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RESEARCH ARTICLE

High urinary oxidative DNA damage in wild chimpanzees ranging in proximity of agricultural fields in Sebitoli area, Uganda

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Abstract

Agriculture expansion is a major cause of habitat loss and exposure to phytochemical pollution for non-human primates. In addition to endocrine disruption, exposure to pesticides may have other sublethal physiological consequences for animals, such as generation of oxidative damage to macromolecules. In this study, we analyzed the pesticides contained in the river water across the home range of wild chimpanzees (*Pan troglodytes*) in Sebitoli area located on the Northern part of Kibale National Park (Uganda). We tested whether levels of three urinary markers of oxidative damage vary among individuals in relation to their ranging patterns, as a proxy for pesticide exposure intensity. To better characterize the foraging habitat use, the trophic level, and the energetic status of study individuals, we also quantified urinary levels of carbon and nitrogen stable isotope signatures and of C-peptide. Among the 511 pesticides screened, 18 compounds including herbicides, insecticides, and fungicides were found in the water sampled in the Western part of the home range of chimpanzees. In this area, chimpanzees used to feed on maize crops. By contrast, in the Eastern part where crop feeding was never observed, we found only seven pesticides. According to their ranging patterns and thus crop feeding frequency, the 139 urine samples collected from 43 Sebitoli chimpanzees were categorized as belonging to low, medium, and high exposure level. Chimpanzees from the high exposure zone had higher oxidative DNA damage (8-OHdG) than chimpanzees from both the low and medium exposure groups, who had similar levels of oxidative DNA damage. In addition, individuals with higher C-peptide tended to have significantly higher oxidative DNA damage and lipid peroxides. The three exposure groups had similar levels of urinary 8-isoprostanes and of urinary lipid peroxides. These results were robust for any potential confounding effect of other variables because neither age category nor sex or isotope levels were significantly associated with markers of oxidative damage. Our study points to genotoxic effects as one potential sublethal consequence of ranging in proximity of agricultural fields owing to exposure to pesticides or other unidentified sources of stress. Given our phylogenetic proximity, this information is relevant for the conservation of this species which is endangered and also sentinel for human health.

Keywords Oxidative stress · Apes · Chimpanzee · Imidacloprid · Glyphosate · DNA damage · One Health · Genotoxicity

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Introduction

Agricultural lands provide important benefits for a variety of species, such as novel habitat or food. Despite these supposed advantages for wildlife, costs of living near agricultural zones may overcome the benefits. Anthropogenic food availability can be synchronous to wild food and/or cannot compensate for the shortage of wild resources. In addition, feeding on anthropogenic food exposes crop feeders to direct threats, such as being chased, injured, and hunted (Carlitz et al. 2016; Hockings et al. 2007; Hyeroba et al. 2011). The human-wildlife contact also triggers indirect health threats via phytochemical pollution of cultivated plants (Krief et al. 2022; Matthiessen et al. 2017; Mendenhall et al. 2014; Spirhanzlova et al. 2019; Van Der Schalie et al. 1999). Actually, in tropical forests, food availability and diversity can unpredictably vary in time and space across seasons and years (Chapman et al. 2005). The current global climate change and human encroachment can further exacerbate seasonal and inter-annual variability in tree phenology (Bush et al. 2020). Such unpredictable fluctuations in dietary intake may cause additional stress to animal species, like primates which are already threatened with extinction and listed as endangered on the IUCN Red list (IUCN 2023). Indeed, primates in tropical forests have to cope with multiple threats, such as loss of habitat, poaching, and infectious diseases (Estrada et al. 2017; Hockings & Humley 2009; Ordaz-Németh et al. 2021). Agriculture expansion is the primary cause of habitat fragmentation, landscape simplification, and reduction of wild food for primates (Estrada 2013; Estrada et al. 2017). The increased proximity of crops to tropical forests provides wildlife with alternative resources, like higher or different nutrient composition when compared to wild plant species (McLennan & Ganzhorn 2017). In addition, anthropogenic food is often found in great abundance throughout the year, usually clustered on the ground and thus easily accessible with low energy expenditure needed (Chiyo et al. 2005; Couturier et al. 2022; Naughton-Treves et al. 1998). In turn, this provides advantages also by reducing within-group feeding competition. However, pesticide exposure is today recognized as a hidden route of a negative impact on wildlife populations, and is driven by agricultural expansion in tropical regions (Costantini 2015). In addition to endocrine disruption, exposure to pesticides also have other sublethal physiological or metabolic consequences for animals. For example, pesticides induce significant effects on the cellular oxidative status, generating oxidative damage to important biomolecules, such as DNA (e.g., Abdollahi et al. 2004; Ledda et al. 2021).

In the vicinity of the forested area in the Kibale National Park, Uganda, where wild chimpanzees (*Pan troglodytes*) live, numerous pesticides are used by farmers (Krief et al. 2017, 2020). Today, an increasing volume of evidence of chimpanzee exposures to chemicals have been recorded. However, invasive sampling of wild chimpanzees (blood analysis) is impossible, as surveying wild chimpanzees is regulated such that all observations must be carried out at over 10-m distance, to prevent any risk of disease transmission (Williamson and Macfie 2014). Thus, by identifying the pesticides that occur in the rivers flowing through the home range of chimpanzees and using urine samples non-invasively collected, we expect a better understanding of the pollutant effects on chimpanzees.

Previous surveys have identified the sources and levels of chemical pollution: glyphosate is commonly used on tea cultivated at its edge, while cypermethrin, profenofos, mancozeb, metalaxyl, dimethoate, chlorpyrifos, and 2,4-D amine are sprayed on the different crops cultivated in farmlands (Krief et al. 2017). A survey of the water collected in 2017 from the rivers in the forested area where Sebitoli chimpanzees live, showed the occurrence of a cocktail of at least 13 different pesticides (Spirhanzlova et al. 2019). Among them, the insecticide imidacloprid, used to coat the maize seeds planted along the Western border of the Park in this area, was also found in wild fish collected in these same rivers within the Sebitoli chimpanzee home range (at 5 km from the Park's border; Krief et al. 2017). The analysis of the chimpanzee hair collected from their night nests revealed the extent of their exposure with the presence of a total of 60 different pesticides. Up to 43 chemicals have been detected in one single individual (Krief et al. 2022). An in vivo study of the river-water collected in the chimpanzee home range showed thyroid and estrogen disrupting properties in laboratory tadpole and fish models (Spirhanzlova et al. 2019). Among the different crops grown at the edge of the Park (e.g., beans, potatoes, sweet potatoes, plantain, tea), Sebitoli chimpanzees only eat maize (*Zea mays*) seeds and stems (Krief et al. 2014a) and they are very rarely observed drinking the river water.

Previous studies also show that there is a gradient in the pesticide exposure related to the agricultural practices, as well as in individual ranging patterns of chimpanzees. The study site is characterised by a polarization in the croplands and in consequences in the use of pesticides: in the Eastern side of the chimpanzee home range, only tea (not eaten by chimpanzees) is grown and just four chemicals were detected in our previous study carried out in 2017, as opposed to 13 found in the Western border's agricultural fields, including crops of both tea and maize (Spirhanzlova et al. 2019). Within the same chimpanzee community, interindividual variability in exposure due to individual ranging patterns may occur. While adult male chimpanzees are traveling the whole home range and patrolling at the boundaries of the home range of the community, adult females and their

dependents may be resident of only a part of it, this area being their individual core area (Couturier et al. 2022; Mitani & Watts 2005; M. E. Thompson & Wrangham 2006; Watts & Mitani 2001; Williams et al. 2002). In addition, individuals residing most of their time near crops also consume the crops more frequently (Couturier et al. 2022).

In this study, we aimed to confirm this East–West difference of exposure among the community of Sebitoli chimpanzees and to test the potential consequences of these differences in pesticide exposure To do so, we screened the river water for presence of pesticides in three different locations of the home range of wild chimpanzees over a longer period (in addition to data obtained in 2017 which were already published in Spirhanzlova et al. (2019), we conducted surveys in 2018 and 2019) and tested whether levels of three urinary markers of oxidative damage vary among individuals in relation to their ranging patterns, as a proxy for pesticide exposure intensity. In addition, when compared with adult females (and their offspring) residing in the Northern-Eastern border or to individuals residing in the Southern part of the home range, we predicted higher levels of oxidative damage in females and offspring residing closer to the Northern-Western forest border (because visiting more the field crops) and in all adult males (because using the whole home range including Northern-Western area).

In the absence of complete and measurable direct feeding observations, the consumption of maize and animal proteins (vertebrates and insects) can be estimated with urinary levels of carbon and nitrogen stable isotope signatures and of C-peptides. First, stable isotope values of nitrogen and carbon in tissues or body products (e.g., blood or fur, respectively) provide information on the trophic position of the animal and the habitat in which they foraged (Deniro and Epstein 1981a, b; Voigt and Kelm 2006). This is because the isotope composition of an animal reflects that of its diet (Deniro and Epstein 1981a, b; Voigt and Kelm 2006). Wild chimpanzees are considered as C3 feeders because they feed on fruits and leaves (Schoeninger et al. 1999; Sponheimer et al. 2006). By contrast, maize is a C4 plant, and animals that eat these plants have more of the heavy isotope of carbon than those that do not consume C4 plants. Second, C-peptide concentration will reflect the proportion of maize in the individual diet: C-peptide is secreted from β cells in the islets of Langerhans following cleavage of proinsulin to insulin and C-peptide. Thus, C-peptide and insulin, a hormone that regulate the metabolism of carbohydrates, are produced in equal amounts. β cells are sensitive to blood sugar levels and high level of insulin reflects high level of blood glucose. Importantly, the urinary concentrations of C-peptide correlate with those in serum. C-peptide in chimpanzee urine could distinguish between fruit poor and fruit rich periods, as well as between more and less productive habitats (Thompson et al. 2009). Because of the high sugar content in maize corn compared to wild fruits as well as the high density of maize plants in a maize garden, we expect a higher C-peptide level in chimpanzees having a significant intake of maize (one ear of maize provides 86 kcal and is particularly high in carbohydrates, i.e., 19.2% compared to the average of 13.9% of 32 wild fruits from Kibale). The consumption of several ears of maize represents a high intake considering that a daily energy intake for Kibale's chimpanzees range from 1206 to 3333 kcal on estimation (Krief et al.2014a, b)).

Material and methods

Study site

Kibale National Park lies in southwestern Uganda between 0°13–0°41 N and 30°19–30°32 E (Fig. 1). The 795-km^2 park is mostly covered by moist evergreen or semi-deciduous forest, at various stages of maturity/regeneration from past human disturbance (Chapman & Lambert 2000). Sebitoli is located at the extreme North of Kibale National Park (Fig. 1). Sebitoli forest was commercially logged in the 1970s, leading to the damage of about 50% of the trees (Struhsaker 1997). Approximately 80–100 chimpanzees range over 25 km^2 within this area. Today, 70% of the Sebitoli chimpanzee home range is composed of regenerating forest and only 14% are old growth forest (Bortolamiol et al. 2014). More than 80% of perimeter of the Sebitoli community's home range are in contact with anthropogenic landscape (mostly farmland and tea estates) (Bortolamiol et al. 2014). In addition, a tarmac road with intense traffic bisects the home range of the Sebitoli chimpanzee community ((Cibot et al. 2015; Krief et al. 2020); Fig. 1). Two rivers flow in Sebitoli chimpanzee home range: Mpanga river and Munobwa river. Mpanga river originates from the foot of the Mountain Rwenzori and flows from Western part to Southern part of the chimpanzee home range, while Munobwa river flows from Eastern edge to the Southern part of their range and joins Mpanga river near the road. Since 2008, individuals from the Sebitoli community are monitored daily by the Sebitoli Chimpanzee Project team (SCP). Sebitoli chimpanzees are monitored without employing invasive methods and without any interactions with SCP team. SCP adhered and followed the guidelines of the Uganda Wildlife Authority (UWA). The SCP research is conducted in the context of the Memorandum of Understanding MNHN/UWA/Makerere University SJ 445–12.

Urine sampling

Observations and urine sampling of chimpanzees were carried out at over 10-m distance as recommended to prevent any behavioral disturbance and any risk of disease transmission or stress caused by human proximity (Williamson & Macfie

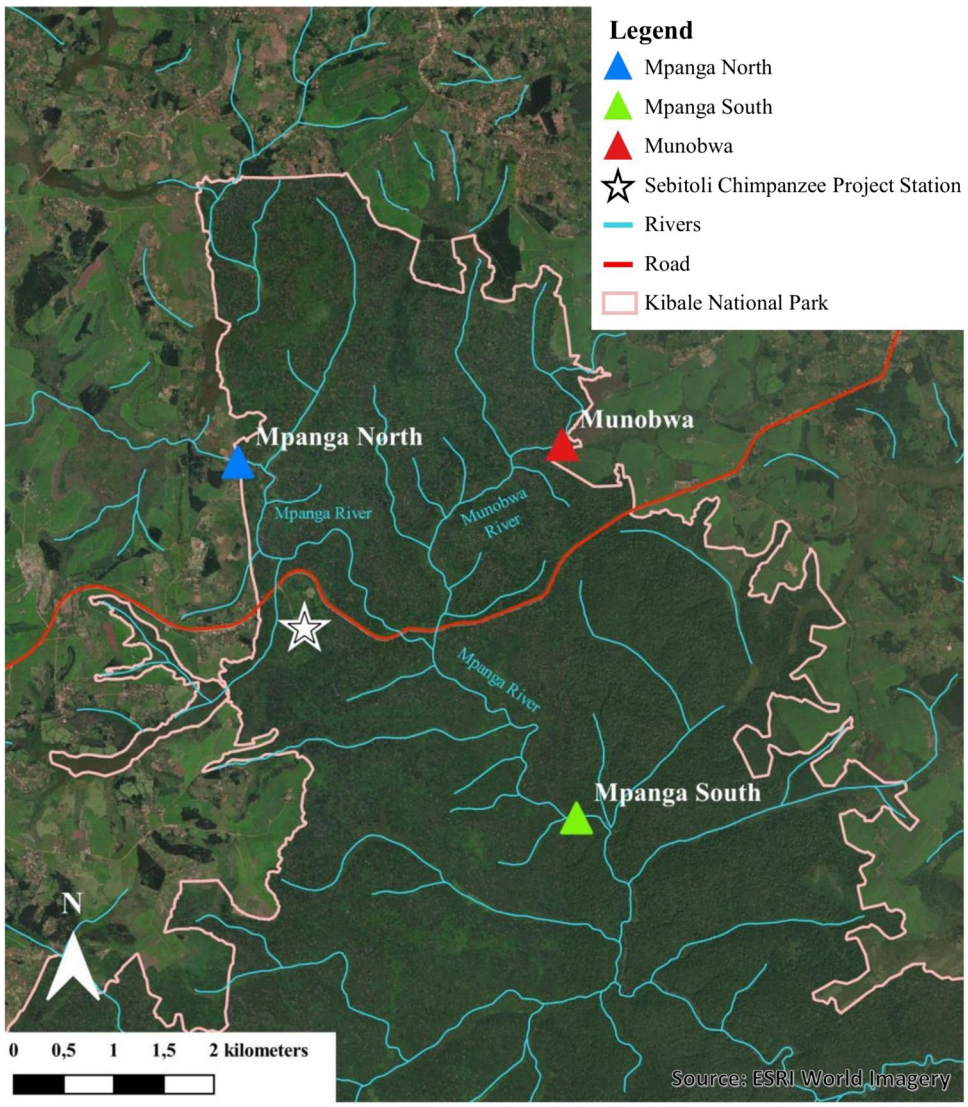

Fig. 1 Map of the Sebitoli area in Kibale national park (Uganda). Water was sampled from two rivers, Mpanga and Munobwa rivers in three locations: in the Northern part of the home range (1) Western area: Mpanga north, (2) Eastern area: Munobwa in Southern part of the home range (3) Mpanga South (adapted from Spirhanzlova et al. 2019)

2014). We collected urine samples from chimpanzees urinating from trees using plastic bag hanging from a forked stick or by pipetting the urine from leaves under them (Krief et al. 2005; Masi et al. 2012). Urine samples were stored in dry clean containers and were placed in a freezer (-5 °C) immediately upon returning to the field station and then at − 80 °C in the laboratory. We analyzed only urine samples not contaminated by feces or soil matter (Krief et al. 2005; Masi et al. 2012) A total of 139 samples were collected between January 2017 and January 2020, during dry and rainy seasons, from 43 chimpanzees (1 to 9 urine sample per individual), including 19 females and 24 males.

Urinary markers of oxidative damage

Three markers of oxidative damage in urine samples were quantified (González et al. 2020; Halliwell & Gutteridge 2015; Martinez-Moral & Kannan 2019): the concentration of lipid peroxides (intermediate products of lipid oxidation) using the URINOX test (Diacron International, Grosseto, Italy); the concentration of 8-isoprostanes (produced by oxidation of arachidonic acid present in membrane phospholipids) using the 8-Isoprostane ELISA Kit (Bertin Technologies, Montigny Le Bretonneux, France); and the concentration of 8-hydroxy-2′-deoxyguanosine (8-OHdG, marker of oxidative DNA damage with mutagenic properties) using the OxiSelect™ Oxidative DNA Damage ELISA kit (Euromedex, Souffelweyersheim, France). We standardized oxidative status markers against the urinary creatinine levels, which is traditionally used as a control for urinary concentration (Creatinine (Urinary) Colorimetric Assay Kit, Bertin Technologies, Montigny Le Bretonneux, France). All analyses were carried out following manufacturer instructions. Each individual sample was assayed in duplicate for each test, and an average value was used in the statistical analyses. Intra- and inter-assay coefficients of variation were

12.0 and 14.2% for lipid peroxides, 8.9 and 13.4% for 8-iso-prostanes, 5.5 and 12.4% for 8-OHdG, and 2.5 and 8.8% for creatinine, respectively.

Urinary C-peptide

We quantified C-peptide levels via enzyme immunoassay using a C-peptide ELISA kit (RE53011, IBL International GmbH Hamburg, Germany), following a method already used in non-human primates (Girard-Buttoz et al. 2011; Higham et al. 2011). Urine samples were used pure without dilution. We standardized C-peptide levels against the urinary creatinine levels, quantified with the Creatinine (Urinary) Colorimetric Assay Kit (Bertin Technologies, Montigny Le Bretonneux, France). C-peptide and insulin are secreted in equimolar amounts because proinsulin is cleaved enzymatically, releasing both insulin and C-peptide. Thus, C-peptide is being used as a proxy of insulin production. Intra- and inter-assay coefficients of variation were 7.5 and 11.3%, respectively. We exclude any influence of sex difference in creatinine concentration as females and males had similar creatinine urinary levels (males: 23.5 ± 3.5; females: 27.1 ± 4.1; linear mixed model: sex, $F = 0.456$, $p = 0.503$).

Urinary stable isotope signatures

Relative abundances of carbon ($^{13}C/^{12}C$) and of nitrogen ($^{15}N/^{14}N$) isotopes were determined using an elemental analyzer (Flash 2000, Thermo Scientific, Milan, Italy) together with an isotope ratio mass spectrometer (Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany). Values were expressed in the δ (or $\delta^{13}C$ and or $\delta^{15}N$ for C and N, respectively) unit notation as parts per mille (‰) deviation from standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N_2 in air for $\delta^{15}N$). Accuracy was checked by replicate measurements of the international standards, and analytical precision was < 0.10 ‰ and < 0.15 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Pesticide exposure

Crops are cultivated uniquely at the Northern and Western borders of the park, i.e., at the edge of Sebitoli chimpanzee home range and not inside it. Sebitoli chimpanzees feed on crops (maize only) only in the Western and Northern parts of their home range. During these events of maize feedings, they are directly exposed through ingestion to chemicals coating maize seeds or sprayed on crops. In the other areas, at the Eastern edge and in the Southern part of their home range, only tea is cultivated; thus, they do not eat crops there.

In order to confirm wildlife exposure to pesticides in Sebitoli area during the study period of oxidative stress markers in the urine samples and to investigate pollution level over time, we sampled and analyzed the water of two rivers collected at three locations during three consecutive years. We used the same location and protocol as the ones used in 2017 and published in Spirhanzlova et al. (2019). In 2018 and 2019, three Polar Organic Chemical Integrative Samplers Hydrophilic Lipophilic Balance (POCIS HLB ATTRACTSPE®Affinisep) and one POCIS GLYPHOSATE (AFFINIMIP®, Affinisep) were placed for 14 days at each of the three river sites (1) in the Mpanga River North-Western at the interface between cropland and forest at the North-Western edge, (2) in the Munobwa River at the interface between tea plantation and forest at North-Eastern edge, and (3) in Mpanga River South, inside the forest at 4910 m from the sampling site Mpanga River North, in the southern part of the Sebitoli home range (Fig. 1). The 12 passive samplers were placed in the flowing water at more than 20 cm from the river bed and more than 20 cm from the surface of the water. In parallel, a field POCIS blank was exposed at each site outside of water, in order to guarantee the absence of contamination during all field operations (deployment and removal), as well as during storage and transport. After the sampling periods, each membrane was wrapped in aluminum foil and stored at − 20 °C until chemical analysis.

The POCIS HLB extraction protocol adapted from Belles et al. (2014) and described in Spirhanzlova et al. (2019) is presented in Supplementary materials. The extracts obtained in 2018 and 2019 and internal standards were analyzed as in 2017 (Spirhanzlova et al. 2019) by liquid phase chromatography coupled to tandem mass spectrometry and gas phase chromatography coupled to tandem mass spectrometry for a set of 509 pesticides. Analyses were managed by a laboratory accredited ISO/IEC 17025, the main standard for recognition of the technical competence for testing and calibration. In order to ensure reliable interpretation of MS-based and effect-based measurements, Quality Controls (QC) as blanks and replicates were implemented. Additionally, compared to sampling carried out in 2017 (Spirhanzlova et al. 2019), we also performed analysis of glyphosate and its metabolite AMPA (aminomethylphosphonic acid) in 2018 and 2019.

The POCIS GLYPHOSATE is based on Molecularly Imprinted Polymer (MIP) sorbent for the selective extraction of glyphosate, AMPA. The specific protocol of extraction was adapted from Berho et al. (2017) and analysis was carried out implementing liquid chromatography coupled to tandem mass spectrometry. Analyses were managed by a laboratory accredited ISO/IEC 17025, the main standard for recognition of the technical competence for testing and calibration.

Chimpanzees' pesticide exposure

The chimpanzees of Sebitoli community were categorized into three groups according to their risk of exposure, i.e., their proximity to the Western-Northern border, and their

frequency of cropland incursion. We used this as proxy for their pesticide exposure level as high, medium, and low. To determine the category of exposure for a given individual, we used both the camera trap frequency of detection at the border between crops and the forest (Couturier et al. 2022) and the long-term observations of the ranging pattern of individuals. We collected 33 urine samples from 4 female and 5 male chimpanzees in the *high exposure* group (5 from juveniles, 12 from subadults, 16 from adults). This included five subadult males born from mothers ranging mostly at the Northern-Western border of the home range and individuals mainly ranging in this area rarely seen in far North-East or Southern part of the home range. Adult females included in this group follow a very similar ranging pattern. 43 urine samples were collected from 6 female and 6 male chimpanzees classed in the *medium exposure* group (6 from juveniles, 4 from subadults, 37 from adults). These individuals are known to use any part of the Sebitoli home range, including both zones considered as high exposure areas (Western and Northern part) and low exposure areas (Eastern part) Among them, we included high ranking males. The *low exposure* group included 63 urine samples (10 from juveniles, 16 from subadults, 37 from adults) from 9 female and 13 male chimpanzees who frequently ranged far from the crops either on Eastern part or Southern part of the community home range. Among them, we included juvenile males who were born from Eastern or Southern mothers and still traveling with their mothers and two adult males staying South of the road.

Statistical analyses

We ran all statistical analyses using RStudio version 1.1.463. Depending on the fitting of the models to our data distribution, we performed general linear mixed models or linear mixed models (package lme4) to test the effects of exposure level and of individual traits on stable isotope signatures and physiological metrics. In the models on stable isotope values, we included exposure group (high, medium, low) as fixed factor, and individual identity and month of sample collection as random factors. In the model on C-peptide, we included exposure group, individual age category, individual sex, nitrogen as fixed factors; individual identity, month of sample collection, and assay number as random factors. In models on metrics of oxidative damage, we included the factors as follows: exposure group, individual age category, individual sex, nitrogen, and C-peptide as fixed factors; individual identity, month of sample collection, and assay number (except for lipid peroxides due to singularity) as random factors. In the models, we included nitrogen and C-peptide because they were similar among exposure groups, so that these metrics enabled us to control for individual trophic niche and nutritional status, respectively. Interactions between exposure group and sex or age were both excluded from the final models because preliminary analyses showed that they were not significant. The Shapiro test showed that residuals of all models were normally distributed. We used the R-package emmeans (estimate marginal means). Values of means and of standard error of each variable are shown in Fig. 2. All physiological metrics were $\log_{10}$ transformed to meet the assumption of normal distribution of model residuals and to improve the fitting of the models.

Results

Water chemical analysis

Among the 511 pesticides screened (509 compounds with POCIS as well as glyphosate and AMPA screened by LC/MS and GC/MS), 18 compounds including herbicides, insecticides, and fungicides were found in the water sampled at the three different sites. We detected 2.4-D amine, acetamiprid, ametryn, isoproturon, metolachlor, terbutryn carbaryl, carbofuran, DEET, dimethoate, dimethylphenylformamid, 2,4-imidacloprid, picaridin, thiamethoxam, carbendazim, glyphosate, and AMPA (Table 1). All 18 substances were detected in the water of Mpanga River North-West, and South. In the Eastern side of the home range, in Munobwa River, only seven substances (ametryn, DEET, metolachlor, picaridin, methamidophos, glyphosate, and AMPA) out of the 18 were detected during the data collection periods. In each of the campaign, between 11 and 15 substances in Mpanga river (North-west and South) and 2 to 4 in Munobwa river (East) were detected according to the sampling periods. Seven substances (2,4-D, ametryn, carbaryl, carbendazim, carbofuran, imidacloprid, terbutryn) were always detected in both sampling periods at the two sites of Mpanga river. In the East, glyphosate and AMPA were detected in Munobwa river in each of the two sampling periods.

Chimpanzee urine sample analysis

Chimpanzees from the low, medium, and high exposure groups had similar nitrogen levels (Table 2, Fig. 2A). By contrast, chimpanzees from the high exposure group had lower carbon level than those from the low exposure group (estimate 1.117 ± 0.466, $p = 0.026$; Table 2, Fig. 2B). Chimpanzees from medium and high exposure group had similar carbon levels (Table 2, Fig. 2B). The concentration of C-peptide was similar among the three exposure groups (Table 2, Fig. 2C) and was not significantly associated with any other factor included in the models, with the exception of a tendency for C-peptide concentration to be higher in individuals with higher nitrogen level (estimate 0.062 ± 0.035, $p = 0.082$, Table 2).

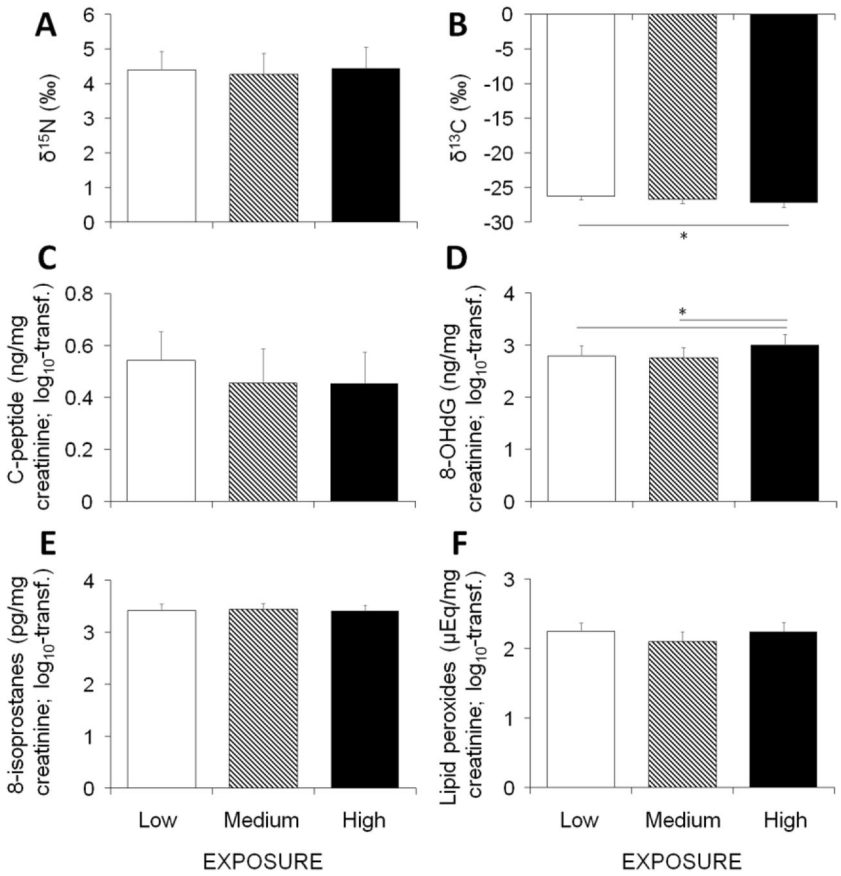

Fig. 2 Estimated marginal means ± standard errors obtained from linear mixed models of the three exposure groups (low, medium, high). Values of all physiological metrics are $\log_{10}$ transformed. 8-OHdG = 8-hydroxy-2′-deoxyguanosine. * indicates significant differences between exposure groups ($p < 0.05$)

Table 1 Chemicals detected in the water sampled with POCIS in the Sebitoli home range of chimpanzees

	Mpanga river (North-West area)			Mpanga river (Southern area)			Munobwa river (Eastern area)		
	2017[a]	2018	2019	2017[a]	2018	2019	2017[a]	2018	2019
2,4-D	x	x	x	x	x	x	0	0	0
Acetamiprid	0	x	0	0	x	0	0	0	0
Ametryn	x	x	x	x	x	x	x	0	0
Carbaryl	x	x	x	x	x	x	0	0	0
Carbendazim	x	x	x	x	x	x	0	0	0
Carbofuran	x	x	x	x	x	x	0	0	0
Diethyl-m-toluamid, N,N- (DEET)	x	0	0	x	0	0	x	0	0
Dimethoat	x	x	0	x	x	0	0	0	0
Dimethylphenylformamid, 2,4-	0	x	0	0	x	0	0	0	0
Imidacloprid	x	x	x	x	x	x	0	0	0
Isoproturon	x	x	0	x	x	0	0	0	0
Methamidophos	0	x	x	0	x	x	0	0	x
Metolachlor	x	0	0	x	0	0	x	0	0
Picaridin	x	0	0	x	0	0	x	0	0
Terbutryn	x	x	x	x	x	x	0	0	0
Thiamethoxam	x	x	x	x	x	x	0	0	0
Glyphosate	NT	x	x	NT	x	x	NT	x	x
AMPA	NT	x	x	NT	x	x	NT	x	x
Number of substances detected	13	15	11	13	15	11	4	2	3

[a]Results from previously published survey (Spirhanzlova et al. 2019)

NT, not tested

Table 2 Outcomes of models used to test the effect of exposure zone and of individual traits on stable isotope signatures and urinary physiological metrics. Significant *p*-values (<0.05) are shown in bold. Sample size: 139 urinary samples from 43 individuals. Residuals of all models were normally distributed according to the Shapiro–Wilk test

Variable	Factor	Reference level	Level	Coefficient estimate	SE	*t*	*P*
Nitrogen	Exposure zone	High	Low	0.209	0.320	0.655	0.518
		High	Medium	0.060	0.346	0.172	0.865
		Low	Medium	−0.150	0.295	−0.508	0.616
Carbon	Exposure zone	High	Low	1.117	0.466	2.395	**0.026**
		High	Medium	0.470	0.504	0.932	0.362
		Low	Medium	−0.647	0.430	−1.505	0.146
C-peptide	Exposure zone	High	Low	0.073	0.121	0.602	0.553
		High	Medium	0.030	0.132	0.226	0.823
		Low	Medium	−0.043	0.113	−0.384	0.704
	Age category			−0.028	0.039	−0.733	0.468
	Sex	Female	Male	0.037	0.098	0.374	0.711
	Nitrogen			0.062	0.035	1.757	0.082
8-OHdG	Exposure zone	High	Low	−0.203	0.086	−2.346	**0.029**
		High	Medium	−0.267	0.094	−2.852	**0.009**
		Low	Medium	−0.065	0.080	−0.804	0.431
	Age category			−0.033	0.028	−1.201	0.239
	Sex	Female	Male	−0.200	0.072	−2.760	**0.011**
	Nitrogen			0.012	0.029	0.418	0.677
	C-peptide			0.007	0.002	2.748	**0.007**
8-isoprostanes	Exposure zone	High	Low	0.031	0.069	0.451	0.656
		High	Medium	−0.029	0.075	−0.384	0.705
		Low	Medium	−0.060	0.064	−0.935	0.360
	Age category			−0.017	0.021	−0.800	0.430
	Sex	Female	Male	−0.004	0.056	−0.073	0.942
	Nitrogen			0.013	0.019	0.677	0.501
	C-peptide			0.002	0.002	1.265	0.209
Lipid peroxides	Exposure zone	High	Low	0.001	0.119	0.005	0.996
		High	Medium	−0.142	0.139	−1.098	0.286
		Low	Medium	−0.143	0.111	−1.288	0.212
	Age category			−0.035	0.038	−0.910	0.371
	Sex	Female	Male	−0.126	0.100	−1.258	0.223
	Nitrogen			0.037	0.031	1.182	0.240
	C-peptide			0.013	0.005	2.679	**0.008**

Chimpanzees from the high exposure zone had higher urinary concentrations of 8-OHdG (marker of oxidative DNA damage) than chimpanzees from both the low and medium exposure groups, whose levels of oxidative DNA damage were similar (Table 2, Fig. 2D). Females (log10 transformed: 2.95 ± 0.20) had higher concentrations of oxidative DNA damage than males (log10 transformed: 2.75 ± 0.19; estimate -0.200 ± 0.072, $p = 0.011$). In addition, individuals with higher C-peptide concentrations tended to have significantly higher levels of oxidative DNA damage (estimate 0.007 ± 0.002, $p = 0.007$) and lipid peroxides (estimate 0.013 ± 0.005, $p = 0.008$). Neither age category nor nitrogen level was significantly associated with oxidative DNA damage (Table 2).

Levels of urinary 8-isoprostanes (Fig. 2E) and of urinary lipid peroxides (Fig. 2F) were similar across the three exposure groups. Both markers were not significantly associated with any other factor included in the models (Table 2).

Discussion

We found that the exposure to chemicals differed among wild chimpanzees in relation to their ranging proximity to croplands. We recorded a higher number of chemical compounds used in agriculture in the Northern-Western side compared to the Eastern side of the chimpanzee home

range. Moreover, during the study period, the number of pesticides found in each of the three sampled locations was rather stable across the three surveyed years. As predicted, isotopic signatures and oxidative stress level differed between wild chimpanzees according to their ranging patterns, i.e., their proximity to crop fields and their maize consumption.

Chimpanzees from the different levels of exposure do not have the same diet composition as confirmed by slightly lower carbon intake for chimpanzees in the highly exposed group. We expected that chimpanzees consuming more maize (C4 plant) would have more of the heavy isotope of carbon than those having a higher proportion of wild fruits and leaves of C3 plants. Our results do not appear to support a strong significant intake of maize in the high exposure zone. However, the variable feeding patterns and diet of chimpanzees might hide any variation in corn intake among sites. This topic needs to be further investigated and compared to other exposure groups to better understand the modified C composition in chimpanzee urine samples. In contrast to carbon, nitrogen level was independent from field proximity and maize consumption, showing that crop consumption does not provide a significant protein contribution to the diet during the study period. In terms of animal proteins, the Sebitoli chimpanzee intake is generally low because they do not feed on insects and rarely hunt vertebrate prey, regardless of their preferred ranging areas (Krief pers. comm.).

The urinary concentration of the DNA metabolite 8-OHdG (marker of oxidative DNA damage) was higher in chimpanzees who were more frequently ranging near the crops, in the Northern-Western areas of their home range (high exposure group). It was also higher in females than in males. Those individuals are thus possibly exposed to genotoxic compounds, and the fact that females are more exposed than males could explain fetal exposure and congenital malformations observed (Krief et al. 2014a, b; 2018). Both isoprostanes and lipid peroxides (markers of oxidative damage to lipids) did not differ among the three exposure groups, indicating that lipid peroxidation was not affected by the differences in the diet. The current results confirmed that feeding on maize does not make a significant difference in the carbohydrates and caloric intake for Sebitoli chimpanzees sampled as shown in a previous study (Couturier et al. 2022).

Although we have not analyzed the presence of natural genotoxic compounds in corn, we previously showed that pesticides like neonicotinoids -imidacloprid- are used locally for coating the maize seeds (Krief et al. 2017, 2020; Spirhanzlova et al. 2019). In this study, imidacloprid was found in each of the three water collection periods. Such family of agrochemicals frequently cause increased oxidative damage, decreased glutathione levels, and altered activity of key antioxidant enzymes (Wang et al. 2018). They may be responsible of an important oxidative stress and toxicity on non-targeted species (Thompson et al. 2020; Wang et al. 2018). Sebitoli chimpanzees are exposed to over 60 pesticides and their metabolites as evidenced by chimpanzee hair analysis (Krief et al. 2022). This study goes further since it confirms a polarization West–East of the exposure over time. Frequent feeding at the Western and Northern parts of Sebitoli area where maize but also soil, water, or other plants are directly contaminated with agrochemicals, is likely responsible for the increase of the oxidative DNA damage marker (8-OHdG) as shown in the high exposure group. In addition, this may likely be responsible for the abnormal congenital facial phenotypes observed in 16 individuals of the community and the absence of sexual cycling observed in three adult females (Krief et al. 2017, 2018; 2014b). The level of exposure and the differences observed in 8-OHdG are consistent with the observed distribution of facial deformities that are more frequent in infants from Northern-Western mothers.

Because chimpanzees from Eastern, Western, and Southern parts of the Sebitoli are likely equally directly exposed to the pollution of the road (i.e., plastics) when crossing it or feeding alongside (Fig. 1), we exclude here that road pollutants may play a role in the abnormal phenotypes. However, indirect exposure through river water flowing from North to South can differ for individuals having core area in the Northern part of the road (not exposed) compared to individuals ranging in South (exposed). Such hypothesis has to be further explored by analyzing plastic pollution in the different sampling points. Chimpanzees may also be indirectly exposed to pesticides when they feed on plants at the edge of the farmlands or growing at the riverbanks, as suggested for mountain gorillas in Western Uganda, who feed on leaves containing detectable levels of ΣDDTs, α-endosulfan, and β-endosulfan (Amusa et al. 2021). The fact that the compounds detected in Mpanga River over the three study years, were consistently found both in the North-Western and Southern sampling points of the river shows that the water is likely constantly polluted by agricultural compounds. Since the water sampled at 5 km from croplands contained the same substances when compared to the water sampled within the croplands, wildlife may be exposed to chemicals also when feeding far away from the croplands and near the riverbanks. The nutritional composition of corn may also have an impact on cellular damage. We found higher levels of the markers of oxidative DNA damage and of lipid peroxidation in individuals with higher C-peptide concentration, indicating that a larger food intake might result in higher molecular oxidation. However, our models about the impact of exposure zone on oxidative DNA damage were robust for this potential effect of nutritional composition because we controlled for C-peptide by inserting it as a covariate.

In conclusion, our study shows that chimpanzees ranging in proximity of agricultural fields have higher levels of a urinary marker of oxidative DNA damage, but not of lipid peroxidation markers. This indicates that chimpanzees might be exposed to genotoxic compounds, which could include pesticides used for crops or other unidentified sources of stress. Further surveys would be necessary to fully understand the effect of agriculture encroachment on natural forested habitat, both on wildlife behavior and health. This may be relevant for the conservation of endangered species, particularly our closest relatives, the great apes who may also serve as sentinels for human health. In that context, our survey points out the necessity to plan actions to reduce the excessive use of pesticides for the health of local human and wildlife populations, in the Kibale National Park and in regions exposed to similar conditions.

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Author contribution Conception and design of the study were performed by Shelly Masi, David Costantini, Sabrina Krief, Petra Spirhanzlova, and Chloé Couturier. Coordination for the data acquisition, administration, and authorization were done by Eric Okwir and Edward Asalu. Data and sample collection were performed by Sabrina Krief and Chloé Couturier. Experiments and analyses were performed by David Costantini and Paco Bustamante. Interpretation of data and draft of the manuscript were done by Sabrina Krief, David Costantini, and Shelly Masi. Chloé Couturier, Eric Okwir, and Paco Bustamante reviewed, commented, and edited on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data could be communicated upon demands to the corresponding author.

Declarations

Ethical approval Sebitoli chimpanzees are monitored without employing invasive methods and without any interactions with SCP team. SCP adhered and followed the guidelines of the Uganda Wildlife Authority (UWA). The SCP research is conducted in the context of the Memorandum of Understanding MNHN/UWA/Makerere University SJ 445–12.

Consent to participate NA.

Consent for publication NA.

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