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Environmental Pollution through Multiple Integrated Hazard Sources in their Shared Environment: A Case Study of Muvumba Community, Rwanda

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Abstract

A community-based One Environmental Health Toxicology (OEHT) study is a holistic approach to examine environmental toxicant exposure in vulnerable communities. It is a novel approach evaluating health effects of environmental toxicant exposure on individuals and/or whole communities, in concert with addressing the health of animals, and of the ecosystem. This approach is novel in Africa. This study first identified a vulnerable community in Rwanda and investigated environmental pollutants impacting the health of the community. The objective of the study was to determine environmental contaminants impacting the Muvumba community, a vulnerable agricultural community in Rwanda. The study was conducted in a vulnerable agricultural community with significant pesticide and fertilizer use and linked to the Akagera-Nile water ecosystem. The Rwanda Institute for Conservation Agriculture (RICA) campus employing conservation agriculture and One Health (OH) principles was selected as a control site. Environmental water was passively sampled for pesticides and harmful algal blooms. Grab drinking water samples were collected from both sites and analyzed for pesticides, organic contaminants, and heavy metals. Rice from Muvumba was analyzed for pesticides and metallic elements. Environmental water samples from Muvumba river tested positive for three organophosphorus pesticides (diazinon, malathion, and profenofos), while imidacloprid was detected in water samples from RICA. Microcystins were not detected in any of the water bodies. Environmental and drinking water samples tested positive for diisononyl phthalate, bis(2-ethylhexyl) phthalate, and ricinine. Lead and arsenic were detected in rice samples from the Muvumba site. The findings of this community based OEHT indicate a significant public health and environmental concern and are vital in advancing a holistic integrated one-health research approach to safeguard human, animal and ecosystem health and ensure sustainable development in Africa.

Keywords: *Vulnerable community, One Health, Environmental Toxicology, Agricultural runoff, Rwanda, Pesticides, Heavy Metals*

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Introduction

The fundamental principle of One Health (OH) is that the health of humans and that of domestic and wild animals, plants, and ecosystem is interlinked. A healthy environment is essential for the health of all biotas. Rooted in a multisectoral, transdisciplinary framework, the OH model seeks to optimize health of people, animals, plants and the environment through transdisciplinary collaborations spanning local, national, and global levels (Pungartnik *et al.,* 2023). Closely linked with OH is the exposome, which refers to the totality of exposures from conception and throughout the lifespan. Exposomics is an emerging area of research with the goal of understanding how environmental exposures impact all species in their shared environment (Vineis *et al.,* 2020). Until recently, studies in environmental toxicology have focused on studying toxic effects of selected toxicants on individual species or on individuals rather than at the population or community level. Moreover, most of that research has focused on understanding exposures to single compounds or to a single class of related compounds on human or animal health (Gao, 2021). However, the reality is that in the environment, people, animals, plants and the ecosystem are co-exposed to multiple toxicants at once and the health outcome of such exposures is likely to reflect potential interactions among the toxicants in the shared environment. Moreover, other environmental stressors such as climate change, also modulate outcomes of toxic environmental exposures, further complicating the picture out health outcomes. This type of environmental exposure, over the lifespan of individuals and communities (exposomics), cannot be easily reproduced in the laboratory and needs to be conducted in the field under natural conditions.

Disproportionately, environmental toxicant exposures impact marginalized and vulnerable communities throughout the world (Moe *et al.,* 2013) . This is partly because of environmental injustice (Gochfeld and Burger, 2011) . Human contact with environmental toxicants occurs through polluted soil, water, air and contaminated food. From the OH perspective, domesticated and wild animals in the same community and the ecosystem are impacted as well. In most cases, animals in the shared ecosystem are impacted earlier and more severely than humans in the shared environment because they happen to be more sensitive or are exposed at a higher rate than humans, thus serving as biosentinels (Shan Neo and Tan, 2017). There are historic examples of this such as the cats of Minamata disease (Eto *et al.,* 2001) . Yet, often, there is a lack of a holistic OH approach in environmental toxicology research. This study investigated the impact of environmental toxicants on the health of the community using a holistic community-based approach, the One Environmental Health Toxicology (OEHT) approach, broadly defined as the health of people, animals, environment and of the ecosystem in a given shared environment. This approach is essential and critical for protecting the environment and transforming the health of the entire community, i.e. people, animals, environment and the ecosystem. This novel approach in OEHT research is particularly relevant for rural and underserved communities throughout the world because such vulnerable communities are likely to be exposed to environmental toxicants in their shared environment from generation to generation because such communities stay permanently in contaminated environments, remaining constantly exposed. It is also essential to ensure sustainable development.

For this study we chose to work in Rwanda. This East African country, often referred to as the "land of a thousand hills," is the most densely populated on continental Africa, with a population of over 13 million people, the majority of whom (80%) depend on agriculture for their livelihoods (Singirankabo *et al.,* 2022). Here there is a critical need to balance food security and environmental sustainability. Like other parts of the world, Rwanda is currently negatively impacted by climate change including droughts. To increase food production, local farmers have resorted to intensive agriculture using irrigation and agricultural chemicals including fertilizers, herbicides, insecticides, and fungicides. These inputs are often misused, resulting in environmental pollution because of agricultural runoff (Ndayambaje *et al.,* 2019). Eutrophication of water bodies from agricultural runoff fertilizer is one major cause of growth of harmful algal blooms (HABs). There is hardly any published literature on HABs and their toxins in Rwanda. This study was also interested in detecting microcystins as these are the HABs toxins most commonly encountered in fresh water bodies around the world. The long-term goal of this project is to conduct a community based OEHT research in a chosen vulnerable community negatively impacted by environmental toxicant exposure in Rwanda for many years to come with the ultimate goal of doing interventions to simultaneously improve the health of people, animals, the environment and the ecosystem in that community. There is scanty information on OEHT or exposomics research in tropical Africa (Ayeni *et al.,* 2022). The goal of this initial study was to identify OH environmental toxicological issues impacting a vulnerable community in Rwanda with a future goal of conducting exposomics research in humans and/or sentinel species.

Materials and Methods

Site Selection

The study site in Rwanda was selected to meet the following criteria: 1) perceived high level pollution of soil, water, the food web, and/or air; 2) the environmental pollution affecting human, animal, plant, and ecosystem health through multiple and integrated sources of hazards in their shared environment; 3) existence of multiple exposure pathways connecting the environmental pollution to human, animal, plant, and ecosystem health; 4) potential for combined health impacts across multiple sectors including the human health, animal health, soil health, crop health, insect health (e.g. honey bees), and fish and wildlife health, and the health of the ecosystem in general; 5) potential for climate change degrading human, animal, plant and environmental health; and 6) signs of community vulnerability e.g. presence of hunger, human and animal diseases and perceived negative health effects on the ecosystem.

The Muvumba rice scheme project site (hereafter referred to Muvumba site) located in the sectors of Tabagwe, Nyagatare and Rwempasha, Nyagatare district, Eastern Province was selected as the vulnerable community and study site (Figure 1). At the Muvumba site, people live in this agricultural community from generation to generation and this makes it a suitable study site to understand cumulative and transgenerational effects of toxic environmental exposures prevalent in this area. This site meets the aforementioned criteria and was selected for this study after a focus group discussion with local OH experts. The Muvumba valley region has been developed into a mega rice growing scheme with goal of increasing food security for Rwanda. It has an area of 10.5 km^2 with an estimated population of 725,068. In addition to rice, some of the community members grow vegetables such as cabbages, tomatoes, and eggplants, while others engage in beef, dairy and apiary farming. Rice hay is used as livestock feed. A major OH concern is the indiscriminate heavy use of pesticides and fertilizers in this region intended to boost agricultural productivity. Runoff from the fields enters and pollutes the Muvumba river. This river is a lifeline for the community and is used for crop irrigation, watering livestock, and for subsistence fishing by the locals. Ultimately, the Muvumba river drains into the Akagera water system, flowing through the Akagera National Park, and ultimately connecting to the Lake Victoria. Drinking water for the Muvumba community is drawn from shallow underground wells (boreholes) located within rice fields and is at risk of groundwater contamination from the heavy fertilizer and pesticide use on the farms. Livestock drink directly from the Muvumba river or from the irrigation canals. Water from the Muvumba river is also used for recreational purposes such as swimming, and for crop irrigation because the region is prone to droughts exacerbated by climate change. Wildlife such as water birds and insects, including honey bees and snails are among the many biota that depend on the ecosystem. Malaria and schistosomiasis are the most rampant human diseases in this community, especially among children. For the control site we selected the campus of Rwanda Institute for Conservation Agriculture (RICA) [hereafter referred to RICA site] in Bugesera district, Eastern Province. The RICA philosophy is based on conservation agriculture and OH principles. RICA has demonstration farms and advocates for the judicious use of pesticides and other agricultural inputs along with other sustainable agricultural practices. RICA grows a variety of crops including beans, alfalfa, and fruit trees such as mangoes. Livestock production at

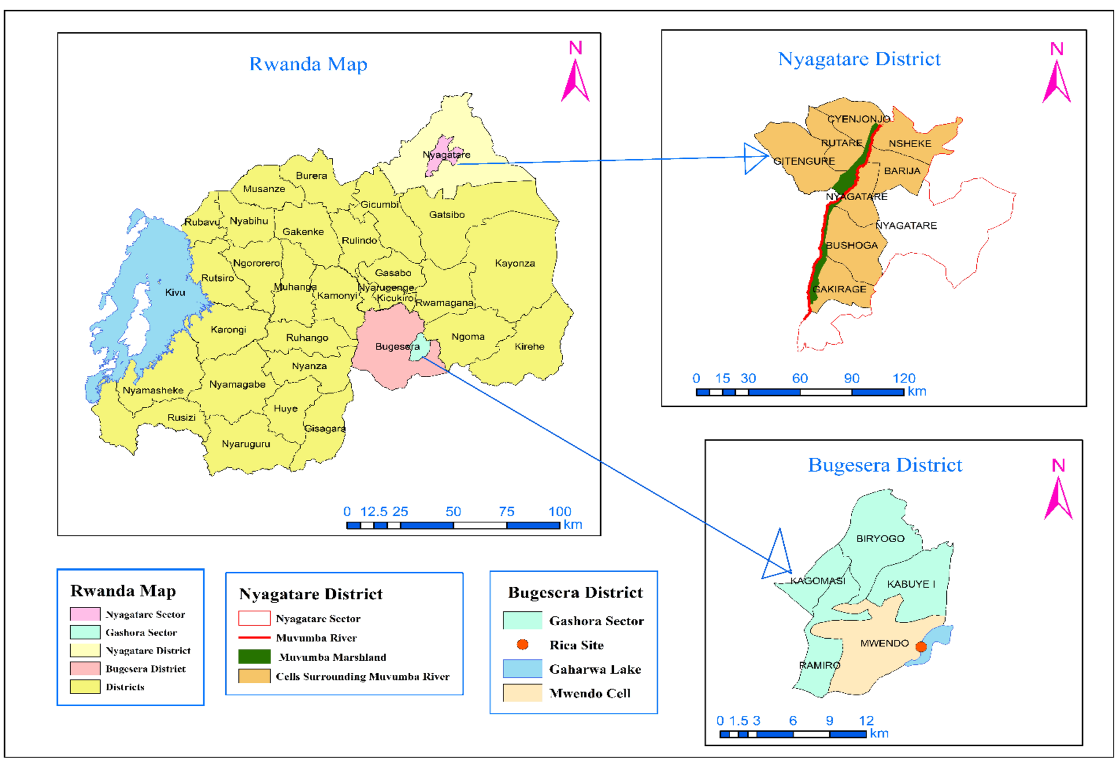
RICA includes dairy cattle and apiculture. Modern irrigation equipment are used at this site. All wastewater from RICA is channeled to a wastewater treatment facility before discharge into Gaharwa Lake through canals/streams.

Environmental sampling for toxicants

The environmental sampling activities were conducted in mid-July 2023, coinciding with a low peak season for pesticide and fertilizer application as it was the harvesting season. The peak season for pesticide and fertilizer application is January throughout March. Water and rice were sampled. Polar Organic Chemical Integrative Samplers [POCIS], (Environmental Sampling Technologies, Inc. (St. Joseph, MO) were used for passive sampling of pesticides in water. POCIS devices were deployed in running water of the Muvumba river for two weeks to passively adsorb waterborne pesticide contaminants from agricultural runoff. The POCIS samplers were placed inside small baskets which were tied to stationary objects to prevent them from being carried away by water currents. Eight POCIS devices were deployed at each of the study sites, i.e. in Muvumba and RICA. At the RICA site, POCIS were submerged in water at the shores of Gaharwa Lake. All devices were submerged at a depth of one meter. After the exposure period, the POCIS devices were carefully removed and rinsed with double distilled water to remove debris. The cleaned POCIS samplers were then wrapped in aluminum foil, placed in their original shipping containers and shipped to the California Animal Health and Food Safety Lab (hereafter referred as Lab) at the University of California – Davis (UC-Davis, California) on ice at approximately 4°C. In the laboratory, POCIS devices were stored at -20°C until extraction for analysis.

Figure 1

Map of the study sites, including the Muvumba site in Nyagatare District, and the RICA site in Bugesera District.



Environmental sampling for harmful algal bloom toxins

For environmental sampling of these toxins Solid Phase Adsorption Toxin Tracking (SPATT) passive sampling devices using Diaion® HP20 (Mitsubishi Chemical Co., Tokyo, Japan) was used as the adsorbent resin. Diaion® HP20 is the most commonly used substrate for binding microcystins (Roué *et al.,* 2018) .

The SPATT devices were assembled in the field as follows: The adsorbent resin (3 g) was placed between two 10.16 cm x 10.16cm pieces of mesh which were secured together using embroidery hoops. These SPATT samplers were activated before deployment by submerging them in methanol (Thermo Fisher Scientific, Waltham, MA) for 24 h. The samplers were then removed from the methanol and thoroughly rinsed in deionized water. This involved repeatedly agitating the samplers in deionized water-filled beakers, replacing the water until no increase in water temperature occurred upon contact with the SPATT resin, indicating complete removal of residual methanol. The activated SPATT samplers were stored in zip lock bags filled with deionized water and refrigerated to maintain stability until deployment. For deployment, the SPATT samplers were placed in small baskets similar to those used for POCIS devices (Figure 2). These SPATT devices were submerged beneath the water surface at the study sites for two weeks. The baskets were secured by tying them to stationary objects. Upon retrieval, the SPATT passive samplers were carefully rinsed in field water to remove silt and debris and then wrapped in aluminum foil. The samplers were then placed into labeled zip lock bags and shipped to the lab on ice (4°C). Upon arrival in the lab, the samplers were store at -20 ^{0}C until extraction for analysis.

Figure 2

Small basket enclosure containing POCIS. For deployment, the basket was tied to stationary objects placed in water to passively adsorb waterborne pesticide contaminants

Sampling for contaminants in human drinking water and rice

Water samples from the study sites, 250 mL each, were collected using plastic water bottles. At the Muvumba site, a single water sample was taken from a shallow well (borehole), while at the RICA control site, three samples were randomly collected from running canal/stream water at various locations, including effluent from the wastewater treatment plant. Those samples from both study sites were analyzed for the presence of chemical pollutants using GC-MS and LC-MS analysis. For rice, approximately 100 grams of rice samples each were collected from various blocks within the Muvumba site during the harvesting period. The samples were collected randomly and stored in sealed plastic bags. Rice was not collected from the RICA control site as it is not grown there. Both water and rice samples were shipped on ice to the lab at 4 ^{0}C and were stored in the lab at -20 ^{0}C until analysis.

Preparation of POCIS samples for LC-MS and GC-MS analysis

In the lab the POCIS assemblies were disassembled, and the resin was transferred to 6 mL Solid Phase Extraction (SPE) cartridges with minimal water. Fortified and unfortified quality control (QC) cartridges were prepared with Oasis HLB resin (Waters Co., USA). After gravity-draining excess water, a 25 µL internal standard (10 µg/mL d10-Diazinon (Syngenta AG, Switzerland) was added. The elution was performed with 30 mL of methanol followed by 10 mL of acetonitrile (Thermo Fisher Scientific, Waltham, MA), and the collected eluents were mixed with 1 g of sodium sulfate (Thermo Fisher Scientific, Waltham, MA), centrifuged, and divided into two aliquots for Liquid chromatography-mass spectrometry (LC-MS) and Gas chromatography–mass spectrometry (GC-MS), analysis. The samples were dried under nitrogen at 40 °C, redissolved in methanol (160 µL) and 0.1% formic acid (Thermo Fisher Scientific, Waltham, MA) in water (340 µL) for LC-MS, or in 500 µL of methanol for GC-MS analysis, followed by filtration through a 0.22 µm syringe filter(Thermo Fisher Scientific, Waltham, MA). LC-MS/MS analysis was done using a Thermo Ultimate 3000 LC system (Thermo Fisher Scientific, Waltham, MA) connected to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) in ESI positive mode, with data searched using an in-house library with over 200 toxins. The GC-MS analysis was done using an Agilent 7890 B GC with a 5977A MSD with data screened using commercial GC-MS libraries.

Preparation of SPATT resins for LC-MS/MS analysis of microcystins

In the Lab the SPATT devices were disassembled, and the resin was transferred into a 20 mL syringe barrels equipped with 1 µm syringe filters and connected to a vacuum SPE manifold. Gravity was used to drain any excess water, leaving the resin wet. Each resin sample underwent three extractions using 10 mL and two 20 mL portions of 50% methanol in water, with the extracts collected separately. The columns were not allowed to run dry until the final extraction. Fortified and unfortified quality control samples were prepared with Diaion HP-20 resin, spiked with 50 ng each of Microcystin-LR, -LA, -YR, and -RR (-RR not detected by this method). The extracted resins were air-dried for 40 h and weighed to determine reporting limits (50 ng/dry resin weight). The extracts were then analyzed using an Agilent 1290 LC System coupled with a SciEx 6500 QTrap mass spectrometer, employing a Polar-RP 150 x 4.6 mm column with mobile phases of 0.1% formic acid in water and 0.01M ammonium acetate in 0.1% formic acid in methanol at a flow rate of 500 µL/min of 75% B for 3 min, a gradient to 95% B over another 3 min, and holding at 95% B for an additional 6.5 min. Enhanced product ion scan mode was used and data were analyzed using SciEx OS software to detect Microcystin-LR, -LA, and -YR through comparison with reference standards.

Preparation of rice samples for trace mineral and heavy metals analysis

For trace mineral and heavy metals analysis by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, Santa Clara, CA), rice samples were homogenized as needed and 0.5g of each sample digested with 3mL of nitric acid following the hot block program protocol (Table 1). After the digestion was completed, 2mL of hydrochloric acid were added and sample settled for 30 min before bringing the volume to 10mL with 18Mohm water. Samples were filtered with 25mm glass fiber syringe filters (1 µm pore size) and 0.25mL of filtrate were removed and diluted to 5mL with a diluent that includes internal standards and acids to matrix match (i.e., ensure that a samples, standards and/or control) have the same chemical composition, physical properties and matrix effects during analysis) the samples to the calibration curve. This ensures the accuracy and reproducibility of analytical results.

The samples were then analyzed using an Agilent 7900 ICP-MS (Agilent Technologies, Santa Clara, CA). Quality control samples were run with each set of samples. These included a method blank, laboratory control spike (fortified method blank), and standard reference material (SRMs). To ensure instrument drift was not more than 10% throughout the run, a continuing calibration verification and blank were run every 10 samples. Samples were prepared in triplicate and results reported as an average of replicates.

Results

Results of pesticides and other chemical pollutant analysis in water samples

Results of POCIS analysis by LC-MS from devices deployed in the Muvumba river and RICA (L. Gaharwa) sites are summarized in Table 2. All POCIS samples from the Muvumba river site tested positive for three organophosphorus pesticide compounds (i.e, diazinon, malathion, and profenofos). In contrast, all POCIS samples collected from the RICA site tested negative for pesticide or other chemical pollutants. Of the three rice samples collected from three different blocks in Muvumba site, only one sample tested positive for only nicotine (Table 2)

Table 1

Hot block program parameters for acid digestion of samples for heavy metals analysis

Temp (°C)	Ramp (°C /min)	Time (min)
50	1	120
80	1	60
200	1	90

Table 2

A summary of pesticide and other chemical pollutant analytical results of samples collected from Muvumba and RICA sites

Sample ID	Collection Site	Results	Analytical procedure
POCIS (2-10)	Muvumba	Diazinon, Malathion, Profenofos,	LC-MS Screen
POCIS (11–18)	RICA	Tested negative	LC-MS Screen
POCIS (2)	Muvumba	Limonen	GC-MS Screen
Rice samples	Muvumba	Nicotine	LC-MS Screen

Table 3

Chemical Pollutants Detected in Water Samples from Muvumba and RICA sites

Sample ID	Collection Site	Analyte	Analytical procedure
W-1	Muvumba	Diisononylphthalate	GC-MS
W-2	RICA	Diisononylphthalate	GC-MS
W-3	RICA	Diisononylphthalate	GC-MS
W-4	RICA	Bis(2-ethylhexyl) phthalate	GC-MS
W-4	RICA	Caffeine	GC-MS
W-2	RICA	Caffeine, Imidacloprid, Ricinine	LC-MS

W-3	RICA	Caffeine	LC-MS
W-4	RICA	Caffeine and theobromine	LC-MS

Table 4

Trace and Heavy metals concentrations in rice samples collected from Muvumba site

Element	Sample 1 (mg/kg)	Sample 2 (mg/kg)	Sample 3 (mg/kg	Reporting limit (mg/kg)
Arsenic	0.2	0.12	0.2	0.1
Barium	2.1	0.51	1.5	0.1
Chromium	0.22	Not detected	Not detected	0.1
Copper	3.4	3.2	1.6	0.1
Iron	110	67	61	0.2
Lead	0.13 (130 ug/kg)	Not detected	Not detected	0.1
Manganese	60	44	50	0.1
Molybdenum	0.52	0.34	0.20	0.1
Nickel	0.23	0.29	0.11	0.1
Selenium	Not Detected	0.34	Not detected	0.1
Vanadium	0.36	0.27	0.29	0.1
Zinc	17	19	14	0.2

With regard to GC-MS test results, one POCIS sample deployed in Muvumba river tested positive for Limonen (Table 2). No other organic toxic compounds were found by GC-MS in POCIS samples deployed at any other sites in the Muvumba river or from those deployed at the control RICA site.

Water (W-1) from the Rwempasha borehole (Muvumba site) used by the community for drinking water tested positive for diisononyl phthalate by GC-MS analysis (Table 3). Also, water collected from the RICA site that discharges into Gaharwa Lake tested positive for various analytes by GC-MS (Table 3). Diisononylphthalate was detected in samples W-2 and W-3 collected from the RICA site (Table 3). Water sample W-4 also from the RICA site tested positive for bis(2-ethylhexyl) phthalate and caffeine. Analysis by of the water samples by LC-MS detected caffeine, imidacloprid, and ricinine in sample W-2 from RICA site. Caffeine was also detected in samples W-3 and W-4 also from the RICA site (Table 3). Sample W-4 also tested positive for theobromine by LC-MS (Table 3).

Results of trace and heavy metal analysis in rice samples

Results of rice samples collected from the Muvumba site in Nyagatare are shown in Table 4. Since rice is not grown at the RICA site, there is no data from the control site. Arsenic was detected in all three samples, with concentrations ranging from 0.12 to 0.20 mg/kg (ppm). Barium was present in all three samples, with the highest concentration in Sample 1 (2.10 mg/kg) and the lowest in Sample 2 (0.51mg/kg). Chromium was detected only in Sample 1 (0.22 mg/kg). Copper was consistently detected in all samples, ranging from 1.6 ppm in Sample 3 to 3.40 mg/kg in Sample 1. Of all the elements analyzed, iron was found in highest concentrations, with concentrations ranging from 61 ppm in Sample 3 to 110 ppm in Sample 1. Lead was detected only in Sample 1 (130 ug/kg [ppb]). Manganese concentrations ranged from 44 mg/kg in Sample 2 to 60 mg/kg in Sample 1, while molybdenum was detected in all samples, ranging from 0.52 mg/kg in Sample 1 to 0.20 mg/kg in Sample 3. Nickel was also present in all rice samples, with concentrations varying between 0.11 mg/kg in Sample 3 and 0.29 mg/kg in Sample 2. Selenium was only detected in Sample 2 at 0.34 mg/kg. Vanadium concentrations were relatively consistently present across all samples, ranging from 0.27 mg/kg in Sample 2 to 0.36 mg/kg in Sample 1. Zinc was detected in all three samples, with the highest concentration in Sample 2 (19 mg/kg) and the lowest in Sample 3 (14 mg/kg).

SPATT microcystin results

All SPATT samples deployed at the Muvumba and RICA sites tested negative for microcystin variants (LA, LR, YR) at the reporting limit of 9.9 to 13 ng/g.

Discussion

A previous report indicated heavy pesticide use in the study site (Ndayambaje *et al.,* 2019). Although the impact of use of pesticides on human, animal, and ecosystem health in Muvumba community is not known, the runoff from the expansive rice farms flows into the Muvumba river, with potential impact on the ecosystem. This river is connected to the fragile Akagera water system, which passes through the Akagera National Park and eventually feeds into Lake Victoria. Pollution in Muvumba can therefore have a significant negative impact on the health of people, domesticated and wild animals and the fragile ecosystems.

The detection of organophosphate pesticides diazinon, malathion, and profenofos in all POCIS samples from the Muvumba site suggests significant organophosphorus pesticide contamination in this area. These potent pesticides, widely used for agricultural purposes, can pose risks to aquatic ecosystems (Gan *et al.,* 2024; Ismail *et al.,* 2017)and human health (Kaur *et al.,* 2024; Kim *et al.,* 2017). Diazinon disrupts aquatic ecosystems (Ghasemzadeh *et al.,* 2015), affects pollinators (Weick and Thorn, 2002), and alters soil microbes (Singh and Singh, 2005), with human exposure causing neurological (Slotkin and Seidler, 2007; Timofeeva *et al.,* 2008) and developmental issues (Saraei *et al.,* 2023). Malathion is toxic to fish (Bharti and Rasool, 2021; Cook and Moore, 1976), amphibians (Relyea *et al.,* 2005), and bees (Tong *et al.,* 2023), disrupting pollination and potentially causing developmental delays and cancer in humans (Calaf *et al.,* 2021). Profenofos severely impacts aquatic organisms (Khan *et al.,* 2018), birds (Kushwaha *et al.,* 2016), and soil health (Mandal *et al.,* 2020), with human exposure leading to neurotoxicity, developmental toxicity, and immunosuppression (Tanveer *et al.,* 2024). The detected pesticides are officially registered for use in Rwanda (Rutikanga, 2015), however their presence in environmental samples suggests that they are potentially misused. Misuse of the pesticides could reduce biodiversity, destabilize ecosystems, and pose health risks for humans and animals, highlighting the need for community education, stricter pesticide regulations, and a need to find safer pest control alternatives. Organophosphorus pesticides were not detected in water samples collected from the RICA control site where pesticides are used judiciously, suggesting that proper pesticide usage and management practices can effectively prevent pesticide residues from contaminating the ecosystem and entering the food chain. This finding illustrates the importance of responsible pesticide stewardship in mitigating environmental pollution and safeguarding both human and wildlife health.

Phthalates, endocrine disruptors, found in water collected at both the study and control sites suggesting a widespread contamination likely from plastic waste disposal, industrial runoff, agricultural inputs, or other unknown sources. The diisononylphthalate detected at Muvumba study site in a shallow water borehole used for human drinking water in Rwempasha sector is an indicator of direct human exposure, as the water source is used for drinking. This shallow water borehole is adjacent to the rice fields. Diisononylphthalate is a plasticizer commonly used in manufacturing flexible plastics (Shaw *et al.,* 2002). Its presence in drinking water indicates contamination, but the source is not clear. Chronic exposure to phthalates can disrupt endocrine functions and has been linked to developmental (Mu *et al.,* 2018), reproductive (Sedha *et al.,* 2021a), and carcinogenic effects in humans (Yang *et al.,* 2022). Therefore, contamination of drinking water by phthalates poses a significant public health concern. At the RICA site, diisononylphthalate was detected in all three canal water samples, suggesting environmental discharge, likely from the waste water treatment plant onsite. RICA collects all waste water and treats it before discharge into Gaharwa lake via canals/streams. One canal sample tested positive for bis(2-ethylhexyl) phthalate, another plasticizer with toxicological implications, including endocrine disruption (Sedha *et al.,* 2021b) and hepatotoxicity (Praveena *et al.,* 2018). In addition, caffeine, a common anthropogenic marker, was also found in multiple canal water samples. The presence of caffeine and theobromine suggests domestic wastewater discharge or ineffective water treatment processes. Caffeine is commonly found in water sources and can negatively impact aquatic ecosystems by interfering with their development, performance or reproduction (Li *et al.,* 2020).

The detection of imidacloprid, a neonicotinoid pesticide, in one water grab sample from RICA site canals indicates agricultural runoff. Imidacloprid is highly toxic to aquatic insects and pollinators (Schmuck and Lewis, 2016), which could disrupt the local aquatic ecosystem and harm biodiversity. Imidacloprid is used as an insecticide on crops and livestock. RICA has livestock, including cattle which require regular use of pest control to prevent tickborne diseases. These results indicate that imidacloprid is a potential environmental hazard on RICA campus even though it was not detected by POCIS sampling in Gaharwa Lake. The other water chemical contaminant found in the grab water samples at RICA was ricinine. Ricinine is an alkaloid derived from castor beans and its presence suggests the likelihood of ricin water contamination. The likely source of the ricinine is seeds of wild castor bean plants (*Ricinus communis*). Ricin is a highly potent toxic compound. Ricin exposure, even in small amounts, can have severe toxic effects on humans and animals, including inhibition of protein synthesis and organ failure (Polito *et al.,* 2019). Ricin (and likely ricinine) is very heat labile and would have been inactivated in the wastewater treatment plant before discharge into the canals. Therefore, this finding suggests that castor bean plants on the RICA campus can naturally contaminate water. Depending on the ricin concentration in water, this toxin could potentially negatively impact the ecosystem (CDC, 2011) .

The trace mineral and heavy metal analytical test results of rice samples collected from the Muvumba rice scheme also revealed significant levels of contamination. Arsenic, detected in all samples at concentrations ranging from 0.12 mg/kg to 0.2 mg/kg (120 to 200 ppb) exceeds the United States Food and Drug Administration (FDA)'s acceptable limit for inorganic arsenic in rice, which is set at 0.1 mg/kg [100 ppb] (US FDA, 2020). Chronic exposure to arsenic through dietary intake poses severe health risks, including increased cancer risk, cardiovascular diseases, and developmental effects, particularly in vulnerable populations such as children and pregnant women (Rasheed *et al.,* 2016). Similarly, the presence of lead (0.13 mg/kg [130 ppb] in one sample) is concerning. The FDA guideline for lead in rice meant for infant children is 20 ppb because lead is a potent neurotoxin with no safe exposure threshold (US FDA, 2023). The high levels of iron (61–110 mg/kg) also raise human health concerns, as these values significantly surpass typical dietary guidelines (2 – 6 mg/kg in unpolished rice) and may indicate environmental contamination, potentially leading to iron overload in individuals consuming this rice as a staple. The FDA guideline for iron in fortified rice is 20-60 mg/kg, which is below the concentration of what was measured is samples from the Muvumba site. Regulatory agencies such as World Health Organization (WHO), United States Food and Drug Administration (FDA) , European Food Safety Authority (EFSA) and others, have established different reference values for heavy metals to guide daily maximum safe exposure levels. These are as follows: inorganic arsenic: 0.3 – 2.14 ug/kg/day, cadmium: 0.1 ug/kg/kg (Wong *et al.,* 2022), vanadium 9 ug/kg/day (ATSDR, 2024). These levels are potentially exceeded in the study area, posing a food safety risk. The detected presence of trace minerals and heavy metals in rice suggests they are present in high concentrations in local geological formations, contamination of agricultural soils, contaminated fertilizers, and/or contaminated Muvumba river water used for irrigation. Unfortunately, this study did not do a heavy metal analysis of the irrigation water. Over time, trace mineral and heavy metal contamination may degrade soil quality, disrupt microbial communities, and reduce agricultural productivity (Fulke *et al.,* 2024).

Microcystins were not found in any of the water samples. The absence of microcystins (LA, LR, and YR) in all samples from both Muvumba and RICA sites suggests a low risk of algal toxin contamination during this sampling period. Results indicate that the water bodies studied were not experiencing harmful algal blooms at the time. However, harmful algal blooms and fish die-offs have been reported in Rwanda. More work is recommended in these areas. Considering the heavy use of fertilizers and the attendant agricultural runoff suggests HABs are likely a problem. The observation period should be extended to include other seasons.

Nicotine was also found in rice samples. Nicotine, a natural alkaloid, occurs in plants of the Solanaceae family (i.e., tomatoes, potatoes, aubergines) (Siegmund *et al.*, 1999). The presence of nicotine in the rice samples suggests a potential contamination from the rotational cropping system involving solanaceae family plants. The probable source of nicotine would be tobacco; however, cultivation of tobacco is prohibited in Rwanda. Therefore, the alternative source in this case might be human contamination from smokers.

This initial pilot study has yielded interesting results which indicate the complexity of environmental pollution in the studied region of Rwanda. Whereas we did not expect to find environmental contaminants at the control RICA site in Bugesera district, the presence of imidacloprid, phthalates, and ricinine was notable. Similarly, the absence of microcystins at the Muvumba site was interesting. These findings emphasize the importance of site-specific environmental monitoring and tailored mitigation strategies.

The study findings should be understood with consideration of some limitations that could impact their application in seeking solutions for pollution of the Mivumba river ecosystem and its impact on the community and downstream ecosystems. Notably, the study was conducted in the second half of July, which was off season for maximum pesticide and fertilizer use both at the control and study locations. The fact that presence of the highly toxic organophosphorus pesticides was detected off season in Muvumba is significant. These organophosphorus pesticides are not persistent in the environment. Therefore, their presence suggests a major contamination problem during the peak season. The second limitation of this study is that the pesticide concentration in water was not quantified because the project that supported the study in initial stages aimed to identify what was present in the environment. Future studies are needed to determine the concentration of these pesticides in the environment and to map their concentration all year long. Quantitative determination of these pesticides in the environment will help the interpretation of the environmental health risks associated with environmental pesticide pollution in Rwanda. A third limitation was the small sample size, especially of the rice and human drinking water samples. The rice farm is expansive and variations in trace and heavy metal contaminants from place to place within the farm is expected. A large study to investigate these issues is needed before final conclusions can be drawn. Nonetheless, results of this study have identified the environmental toxicants of concern for future exposome studies.

Conclusion

The study identified a community in Rwanda which is vulnerable to exposure to agricultural pollutants from agricultural runoff from the expansive rice fields in Muvumba site. This site is ideal for OEHT research as agricultural inputs likely negatively impact human, animal, plant and ecosystem health. It is an ideal site for OH interdisciplinary research on factors negatively impacting the health people, animals, plants, and the ecosystem. The study has shown that agricultural runoff is contaminating the Muvumba river with organophosphate pesticides. Water from this river is used for crop irrigation, for watering livestock, for fishing and recreational swimming meaning contamination with toxic substances could have disastrous effects on the health and welfare of the community. The drinking water used by the community in Muvumba site is contaminated with phthalates which are well known endocrine disruptors. It was also interesting that some of the rice was contaminated with arsenic and lead at levels that exceed FDA guidelines. Other elements such as iron and vanadium also exceeded FDA regulatory guidelines. This vulnerable community is likely impacted by multiple toxicant exposures through food, water and recreation. The findings serve as a foundation for advancing an integrated OH research approach to safeguarding human, animal, plant, wildlife and ecosystem health. It was also notable that the control RICA site in Bugesera district was also susceptible to environmental pollution from natural toxins like caffeine, theobromine, ricinine, and from the highly toxic neonicotinamide imidacloprid. This is the first research project at these community sites and additional studies are recommended to confirm these results before regulatory action can be taken. Future studies should also include human and animal exposome studies to understand the lifelong health impact of these environmental contaminants.

Ethical Approval

This study was conducted in compliance with institutional ethics requirements of the University of Rwanda (003/2023/DRI).

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