfan in the soils. α- and β-endosulfan isomers can easily be degraded to endosulfan sulfate by microbes in the soils to form the endosulfan sulfate which is resistant to further degradation (Qiao et al., 2010). In addition to the continuous microbial metabolism, the very low levels of endosulfans observed in the soils could also be attributed to the plant-facilitated mobilization of the endosulfans (Mitton et al., 2016). The α-/β-endosulfan ratio of 0.22 < 2.3 in soils from Nkuringo site suggests historical application of endosulfan in this area (Yadav et al., 2016).

3.3.2. Plant leaves

In all the three study sites, 36.1%, 44.4% and 13.9% of all the samples of leaves analysed showed detectable levels of ∑DDTs, α-endosulfan and β-endosulfan, respectively. The mean concentrations of ∑DDTs, α-endosulfan and β-endosulfan in the samples of leaves ranged from 0.67 to 1.38, 0.6 to 1.86 and 0.29 to 0.85 mg/kg d.w, respectively (Table 2). No data from our literature review were available pertaining to the residues of DDT (and its metabolites) and endosulfans within leaves consumed by mountain gorillas. However, several studies have reported the levels of DDT (and its metabolites) and endosulfans in leaves from other plant species and vegetables (Ligani and Hussen, 2014; Łozowicka et al., 2016; Luo et al., 2020; Luo et al., 2019; Marco and Kishimba, 2007; Qiu et al., 2019; Singh and Singh, 2014; Witczak et al., 2018; Yang et al., 2007; Yi et al., 2013; Zhang et al., 2015).

In general, the mean concentration of ∑DDTs in samples of leaves in our study was higher than that reported in tea leaves from China (0.003 to 0.004 mg/kg) (Yi et al., 2013) and Poland (Witczak et al., 2018), mangrove leaves from South China (0.0027 mg/kg) (Qiu et al., 2019), leaves of *Mangifera indica* (0.0061 to 1.242 mg/kg) from Tanzania (Marco and Kishimba, 2007), and leaves of khat (*Catha edulis*) plants from Ethiopia (0.0167 to 0.0448 mg/kg) (Ligani and Hussen, 2014). Similarly, the mean concentration of ∑endosulfans (1.68 mg/kg d.w) in samples of leaves in our study was higher than that reported for the leaves of several plant species from India (Singh and Singh, 2014). The high levels of DDTs in our study could be attributed, in part, to the poorly regulated use of pesticides in Uganda. It is possible that traders still import DDTs and sell them to the farmers due to their effectiveness.

In the present study, α- and/or β-endosulfan isomers were detected in the majority of the samples of leaves collected from all the three study sites. The levels of α-endosulfan in samples of leaves ranged from 1.1 to 1.36, 4.11 to 5.61 and 1.93 to 2.42 mg/kg d.w in Ruhija, Nkuringo and Buhoma sites respectively while the levels of the β-endosulfan isomers varied from 1.26 to 2.26, n.d to 10.2 and 3.24 to 3.34 mg/kg d.w in the three study sites, respectively. The relatively higher concentration values of β-endosulfan observed in the leaves in our study could be attributed to the thermodynamic stability of the β-endosulfan isomer under aerobic conditions and its equatorial configuration of the chlorines which favors physical and metabolic stability, storage in biological media and affords its resistance to hydrolysis and enzymatic degradation (Mitton et al., 2016; Singh and Singh, 2014).

3.3.3. Possible sources of DDTs

In this study, there was a weak positive correlation in the levels of the seven OCP residues between the samples of soil and leaves based on Spearman's rank correlation ($r_s = 0.39$, $n = 7$, $p < 0.01$). In our study, soil samples were collected 30 m outside and 30 m inside the forest while all the samples of leaves were collected from inside the forest. Therefore, the enhanced deposition of the less-volatile OCPs like DDT and its metabolites in leaf samples could be attributed to the higher capacity of the Bwindi rainforest to enrich OCPs from the atmosphere (Luo et al., 2019) in addition to the specific biochemical composition of the plant and its bioaccumulation efficacy (Waliszewski et al., 2008). According to Arinaitwe et al. (2016), recent exposure to OCPs like DDTs

is majorly attributed to atmospheric deposition since these contaminants have the potential to undergo long range atmospheric transfer from their sources of emission elsewhere. In 2001, more than 100 countries signed the Stockholm Convention and agreed to phase out the use of 12 persistent toxic compounds including DDT whose use was only restricted to the control of malaria vector. Since then, nine nations including Uganda notified about their continued use of DDT (as cited in Krief et al., 2017). Concomitantly, in 2008, there was a public health campaign against malaria vector in Uganda through indoor spraying of DDT (Mukasa et al., 2013) and approximately 24 metric tons of DDT were used (Van Den Berg et al., 2017). In the surrounding areas of the Bwindi park, DDT and its metabolites have been reported in soils from Kihiihi sub-county in Kanungu district (Ssebugere et al., 2010). Similar studies have also reported excess levels of DDT and its metabolites in the locations near Kibale National Park, which is inhabited by the endangered chimpanzee (Krief et al., 2017).

DDTs/(DDEs + DDDs) ratios can be used to indicate whether DDT is from an old or recent input with a high ratio suggesting a recent input (Qiu et al., 2019; Zheng et al., 2020). In our study (Fig. 2), higher percentages of ∑DDT metabolites (DDEs + DDDs) compared to the ∑DDT isomers in the samples of leaves collected from all the three study sites suggested old input of DDT into the Bwindi rainforest.

Furthermore, the *o,p′*-DDT/*p,p′*-DDT ratios can be used to distinguish whether the DDT source in the environment is from the use of technical-DDT type (about 0.2–0.26) or dicofol-DDT type (about 7.0) (Qiu et al., 2005; Qiu et al., 2019; Yang et al., 2013; Zheng et al., 2020). While technical-DDT type contains less *o,p′*-DDT (about 15%) than *p,p′*-DDT (about 85%), the composition of dicofol (2,2,2-trichloro-1,1-bis-(*p*-chlorophenyl)ethanol) has the reverse pattern (Qiu et al., 2005). In the present study, *o,p′-DDT*/*p,p′*-DDT ratios of 0.63, 0.5 and 0.61 were observed for the samples of leaves from Ruhija, Nkuringo and Buhoma sites, respectively. All these ratios were much lower than 7.0, but were still higher than that in technical-DDT type, suggesting that the DDT profile in the Bwindi rainforest is possibly from a mixture of technical and dicofol-DDT type though dicofol-DDT type is much less frequent in the mixtures. In the environment, *o,p′*-DDT metabolizes more quickly than *p,p′*-DDT resulting into observed lower *o,p′*-DDT/*p,p′*-DDT ratios than those of the product formulas. Li et al. (2006) indicated that the mixing of technical and dicofol-DDT types could also result in an observed ratio of *o,p′*-DDT/*p,p′*-DDT between the two origins. Interestingly, H. Huang et al. (2018) for the first time reported *p,p′*-(dicofol + DBP) as metabolites of *p,p′*-DDT in field soils rather than formulated dicofol.

In a related study, Krief et al. (2017) reported an *o,p′*-DDT/*p,p′*-DDT ratio of 0.3 for maize stem samples collected from the Sebitoli area of Kibale National Park. The authors commented that DDT pollution in that area was due to recent use of technical-DDT and not the dicofol-DDT type. Krief's findings and ours suggest the use of different formulations of DDT in the different areas surrounding Bwindi Impenetrable National Park and Kibale National Park. A previous study by Ssebugere et al. (2010) suggested recent input of technical-DDT type in soils from Kihiihi. Interestingly, the *o,p′*-DDT/*p,p′*-DDT ratios (0.43–1.9) observed for the 4 sampling sites in their Kihiihi soil study implicated input of a mixture of technical and dicofol-type DDT in that area. These findings point arrows to the illegal use of both technical and dicofol-DDT types in different parts of Uganda, and it's imperative to note that the marketing and use of dicofol had not been halted in China until Makombo, 2018 (Zheng et al., 2020).

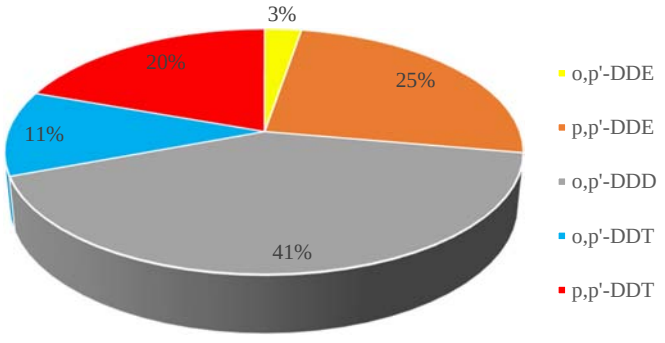

Fig. 2. Percentage contribution of DDT isomers and its breakdown metabolites to the ∑DDT residues in samples of leaves collected from all the three study sites.

Given the rich history of intensive use of DDT in Kihiihi environment (Bimenya et al., 2007; Ssebugere et al., 2010) and its proximity with Bwindi Impenetrable National park, we hypothesize that the observed DDT profiles in the Bwindi park is to a greater extent influenced by atmospheric deposition. In a study on the DDT profiles in air samples collected near the northern shoreline of Lake Victoria, Ugandan side, Arinaitwe et al. (2016) implicated fresh emissions of dicofol in their atmospheric samples as a possible contributor to the observed DDT profiles. In another study on OCPs in the arctic atmosphere, Becker et al. (2012) reported a significant decline in the *p,p′*-DDT isomer and an increasing trend in the ratios of *o,p′*-DDT/*p,p′*-DDT, reflecting a shift from the use of technical-DDT type to dicofol. In their study, the researchers argued that the observed ratios might reflect ongoing reemission of the compounds from the soils and other factors.

3.4. Possible health risks to the mountain gorillas

The detection of OCPs in samples of leaves from plants consumed by mountain gorillas from the different locations of the park suggests that these gorillas could be continuously exposed to these chemical toxins through the food chain. The European pharmacopeia (EP) and United States pharmacopeia (USP) recommended maximum residue limits (MRLs) for DDTs and endosulfans in medicinal plants including leaves are 1.0 mg/kg and 3.0 mg/kg respectively (WHO, 2007). It should be emphasized that these set MRLs are for humans and not great apes like mountain gorillas. From our literature reviews, there is insufficient quantitative data exploring the exposure of mountain gorillas to pesticides and there are no reported MRLs specific to the plant species consumed by mountain gorillas. Mountain gorillas live in groups and are highly social (Robbins and Robbins, 2018). They are also genetically close to humans (Steiper and Young, 2006) and are thus susceptible to the same diseases as humans (Gilardi et al., 2015). It is therefore plausible to use the set MRLs for risk assessment in great apes like mountain gorillas as well. The mean concentrations of the ∑DDTs observed in samples of leaves from plants consumed by mountain gorillas ranged from 0.67 to 1.13 mg/kg (total mean = 1.07 mg/kg) from all the three study sites, which were higher than the set MRL (1.0 mg/kg).

Moreover, the average EDIs of DDTs and endosulfans in our study exceeded the acceptable daily intakes (ADIs) (Table 4). The calculated HI values for the silverbacks (36.35), females (57.54) and juveniles (77.04) were far above the threshold value of one (1). These findings suggested that consumption of leaves contaminated with OCPs poses serious health risks to the mountain gorillas. In a related study, Krief et al. (2017) reported that excess levels of DDT and its metabolite DDE in the vicinity of Kibale National Park were associated with facial dysplasia in chimpanzees and baboons. In general, exposure to elevated levels of DDTs has been linked with several human health effects including, liver cancer, breast cancer, diabetes, neurodevelopmental deficits, reproductive abnormalities and asthma (Eskenazi et al., 2009). Whether or not the same effects could be elicited in human-like apes such as mountain gorillas needs further investigation.

3.5. Strengths, limitations and future recommendations

To the best of our knowledge, this is the first study to investigate the distribution of pesticide residues like OCPs and cypermethrin in soils and plant leaves from Bwindi Impenetrable National park. This paper has also generated a very important baseline data for future

Table 4
Average concentration (mg/kg d.w) of pesticide residues in samples of leaves and calculated EDI, cHQ, and HI in a silverback, female and juvenile.

Pesticide residues	Average concentration (mg/kg d.w)	EDI (mg/kg bw/day)			cHQ		
		Silverback	Female	Juvenile	Silverback	Female	Juvenile
p,p′-DDT	0.21	0.020	0.031	0.042	2.00	3.10	4.20
o,p′-DDT	0.12	0.011	0.018	0.024	1.10	1.80	2.40
o,p′-DDD	0.44	0.041	0.066	0.087	4.10	6.60	8.70
p,p′-DDE	0.27	0.025	0.040	0.054	2.50	4.00	5.40
o,p′-DDE	0.03	0.003	0.004	0.006	0.30	0.40	0.60
α-endosulfan	1.12	0.105	0.167	0.223	17.5	27.8	37.2
β-endosulfan	0.56	0.053	0.083	0.111	8.83	13.8	18.5
Cypermethrin	0.01	0.001	0.002	0.002	0.02	0.04	0.04
HI					**36.35**	**57.54**	**77.04**

EDI-Estimated daily intake; *c*HQ-chronic/long-term hazard quotient; HI-hazard index.

assessments of cypermethrin and OCPs like DDTs and endosulfan within the Bwindi Park. Nonetheless, there were some limitations to the study. Firstly, the sample sizes ($N = 24$ for soils and $N = 36$ for leaves) were small and therefore, did not provide sufficient statistical power to allow generalisations and detection of potential subtle effects from low pesticide residue levels. Secondly, the present study investigated very few pesticides in totality (α-endosulfan, β-endosulfan, *o*,*p*′-DDT, *p*,*p*′-DDT, *o*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDE and cypermethrin) which are not sufficient for conclusive risk assessment in the complex Bwindi Park. We strongly recommend future studies to focus on (i) the nature, spatial distribution, and potential sources of OCPs and other contaminants within the ecosystem of Bwindi Impenetrable National Park, (ii) a detailed investigation into the bioaccumulation of the OCP residues and other contaminants within Bwindi Impenetrable National Park and an assessment of the associated toxicological risks to both biological ecosystems and human health.

4. Conclusion

The present study has revealed the occurrence of DDT (and its breakdown metabolites) and endosulfan in samples of soils and leaves from Bwindi Impenetrable National park in Uganda. Detectable levels of ∑DDTs, α-endosulfan and β-endosulfan were found in 2.5%, 8.3% and 4.2% of all the soil samples and 36.1%, 44.4% and 13.9% of all the leaf samples respectively. In spite of the conservation status of the Bwindi Park and the banning of many of these OCPs many decades ago, their residues especially DDTs and endosulfans were widely found in the plant leaves consumed by mountain gorillas. Mean concentration of ∑DDTs (1.07 mg/kg) exceeded the European pharmacopeia and United States pharmacopeia recommended MRL (1.0 mg/kg) for DDTs in medicinal plants. Furthermore, calculated HIs for the silverbacks (36.35), females (57.54) and juveniles (77.04) were far above the threshold value of one (1), which raises serious concerns regarding their toxicological risks to the endangered mountain gorillas. The presence of these contaminants is likely to have adverse impacts on both the ecological environment and human health. This indicates the urgency for further monitoring and the present study provides a very important baseline data for future assessments and policy formulation by the Uganda Wildlife Authority.

CRediT authorship contribution statement

Chemonges Amusa: Conceptualization, Methodology, Formal analysis, Investigation, Methodology, Writing - original draft. **Jessica Rothman:** Writing - review & editing, Supervision, Funding acquisition. **Silver Odongo:** Conceptualization, Writing - review & editing. **Henry Matovu:** Conceptualization, Writing - review & editing. **Patrick Ssebugere:** Writing - review & editing, Supervision, Funding acquisition, Data curation. **Deborah Baranga:** Conceptualization, Writing - review & editing. **Mika Sillanpää:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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