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Heavy metal contamination in urban agriculture and human health risk in Yaoundé, Cameroon

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Abstract

Heavy metals are ubiquitous in the environment. The sources of heavy metals are both from natural and anthropogenic activities. Urban growth, coupled with increasing demand for vegetables, has led to the utilization of lowlands for agricultural production in Cameroon and beyond that increased the level of heavy metals in environmental media. This study assessed the seasonal characteristics of heavy metals in foods and their potential health risks in the main areas of urban agriculture in Yaoundé, Cameroon. A cross-sectional survey of 130 vegetable producers in six lowland areas was conducted to gather insightful information about their farming practices. Heavy metals (i.e., Cr, Cu, Zn, Ni, Cd, and Pb) contamination in irrigation water, soil, and vegetables was investigated in four cultivated lowland areas in and around Yaoundé during the dry and wet seasons. The degree of contamination of the vegetable, using the leafy vegetable *Solanum nigrum* as a model species, was evaluated using the crop pollution index (Pi), and the bioaccumulation potential of metals was calculated using the bioaccumulation factor (BAF). The potential human health risk was assessed using the hazard quotient (HQ), hazard index (HI), and target cancer risk (TCR). The study found that the farming practices, specifically the use of pesticides and both organic and mineral fertilizers, were similar in all study areas. Except for the Mokolo area, all farmers utilized surface and groundwater for crop irrigation. Although the heavy metals concentration in the irrigation water was higher in the dry than wet season, all six heavy metals were found at levels below the WHO/FAO recommended limits, suggesting that the water was safe for crop irrigation. The soil analyses revealed that Cr concentration exceeded the permissible limit, while the Ni concentration was within the WHO/FAO limit in both seasons in all study areas. Cu, Zn, and Pb in agricultural soils were below the thresholds except for the Mokolo area. Contamination by heavy metals in plant and soil samples was higher than in irrigation water and was higher in plants than in soil samples. Most of the studied heavy metals exceeded the FAO/WHO thresholds in all study areas. Cd had the highest Pi, and Zn the highest BAF in both seasons and all studied areas. The HQ of Cd, Cr, and Cu for all areas, as well as Zn for Mokolo was >1, suggesting the probability of adverse effects. The HI >10 for most areas indicates a high probability of health risks that can be chronic or acute. The TCRs for Cd, Cr, and Ni for all areas as well as Pb for the Mokolo area through the consumption of *S. nigrum* also exceeded the recommended WHO thresholds. The magnitude of human health risks per area was Mokolo > Ekoumdoum > Ekounou > Nkolbisson. These results indicate that the edible parts of *S. nigrum* produced in these lowlands were unsafe for human consumption and might lead to acute or chronic health risks. This study highlights the importance of seasonal and site-specific health risks regarding heavy metals exposure for urban consumers in Yaoundé. It serves as a case study for short-term and indicates the possibility for long-term adverse effects for other contaminant incidents. It calls for more attention on human health hazards via the food chains and more efforts to solve the outstanding environmental issues affecting population health and well-being of urban communities in Africa.

Zusammenfassung

Schwermetalle sind allgegenwärtig in der Umwelt. Die Quelle für Schwermetalle sind sowohl natürlich als auch von Menschen verursachte Aktivitäten. Das Wachstum der Städte und die damit einhergehende steigende Nachfrage nach Gemüse haben dazu geführt, dass u.a. Tieflandgebiete in Kamerun immer stärker für die landwirtschaftliche Produktion genutzt werden, was zu einem Anstieg der Schwermetallkonzentration in der Umwelt geführt hat. In dieser Studie wurden die saisonalen Merkmale von Schwermetallen in Lebensmitteln und ihre potenziellen Gesundheitsrisiken in den wichtigsten landwirtschaftlichen Anbaugebieten in Yaoundé, Kamerun untersucht. Es wurde eine Querschnittsbefragung von 130 Gemüseproduzenten in sechs Tieflandgebieten durchgeführt, um Informationen über ihre Anbaupraktiken zu erhalten. Die Verunreinigung mit Schwermetallen (d.h. Cr, Cu, Zn, Ni, Cd und Pb) im Bewässerungswasser, im Boden und im Gemüse wurde in vier Tieflandgebieten in und um Yaoundé während der Trocken- und Regenzeit untersucht. Der Kontaminationsgrad des Gemüses wurde bei der Blattgemüseart *Solanum nigrum* als Modellpflanze mit Hilfe des Verschmutzungsindex (Pi) bewertet, und das Bioakkumulationspotenzial der Schwermetalle wurde mit dem Bioakkumulationsfaktor (BAF) berechnet. Das potenzielle menschliche Gesundheitsrisiko wurde anhand des Gefahrenquotienten (HQ), des Gefahrenindex (HI) und des Zielkrebsrisikos (TCR) bewertet. Die Studie ergab, dass die landwirtschaftlichen Praktiken, insbesondere der Einsatz von Pestiziden sowie von organischen und mineralischen Düngemitteln, in allen Untersuchungsgebieten ähnlich waren. Mit Ausnahme von Mokolo nutzten alle Bauern Oberflächen- und Grundwasser für die Bewässerung ihrer Felder. Obwohl die Schwermetallkonzentration im Bewässerungswasser in der Trockenzeit höher war als in der Regenzeit, lagen die Werte aller sechs untersuchten Schwermetalle unter den von der WHO/FAO empfohlenen Grenzwerten, was darauf hindeutet, dass das Wasser für die Bewässerung von Nutzpflanzen sicher war. Die Bodenanalysen ergaben, dass die Cr-Konzentration den zulässigen Grenzwert überschritt, während die Ni-Konzentration in beiden Regenzeiten in allen Untersuchungsgebieten innerhalb der Grenzwerte lag. Die Cu-, Zn- und Pb-Konzentrationen in landwirtschaftlich genutzten Böden lagen unter den Grenzwerten, außer in Mokolo. Die Schwermetallbelastung in Pflanzen- und Bodenproben war höher als im Bewässerungswasser und in den Pflanzen- höher als in den Bodenproben. Die meisten der untersuchten Schwermetalle überschritten die WHO/FAO-Grenzwerte in allen Untersuchungsgebieten. Cd wies in beiden Regenzeiten und in allen untersuchten Gebieten den höchsten Pi-Wert und Zn den höchsten BAF-Wert auf. Der HQ von Cd, Cr und Cu für alle Gebiete, sowie der HQ von Zn für Mokolo war >1 , was auf mögliche schädliche Auswirkungen hinweist. Der HI-Wert >10 für die meisten Gebiete deutet auf eine hohe Wahrscheinlichkeit von Gesundheitsrisiken hin, die chronisch oder akut sein können. Die TCRs für Cd, Cr und Ni für alle Gebiete sowie Pb für Mokolo durch den Verzehr von *S. nigrum* überstiegen ebenfalls die empfohlenen WHO-Grenzwerte. Die Reihenfolge des Ausmaßes von menschlichen Gesundheitsrisiken in den Untersuchungsgebieten war Mokolo $>$ Ekoumdoum $>$ Ekounou $>$ Nkolbisson. Diese Ergebnisse deuten darauf hin, dass in diesen Tiefebene(n) produzierte *S. nigrum*, für den menschlichen Verzehr nicht geeignet ist und zu akuten oder chronischen Gesundheitsrisiken führen kann. Diese Studie unterstreicht die Bedeutung der saisonalen und

ortsspezifischen Gesundheitsrisiken durch Schwermetalle für die städtische Bevölkerung von Yaoundé. Sie dient als Fallstudie für kurzfristige und zeigt die Möglichkeit langfristiger schädlicher Auswirkungen bei anderen Kontaminationsereignissen auf. Sie unterstreicht die Gefahren für die menschliche Gesundheit über Kontaminationen in den Nahrungsketten. Gleichzeitig fordert sie zu mehr Anstrengungen auf, um die pressierenden Umweltprobleme zu lösen, die die Gesundheit und das Wohlergehen der städtischen Gemeinschaften in Afrika bedrohen.

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List of Acronyms and Abbreviations

ACHHRA	Australian Centre for Human Health Risk Assessment
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	Bioaccumulation factor
CEC	Cation exchange capacity
CDC	Centers for Disease Control and Prevention
CGIAR	Consultative Group on International Agricultural Research
CHP	Centre for Health Protection
EC	Electrical conductivity
EDI	Estimate daily intake
Eh	Redox potential
FAO	Food and Agriculture Organization of the United Nations
HCA	Hierarchical Cluster Analysis
IARC	International Agency for Research on Cancer
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IITA	International Institute of Tropical Agriculture
INS	National Institute of Statistics
IRIS	Integrated Risk Information System
IMSFH	Institute of Medicine Shaping the Future for Health
JECFA	Joint Expert Committee for Food Additives
HI	Health index
OHS	Occupational health and safety
OM	Organic matter
PC	Principal components
PCA	Principal component analysis
PPE	Personal protective equipment
RfD	Reference dose
SF	Slope factor
TCR	Target cancer risk
THQ	Target hazard quotient
UN	United Nations
USEPA	United States Environmental Protection Agency
USDHHS	United State Department of Health and Human Services
USGS	United States Geological Survey
WHO	World Health Organization
WWRU	Waste Water Research Unit

Introduction

The world population has significantly increased over the past decades, and so has urbanization. Today, 54% of the world's population lives in cities compared to only 43% in 1990, and the population is expected to rise to about 66% by 2050 (UN-Habitat, 2018). This implies that about 96% of global population growth from 2018 to 2050 will happen in urban areas of less developed countries, including many in Africa (UN-Habitat, 2020). The urban population in Africa is expected to rise from 400 million in 2010 to 1.26 billion in 2050 (Beegle et al., 2020). These rapidly growing cities in developing countries are often characterized by the emergence of informal settlements coupled with severe poverty leading to lack of sanitation facilities and services, lack of access to potable water, high levels of unemployment, insecurity, environmental degradation, pollution, and natural hazards (Cobbinah et al., 2015; Cohen, 2006). According to Orsini et al. (2013), urbanization comes with a series of challenges, including creating urban and peri-urban areas where poverty is condensed, and socio-economic constraints are exalted. Other challenges include deforestation, reduction of soil fertility, reduced drainage of rainfall, and air and water contamination.

Food insecurity is one of the most critical issues resulting from rapid urbanization (Magnusson et al., 2014). The majority of the urban population disburses approximately 60 to 80% of their incomes to meet their food needs (Magnusson et al., 2014; Orsini et al., 2013). Nevertheless, food consumption remains insufficient in quality and quantity (FAO, 2020a). According to WHO (2003), in many developing countries, the consumption of fruits and vegetables falls far short of the minimum daily intake of 400 g per person per day, or about 150 kg per person per year as recommended by the joint WHO-FAO Expert Consultation on Diet, Nutrition and Prevention of Chronic Diseases for the prevention of noncommunicable diseases and micronutrient and vitamin deficiencies (CHP, 2021; FAO, 2020b; Mason-D'Croz et al., 2019). About 3.9 million deaths worldwide are attributed to not eating enough fruit and vegetables (FAO, 2020). According to Okop et al. (2019), insufficient intake of fruits and vegetables is estimated worldwide to cause around 14% of gastrointestinal cancer deaths, about 11% of ischemic heart disease deaths, and about 9% of stroke deaths. In addition to their dietary benefits, vegetables and fruits are high-value crops that can often generate excellent income opportunities for small-scale farmers (FAO, 2020b). Growing and consuming fruit and vegetables could thus also help reduce the producers' poverty and improve consumers' health. Urban dwellers adopt alternative livelihood strategies such as urban agriculture that often encroach upon and deplete ecologically sensitive areas (Cobbinah et al., 2015; UN-Habitat, 2010). The development of agriculture in towns and cities is recognized as one of the major strategies for developing countries to address food insecurity, alleviate urban poverty, and improve the well-being of city dwellers (FAO, 2014; Orsini et al., 2013).

Urban agriculture can be defined as "the production, processing, and distribution of foodstuff from crop and animal production, fish, ornamental plants and flowers within and around

urban areas" (Mougeot, 2000). It is "one mechanism that plays a role in enhancing access to and distribution of food in urban areas and, thus, filling the hunger gap" (Lee-Smith, 2010). Besides food provision, urban agriculture provides a series of socioeconomic and ecological benefits for urban dwellers (Hallett et al., 2016; Noubissié et al., 2016). It is practiced by 800 million people worldwide, including more than ten percent of the urban population (around 11 million people) in sub-Saharan Africa (SSA) (FAO, 2007). Urban agriculture can positively and negatively influence the urban ecology, become part of the urban food system, and help to generate income while reducing food costs (Siegener, 2018). It benefits urban and peri-urban farmers and traders, input suppliers, and other service providers involved in agricultural value chains (Lagerkvist, 2014). Horticulture is the main component of the agricultural activities in cities that vary from vegetables (fruits, herbs) and mushrooms to ornamental plants that can easily grow in a city and its surroundings (Khan et al., 2020). Providing city-dwellers with fresh food is challenging, especially in SSA, where poor roads lead to heavy losses of vegetables in transit from rural areas within a few days of harvesting. Moreover, fresh food often quickly deteriorates once in the city due to the lack of cold storage facilities, both in markets and in most urban households. These issues lead to shortages and high prices of vegetables and fruits for urban consumers (FAO, 2012).

The urban production of vegetables can provide rich sources of vitamins, minerals, and phytochemicals to consumers that are essential for good health. For example, dark green leafy vegetables and yellow-fleshed fruits are recommended in people having Vitamin A deficiency, a significant cause of blindness in African children. Agricultural production in urban areas not only increases the city's food supply and household incomes but also helps to recycle water and nutrients from urban waste products (Wielemaker et al., 2018). FAO has estimated five major benefits of urban agriculture: food and nutrition security, sustainable livelihoods, a safe, clean environment, good governance, and healthy communities (FAO, 2012). Despite these universal advantages, urban agriculture faces many constraints. Some of these constraints are land tenure insecurity, rudimentary farming methods, limited space availability, fertilizers overuse, improper use of pesticides, and irrigation with untreated wastewater. Without appropriate knowledge, these farming practices may cause heavy metals and pesticides to contaminate the environment, and the products, thus negatively affecting human health (Selvi et al., 2019; AlKhader, 2015).

Human activities are the primary sources for heavy metal contamination of agricultural soils. These activities include emission from diverse sources (industrial, traffic, domestic), weathering of building and pavement surface, mining, smelting, atmospheric deposits, vehicle exhausts, urban effluent, waste disposal, sewage sludge, pesticides, and fertilizer application (Gupta et al., 2019; Zheng et al., 2020). Heavy metals can be classified as essential and non-essentials based on their roles in biological systems (Ali et al., 2019). Essential heavy metals such as Manganese (Mn), Iron (Fe), Copper (Cu), and Zinc (Zn) are critically important for living organisms at low concentrations. The heavy metals Mn, Fe, Co, Ni, Cu, and Zn, are micronutrients or trace elements for plants (Ali et al., 2019; Alotaibi et al., 2021). Meanwhile, Cadmium (Cd), Lead (Pb), Chromium (Cr), and Mercuric (Hg) are toxic and have no known biological roles. Since

heavy metals are toxic, cannot be degraded, and persist in the environment, they either accumulate in biota or leach down into the groundwater (Ali et al., 2019; Chandel et al., 2021; Islam et al., 2018). The pollution path may be horizontal (by streaming water) or vertical by mobility, such as migration into the depths by scrubbing. Heavy metal contamination of water resources is a critical environmental issue that negatively affects plants, animals, and human health (Rezania et al., 2016).

Heavy metal pollution in irrigation water and urban soil can directly affect human health because vegetables grown in contaminated soil and irrigated with untreated wastewater can accumulate a higher amount of heavy metals than those cultivated in unpolluted soils (Ali et al., 2019; Ugulu et al., 2019). The efficiency of different crops to absorb metals is determined by plant metal uptake or the transfer factor of metals from the substrate to plants (Rattan et al., 2005). Vegetative organs, including leaves and stems, can accumulate more heavy metals than reproductive organs such as grains and fruits (Singh, 2012). Heavy metal uptake via plant roots and direct pollution from the atmosphere onto plant surfaces can pose potential health risks to animals as well as humans (Kumar et al., 2019). Although the human body can take up heavy metals by inhaling soil particles and dermal contact, dietary intake remains the main route of exposure to heavy metals for humans (Ahmed et al., 2019; Ali et al., 2019).

Generally, people have little awareness and knowledge about heavy metals and their consequences for human health, especially in developing countries (Ali et al., 2019; Eqani et al., 2016). Growing food in heavy metal-contaminated media leads to bioaccumulation of these elements in the food chains (Ali et al., 2019). Due to biomagnification, higher concentrations of heavy metals in organisms of higher trophic levels can pose a health risk to them or their human consumers (Barwick & Maher, 2003). Heavy metals have been reported to be carcinogenic, mutagenic, and teratogenic (Godson et al., 2018; Manzoor et al., 2018; Yuan, Xiang et al., 2019). Excessive exposure to heavy metals has been shown to cause various diseases, including cancer, brain and kidney damage, heart problems, liver dysfunction, damage to the reproductive system, memory impairment, even death, and can cross the placental barrier, with potential toxic effects on the fetus (Järup, 2003; WHO, 2007; Solomon F., 2008; Steenland et al., 2017, 2019). Food chains should be constantly monitored for bioaccumulation of heavy metals to protect human health from the harmful effects of toxic heavy metals (Ali et al., 2019; Ramezani et al., 2021). Information about heavy metal concentrations in food products and their dietary intake is essential for health risk assessment.

Problems statement

Yaoundé, Cameroon's second-largest city, has faced rapid demographic growth after the end of the economic crisis in the 1980s (INS, 2017). This rapid population growth of 4% over the years has affected the city's spatial evolution, contributing to the increase of flood frequency in and around the town (Defo et al., 2015; Yongsu, 2010). Intensive human interventions like unplanned urbanization and population pressure have led to the rise of impermeable surfaces, the

increase of municipal waste, improper waste dumping sites, but also to the extension of urban agriculture. Consequently, these activities have led to changes in the peripheral rivers of the city, resulting in a deterioration of water and soil qualities (Defo et al., 2015; Kwon et al., 2012). Furthermore, Yaoundé faces a progressively increasing vehicular pollution rate due to the often heavily congested urban circulation (Matcheubou et al., 2009). Large amounts of solid, liquid and gaseous waste are discharged into the nature without any treatment, leading to the accumulation of heavy metals and other toxicants in water and soil, which ultimately end up in the food chain (Nazir et al., 2015; Noubissié et al., 2016).

Agricultural practices, especially the misuse of fertilizers and pesticides, are responsible for the further accumulation of heavy metals in the environment (Asongwe & Yerima, 2016; Benoi et al., 2016). Lowland areas in Yaoundé, located at the bottom of the valleys and the main urban agriculture sites, are the receptacle of various municipal waste due to the absence of waste treatment technologies. Farmers in Yaoundé lowlands often use untreated solid and liquid waste for crop production, resulting in heavy metal accumulation in the soil and the plants that grow in it. Heavy metals in irrigation water and soils can accumulate in crops and get in the food chain, posing a threat to human health (Gebeyehu et al., 2020; Gupta et al., 2019; Sultana et al., 2019). There are very limited studies on the heavy metal contamination in irrigation water, agricultural soil, and vegetable crops in Yaoundé. One of the few studies on heavy metal pollution of soil and groundwater in Yaoundé reported that the contamination was substantially higher than the WHO thresholds (Defo et al., 2015). In addition, a recent study conducted in two Yaoundé communities reported that there is a probability of adverse health risks likely to occur for consumers of heavy metals contaminated vegetables (Aboubakar et al., 2021). Increasing awareness of the importance of vegetables to the human diet calls for more frequent monitoring of heavy metals in food crops. However, limited data are available in most developing countries like Cameroon on the potential human health risks, especially cancer, due to consuming vegetables contaminated with heavy metals. Therefore, this study was conducted to comprehensively assess seasonal characteristics of heavy metals in foods and their potential human health risk in the most important areas for urban agriculture in Yaoundé, Cameroon.

The purpose of this thesis

The overall focus of this study is to identify the farming practices potentially causing heavy metal contamination in the environment and the resulting health hazards, especially increased cancer risks associated with the consumption of contaminated vegetables in the context of Yaoundé.

Specifically, the objectives are:

- (1) to describe the existing farming practices, especially the use of agricultural inputs in some cultivated lowlands of Yaoundé;
- (2) to investigate the concentration of six heavy metals (Cr, Ni, Cu, Cd, Pb, and Zn) in irrigation water, soils, and foodstuffs and their seasonal variation;

- (3) to assess the crop pollution index and the bioaccumulation of these metals; and
- (4) to assess human health hazards of heavy metal contamination of agricultural produces.

Research hypotheses

The study aims at testing the following hypotheses:

- (1) Heavy metals in the environment of Yaoundé result from agricultural inputs such as fertilizers, pesticides, and contaminated irrigation water.
- (2) Heavy metal concentrations in water, soil, and plants are site-specific and seasonal.
- (3) The bio-availability of metals is not only a function of the metal concentration but also equally depends on the site-specific and the species of metals.
- (4) The consumption of edible parts of vegetables cultivated under urban agriculture in Yaoundé can harm human health.

Study Framework / Overview

The term "heavy metals" refers to the group of metals and metalloids of relatively high atomic mass ($>4.5 \text{ g/cm}^3$), such as Pb, Cd, Cr, Hg, Ni, and Zn, that are toxic to humans and the environment (FAO, 2018). Some lighter metals such as Arsenic (As), Selenium (Se), and Aluminum (Al) are also toxic. Heavy metals are everywhere in the environment. They originate from a lithogeny background (natural) and anthropogenic activities (untreated waste disposal, industries, fertilizers, pesticides, vehicle fumes). Heavy metals are stable, cannot be degraded or destroyed, and tend to accumulate in the environment (Walker et al., 2012; Alotaibi et al., 2021; Briffa, Sinagra, & Blundell, 2020). Concerns on the contamination of toxic elements in the environment and their associated public health risks have increased over the years. Heavy metals are some of the most persistent and complex pollutants that are difficult to remediate in nature. They negatively affect the quality of the atmosphere, water, and food crops. Metals threaten the health and well-being of animals and humans because they are not subject to metabolic breakdown and stay in the tissue of living organisms, unlike most organic compounds.

The human health risk assessment model for heavy metal contamination in Yaoundé lowlands (Figure 1) in this study is based on an established sources-pathways framework (Masindi & Muedi, 2018; USEPA, 2006).

The health risk assessment model consists of four main steps: hazards identification and characterization, dose-response, and exposure assessment. The first step, hazard identification, aims to identify the potential sources of heavy metals in the environment. This study focuses on urban agriculture and associated anthropogenic activities and less on the natural origin of heavy metals. Heavy metals accumulated in the environment may cause potential health risks once they enter the food chain from the water and soil to the plants (Briffa et al., 2020; Kumar et al., 2019). Heavy metal contamination poses threats to food quality and food safety. Vegetables cultivated near industrial sites and irrigated with untreated wastewater are likely to be contaminated by toxic metals, especially Cd, Cr, and Pb, that can harm human health (Chaoua et al., 2018; Ahmed et al.,

2019; Muhammad et al., 2020). Essential elements in moderate amounts, including Cu and Zn, are necessary for plant growth. However, in high concentrations, also these metals can be toxic to plants (Ashraf et al., 2021; Chaoua et al., 2019; WHO, 1996).

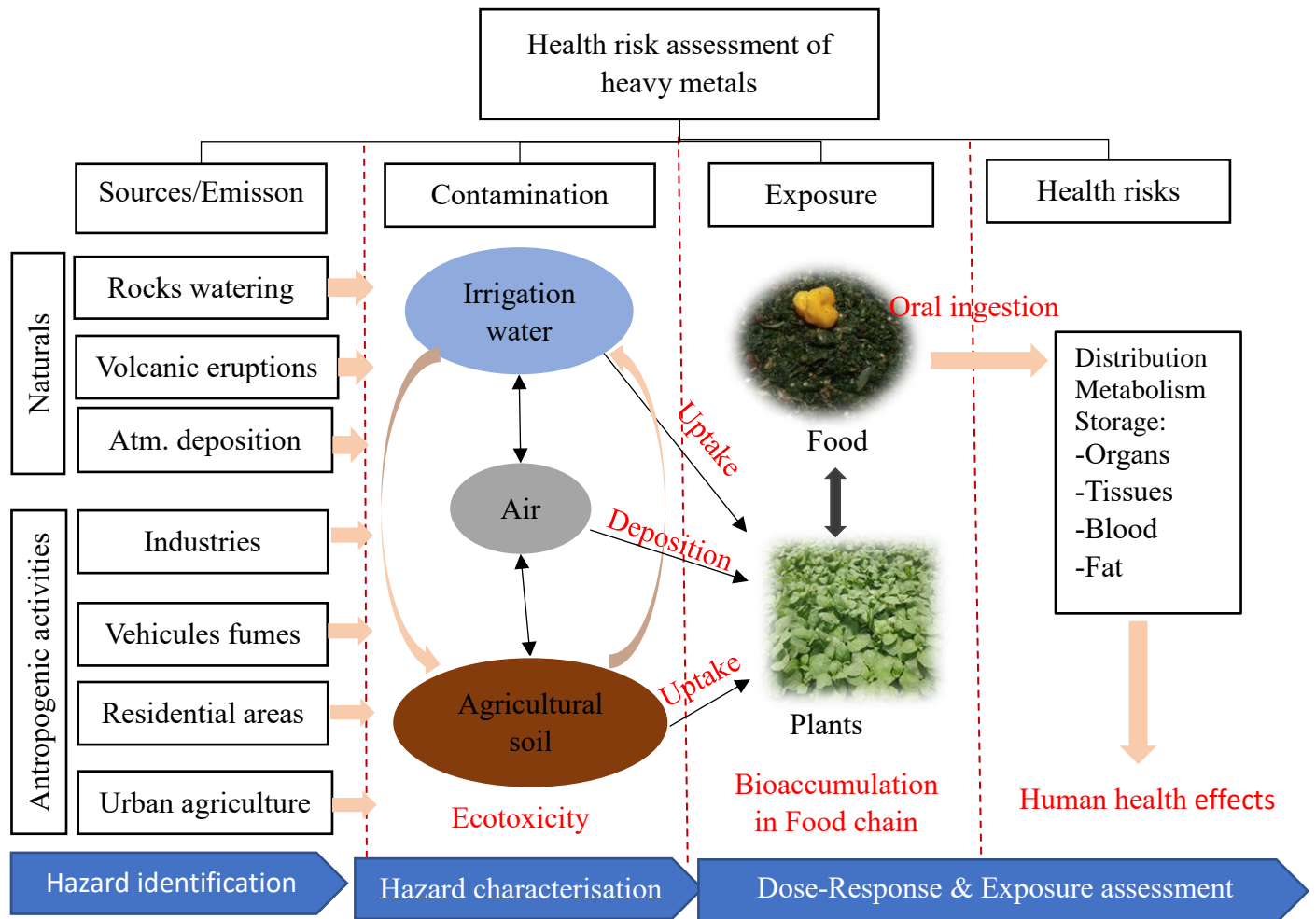


Figure 1 Conceptual framework of human exposure to heavy metals via the food chain
 Source: Author's construction from several studies including Masindi & Muedi, (2018) and USEPA, (2006)

In the hazard characterization step, the concentration of heavy metals (Cd, Cr, b, Cu, Zn, and Ni) in the environmental media (irrigation water, agricultural soil, and plants) are measured. Based on different thresholds specific to each medium, they are classified as ecological toxic and a potential threat to human health. The air medium in the framework highlights the possibility of water, soil, and plants being polluted by the heavy metal present in the air. However, this study does not assess the amount of heavy metals in the air. Heavy metal absorptions in vegetables and nutrition negatively affect various components of vegetables such as protein, fat, and carbohydrate (Ashraf et al., 2021). Therefore, it is necessary to assess and monitor pollution sources. In the third and fourth steps (Dose-Response & Exposure assessment), some factors such as the bioaccumulation factor (BAF) and the estimated daily intake (EDI) of the aforementioned heavy metal through the consumption of *S. nigrum*, one of the most important vegetable species grown

in the lowlands of Yaoundé, are calculated. Long-term accumulation of these heavy metals in vegetables has been shown to threaten human health (Bayissa & Gebeyehu, 2021; Zheng et al., 2020). The health risk based on the chronic (a significant part of a lifetime or an entire lifetime) exposure duration is then calculated as a target hazard quotient (THQ) and health index (HI) for exposure to more than one heavy metal. The target cancer risks (TCR) for Cd, Cr, Ni, and Pb are also estimated to determine the risk level for humans. Previous studies have reported that heavy metals are most toxic to fetal and childhood development while also being harmful during aging (ACHHRA, 2012; Baltas et al., 2020; Hu et al., 2017). This study, however, mainly focuses on the adult population because of its importance as the main actor of urban agriculture in the selected areas.

Dissertation outline

This dissertation is comprised of five chapters. The first chapter is an introduction that presents the investigated contaminants and the research's objectives and hypotheses. Pertinent literature regarding the sources and distribution of heavy metals in the environment, their transfer mechanisms, and the health effects from metals exposure are reviewed in Chapter 2. The research carried out is described in detail in Chapter 3, including descriptions of the study areas, sample population, and key characteristics. It is followed by the methods used to achieve the specific objectives and activities. In Chapter 4, the research results and discussion are presented in four separate sections. The first gives information on farming practices and the relationship between socio-economic characteristics and agricultural inputs. The second presents heavy metal concentration in the irrigation water, cultivated soils, and vegetables. It also includes the Pearson correlation findings concerning the heavy metal characteristic in water, soil, and vegetables and results from the multivariate analysis. The third section assesses the crop pollution index and the bioaccumulation capacity of the studied vegetable (*S. nigrum*). The last section outlines the health hazards (non-carcinogenic and carcinogenic risks) due to the consumption of *S. nigrum* grown in the study areas. In the final Chapter 5, the main results are summarized and interpreted to draw a conclusion and answer the main research questions. Future research suggestions and recommendations are also provided.

1. Literature Review

1.1. Sources of heavy metal in the environment

Heavy metals are ubiquitous. Anthropogenic activities and natural/geogenic/lithogenic backgrounds generate heavy metals in the environment (Figure 2).

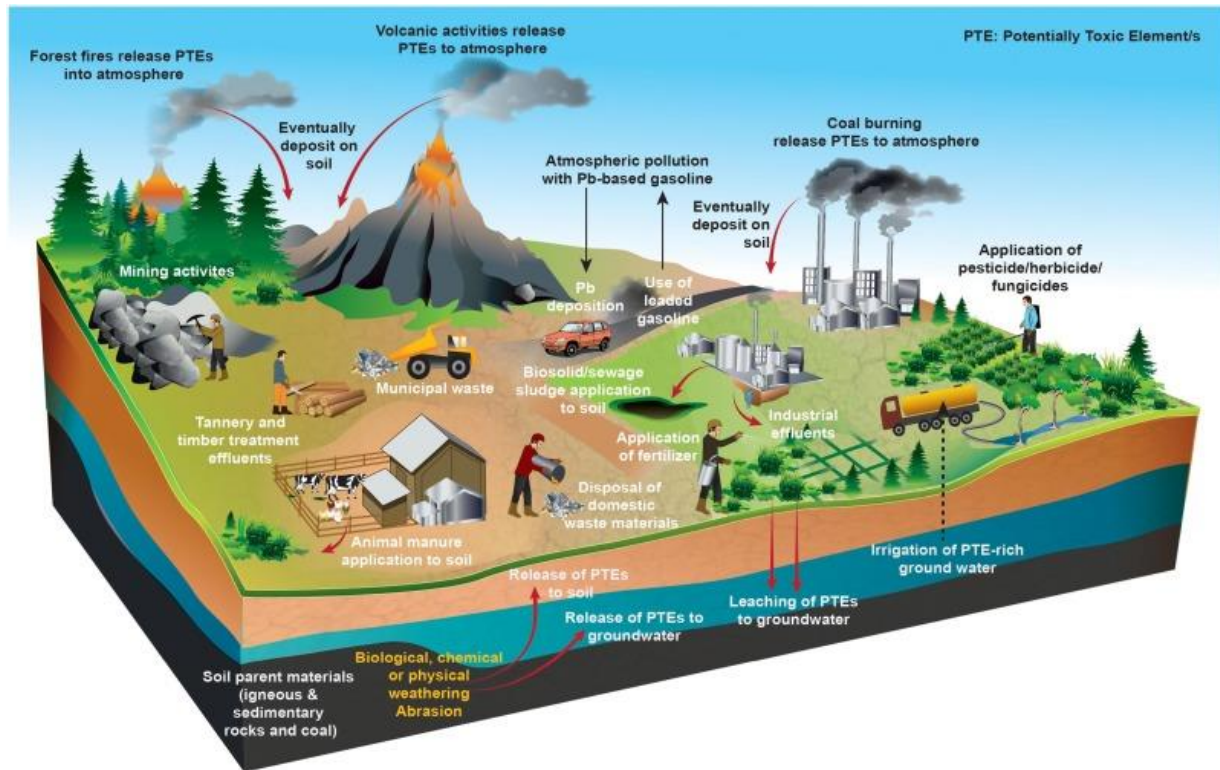


Figure 2 Potential sources of heavy metal contamination in the environment
 Source : Palansooriya et al. (2020)

Natural sources

Heavy metals can be found in hydroxides, oxides, sulfides, sulfates, phosphates, silicates, and organic compounds. Rocks and parent materials are the primary sources of metals, both from weathering processes and volcanic eruptions. Natural weathering processes can lead to the release of metals from their endemic spheres to different environmental compartments. Depending on the geographic region, the environment can harbor higher heavy metal concentrations than parent rock (Forstner Bradl, 2005). Geological materials generally have high concentrations of Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, and Pb (Srivastava et al., 2017). Soil formation from sedimentary rocks is also a small source of heavy metals. Sedimentary rocks, such as shale, limestone, and sandstone, contain high concentrations of Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, and Pb (Nagajyoti et al., 2010). High levels of Al, Zn, Mn, Pb, Ni, Cu, and Hg are also found in volcanic emissions, along with toxic and harmful gases (Nagajyoti et al., 2010; Sharma & Agrawal, 2005). Similarly, marine aerosols, forest fires, and natural vegetation also contribute to the leaching, decomposition, and volatilization of heavy metals in the environment. Likewise, oceanic activities produce sea sprays and aerosols that contribute heavy metals into inland coastal areas (Srivastava et al., 2017). The most common heavy metals in the environment are Pb, Ni, Cr, Cd, As, Hg, Zn, and Cu (Masindi & Muedi, 2018).

Anthropogenic sources

Anthropogenic activities are the most significant contributor to heavy metal pollution in the environment, primarily due to industrial activities, such as mining, smelting, foundries, and agricultural activities. A heavy metal element can be released from various sources, and a source may release more than one metal element. Therefore, metals accumulated in the environment may originate from specific local industrial sources such as discharge from smelters (Cu, Pb, Ni), metal-based industries (Zn, Cr, and Cd), paint and dye formulators (Cd, Cr, Cu, Pb, Hg, Se, and Zn), petroleum refineries (AS, Pb), and effluents from chemical manufacturing plants (Harikumar et al., 2009).

Domestic effluents constitute the largest single source of heavy metal contamination in rivers and lakes in general. Several studies have found that most enzyme detergents contain trace amounts of Cd, Mn, Cr, Co, and Zn (Soylak et al., 2013; Abulude et al., 2010). Urban runoff presents a severe problem of heavy metal pollution (Srivastava et al., 2017). Other contributors of heavy metals in the environment are vehicle traffic that produces Cr, Zn, Cd, and Pb, and coal combustion that produces Cd, Pb, Hg, and As (Nabulo et al., 2010; Ali & Khan, 2018; Xu et al., 2019). One of the most harmful metals, Pb, is considered a good indicator of urban pollution caused by runoff water (Islam, 2014). Pb is mainly added to the environment from various sources, including acid batteries, old plumbing systems, lead shots used for hunting game birds, and combustion of leaded gasoline (ATSDR, 2017).

Agricultural practices are one of the main heavy metal contamination factors and can strongly influence element concentrations in the soil environment (Alves et al., 2016). The agricultural sources of heavy metals are summarized in Table 1.

Table 1 Heavy metal sources in agricultural soils

Agricultural sources of heavy metals			
Source	Heavy metal input		References
Fertilizers	Phosphate fertilizers, nitrate fertilizers, potash fertilizers, lime	Cr, Cd, Cu, Zn, Ni, Mn, and Pb	AlKhader, 2015; Kelepertzis, 2014; Atafar et al., 2010
Pesticides	Herbicides, insecticides, fungicides	Primarily Cu, Zn, Cd, Pb, and As	Tóth et al., 2016; Kelepertzis, 2014; Walker et al., 2012
Biosolids and manure	Livestock manures, composts, sewage sludge, fly ash	Cr, Cd, Cu, Zn, Ni, As, Hg, and Pb	Bolan et al., 2010; Dwivedi et al., 2014; Sharma et al., 2017
Waste water	Irrigation with municipal waste water, industrial waste water	Cr, Cd, Cu, Zn, Ni, As, Hg, and Pb	Walker et al., 2012; Khan et al., 2013; Balkhair & Ashraf, 2016; Woldetsadik et al., 2017

Atmospheric deposition	Mining, metal smelting, and refining processes, transport, and waste incineration.	Primarily Ni, Cd, Pb, Cu, Zn, Hg, and Cr	Liu et al., 2014; Xu et al., 2014; Deng et al., 2016
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Source: Author's construction from Srivastava et al. (2017)

Heavy metals contamination of agricultural soil may also originate from irrigation water sources such as deep wells, rivers, lakes, and irrigation canals (FAO, 2017). While continuous irrigation can lead to the accumulation of Pb and Cd in the cultivated soils (Chaoua et al., 2019), inorganic and organic fertilizers are the most important causes of heavy metals in agricultural soil through liming, sewage sludge, and irrigation waters (Khan et al., 2017). Several synthetic pesticides are also major sources of heavy metal contamination in agricultural fields (Tóth et al., 2016). Depending on their sources, fungicides, inorganic fertilizers, and phosphate fertilizers have varied concentrations of Cd, Cr, Ni, Pb, and Zn (Nagajyoti et al., 2010). Furthermore, phosphate fertilizers (triple superphosphate, TPS) and pesticides are the main sources of heavy metals (AlKhader, 2015). Although the natural levels of heavy metals are not high in agricultural soil, the repeated applications of fertilizers containing trace heavy metals, particularly Cd and Pb, can accumulate heavy metals in the soil (Atafar et al., 2010; Wuana & Okieimen, 2011). Major contributors of Zn and Cd are phosphate fertilizers in their different formulations, and these fertilizers inputs are directly proportional to the concentration of heavy metals. Applying pesticides in agriculture adds elements such as Pb, Hg, and As. (Selvi et al., 2019). The concentrations of heavy metals in various agricultural inputs are summarized in Table 2.

Table 2 Element concentrations ($\mu\text{g/g}^{-1}$) in agricultural amendments

Metals	Pesticides	Lime	Nitrate fertilizers	Phosphate fertilizers	Farmyard manure	Compost	Sewage sludge
Cr	-	10-15	3.2-19	66-245	1.1-55	1.8-410	8.40-600
Ni	-	10-20	7-34	7-38	2.1-30	0.9-279	6-5,300
Cu	-	2-125	-	1-300	2-172	13-3,580	50-8,000
Zn	-	10-450	1-42	50-1,450	15-556	82-5,894	91-49,000
Cd	-	0.04-0.1	0.05-8.5	0.1-190	0.1-0.8	0.01-100	<1-3,410
Pb	11-26	20-1,250	2-120	4-1,000	0.4-27	13-2,240	2-7,000

Source: Author's construction from Srivastava et al. (2017)

Phosphate fertilizers cause an unavertable soil accumulation of Cd and Pb. Many commercial phosphate fertilizers contain carcinogenic heavy metals such as Cd, Pb, and As (AlKhader, 2015). The uses of traditional organic amendments such as farmyard manure, hen droppings, cow dung, compost or pig manure, and municipal sewage sludge may also contribute to the increase in heavy metal content in the soil, including Cd, Cr, Pb, Ni, Cu, and Zn (Bolan et al., 2010). Manures can contain high concentrations of Cu and Zn, especially animal manures from a commercial pig or poultry farms due to the increased use of Cu and Zn as additives to animal

diet, noted that 80% of the added metals are excreted by the animals (He et al., 2005). Applying these manures as fertilizers may considerably increase the Cu and Zn content in agricultural soils. Municipal sewage sludge used as fertilizers may contain Ni, Cd, Cr, Zn, Cu, or Pb, depending on natural and industrial activities. Some metals, including As, Cu, Pb, and Cr, may still be found in some areas where pesticides had been used in the past (Walker et al., 2012). The discharges of industrial effluents, domestic sewage, and other wastewaters increase heavy metals in the environment (Ali et al., 2019). These metals end up in different environmental compartments such as soils, water, air, and interfaces (Masindi & Muedi, 2018).

1.2. Heavy metals in the environment and toxicity

Heavy metals persist in the environment, and their levels in different ecological compartments from water, soil, food, to air indicate the level of ecosystem contamination (Manzoor et al., 2018). Due to their high propensity in nature, heavy metals accumulate in various environmental matrices, resulting in concentrations exceeding the common safety guidelines (Rzymiski et al., 2014). The behavior of trace elements in each ecosystem is complex and has usually been studied separately for each ecological compartment (Kabata-Pendias, 2011). Except for mercury, most heavy metals go into the atmosphere as aerosols and deposits to the soil through natural sedimentation and precipitation (Su et al., 2014). The soil is the main repository of heavy metal contaminants in terrestrial ecosystems due to its various anthropogenic activities. Similarly, sediments serve as the ultimate sink for heavy metals (Emenike et al., 2018; Lian et al., 2019). Thus, their accumulation in the environment varies according to the media and the metals. For instance, Cd is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. The highest level of Cd is accumulated in sedimentary rocks, while marine phosphates contain about 15 mg/kg of Cd (FAO, 1985). Cd is found from 0.02 to 0.2 mg/kg in igneous and metamorphic rocks and 0.1 to 25 mg/kg in sedimentary rocks. The levels of Cd contents measured in water, rain, fresh, and surface in urban and industrialized areas are highly varied, ranging from 10 to 4,000 ng/L depending on the locations (WHO, 1992). The levels of Cd in rural areas have generally been estimated between 0.1 and 5 ng/m³, 2 to 15 ng/m³ in urban, and 15 to 150 ng/m³ in industrialized areas (Morrow, 2009; WHO, 1992). The levels of Cd is estimated from 0.1–5 mg/kg in raw materials for iron and steel production, at 2 mg/kg for cement production, from 0.5–1.5 mg/kg in fossil fuels, and 10 to as high as 200 mg/kg in phosphate fertilizers (Morrow, 2009). Over 90% ($5.6\text{--}38 \times 10^6$ kg/year) of Cd is released into the environment from anthropogenic sources, including the use of phosphate fertilizers, fossil fuel combustion, metallurgical works, wastes from the cement industry, sewage sludge, municipal and industrial wastes, and mining, smelting, and metals ore processing (Khan et al., 2017). Globally, about 50,000 and 60,000 t/year of Cr and Ni, respectively, may be emitted from coal combustion, wood burning, and refuse incineration. About 7,000 t/year of Cd may be emitted purely from coal combustion (Merian, 2015). Table 3 summarizes some metal emissions into the environment.

Table 3 Global sources for heavy metal inputs into the environment (crude estimates in t/a)

Variables	Cr	Ni	Co	Be	As	Cd	Se
Natural inputs							
Volcanic emissions	Small	small	small	small	3,000 1%	500 1%	small
Biological cycle (Extraction from soil by plants)	100,000 15%	100,000 30%	30,000 60%	5,000 36%	100,000 35%	30,000 50%	10,000 15%
Weathering of rocks and soils	100,000 15%	50,000 15%	10,000 20%	small	45,000 16%	2,000 4%	3,000 8%
Anthropogenic emissions into air, waters, and soil							
Emissions from general ore and metal production	20,000 3%	5,000 3%	little	little	50,000 17%	10,000 17%	12,000 33%
Emissions from metal use	400,000 60%	100,000 30%	3,000 6%	200 2%	40,000 14%	10,000 17%	400 2%
Emissions from coal burning and other combustion processes	50,000 7%	75,000 23%	5,000 10%	8,000 57%	50,000 17%	7,000 12%	10,000 28%
Total inputs (100%)	670,000	330,000	50,000	14,000	288,000	59,500	36,000
(For comparison: Global Metal Production (incl. alloys)	7,000, 000	700,000	30,000	1,000 or more	50,000	17,000	1,400

Source: Author's construction from Merian, 2015.

Cr background concentrations in environmental components have been estimated to be <10 ng/m³ for the atmosphere; <500 mg/kg for soil; <0.5 mg/kg for the vegetation; <10 µg/L for the freshwater; <1 µg/L seawater and <80 mg/kg for the sediments (Rieuwerts, 2017). Tests on soils have shown Cr concentrations ranging from 1 to 1,000 mg/kg, with an average concentration ranging from 14 to about 70 mg/kg (WHO, 2000). Cr enters the air, water, and soil in the Cr (III) and Cr (VI) while plants contain systems that lower the Cr uptake.

Pb rarely occurs in the environment in its native metallic form, and its abundance is 14 mg/kg in the Earth System (Davison et al., 2014). Global Pb resources exceed two billion tons (USGS, 2020). Pb contamination increases with population and economic growth from an estimated 10 tons per year to 1,000,000 tons per year (Tiwari et al., 2013). However, typical background concentrations of Pb are < 0.1 µg/m³ in the atmosphere; 100 mg/kg in the soil; and 5 µg/L in fresh- and seawater (Rieuwerts et al., 2015).

Cu occurs in the environment in a directly usable metallic form (native metal). It constitutes 50 mg/kg of the Earth's crust and is present to the extent of 0.020-0.001 mg/kg in seawater. Cu

concentration in the soil ranges from 10 to 50 mg/kg (Oorts, 2013). The identified resources contain 2.1 billion tons of Cu, and undiscovered resources contain an estimated 3.5 billion tons (USGS, 2021a).

Despite being the fifth most common element on earth, Ni is inaccessible due to its location within our planet's core (Garsida, 2021). Identified land-based resources estimate the presence of Ni of at least 0.5% or approximately 300 million tons worldwide, about double the known reserve. Approximately 60% of this amount is found in Laterites and 40 % in Sulfide deposits (McRae, 2021). More than 2.5 million metric tons of Ni per year are mined globally, with Indonesia (760,000 t) as the largest Ni producer as of 2020 (Garsida, 2021; USGS, 2021b).

Zinc (Zn) naturally occurs in the environment associated with other base metals (Cd and Pb), primarily through the weathering of rocks. Seventy-five (75) ppm (0.0075 %) of Earth's crust is Zn. Soil contains 64 mg/kg of Zn on average. Meanwhile, there are 30 ug/L of Zn in seawater and 0.1-4 $\mu\text{g}/\text{m}^3$ in the atmosphere (Emsley, 2011). The world resources of Zn are estimated at 1.9-2.8 billion tons (USGS, 2021c).

Metals toxicity in the environment

In aquatic ecosystems, heavy metal contamination is one of the main pollution types that stress the biotic community (Ndeda & Manohar, 2014). For instance, high Ni concentrations in surface water can diminish the growth rates of algae. The aquatic media are more susceptible to the harmful effects of heavy metals pollution because aquatic organisms are in prolonged contact with soluble metals (Kwon et al., 2012). High concentration of heavy metals in the soil results in high soil toxicity (Su et al., 2014). Metal toxicity affects soil microbial compositions and activities (Xie et al., 2016). According to Chander et al. (1995), the enzyme activities in heavy metal polluted soil decrease significantly (about 10–50 times) compared to those in noncontaminated soils. When heavy metal concentrations in environmental components exceed recommended thresholds, they can be a significant source of many human chronic health risks (Muszyńska & Hanus-Fajerska, 2015). Heavy metals' properties, uses, and effects on human health vary from one metal to another (Table 4).

Table 4 Summary of some selected heavy metals properties uses and effects on humans

Heavy metals	Properties	Uses	Effects on human	Food sources
Cadmium (Cd)	<p>Density: 8.69 g/cm³</p> <ul style="list-style-type: none"> - 64th most abundant metal - Found frequently in combination with Zinc - The only mineral found is Greenockite which is made up of Cadmium and Sulfur - Silvery bluish tint metal 	<ul style="list-style-type: none"> - Phosphate fertilizer - Pesticides - Nickel–Cadmium batteries - Glassware pigmentation - Corrosion-resistant plating - Stabilizer in plastic production - Nuclear reactors 	<ul style="list-style-type: none"> - Kidneys are primarily affected, causing nephrotoxicity - Infertility is caused by a reproductive system failure - Calcium metabolism alterations; bone fracture - Psychological disorders - Gastrointestinal disorders - Central nervous system complications - Immune system deficiencies - DNA impairment, cancer - Itai-Itai disease; osteoporosis, renal dysfunction - Reported to be genotoxic and ecotoxic in animals 	<ul style="list-style-type: none"> - Shellfish - Mussels - Dried seaweed - Shrimps - Mushrooms - Liver
Chromium (Cr)	<p>Density: 7.15 g/cm³</p> <ul style="list-style-type: none"> - 21st most abundant element in the Earth's surface - Extracted as a chromite ore known as Siberian red lead, hard shiny, steel-grey - Fairly active metal - Reacts with most acids - Forms a layer of Chromium (III) oxide making the metal less corrosive 	<ul style="list-style-type: none"> - Alloys, metal ceramics - Electroplating - Leather tanning - Manufacturing of synthetic rubies - Dye paints - Chromium salts are used to color glass green 	<ul style="list-style-type: none"> - Oral intake of Chromium (VI) usually causes acute poisoning and various symptoms including gastrointestinal ulceration, nausea, and vomiting, fever, diarrhea, vertigo, toxic nephritis, liver damage coma, death (usually at 1–3g) - Inhalation of Chromium (VI) or having repeated skin contact will cause chronic poisoning. Chromium (VI) can cause allergic contact dermatitis and eczema, gingivitis, irritation of mucous membranes, bronchitis, liver and kidney disease, sinusitis, pneumonia, lung cancer, chrome holes, especially in the forearms, hands, fingers, and nose. 	<ul style="list-style-type: none"> - Fruits and vegetables - Yeasts - Meats - Shellfish - Grains
Nickel (Ni)	<p>Density: 8.9 g/cm³</p> <ul style="list-style-type: none"> - 22nd most abundant metal 	<ul style="list-style-type: none"> - Jewelry - Coins 	<ul style="list-style-type: none"> - Lung embolisms - Asthma - Allergic reactions (jewelry) 	<ul style="list-style-type: none"> - Baked beans, kidney beans, soybeans

Heavy metals	Properties	Uses	Effects on human	Food sources
	<ul style="list-style-type: none"> - Minerals found are pentlandite which is an iron-nickel sulfide; garnierite, which is a green hydrous nickel silicate compound - Silvery metal - Resists corrosion at high temperatures - High amount of Ni came from meteorites 	<ul style="list-style-type: none"> - Plating other metals to avoid corrosion - Stainless steel alloy - Welding - Armor plating - Oat propeller shafts - Rocket engines - Nichrome alloy is used in appliances that use heat while remaining non-corrosive 	<ul style="list-style-type: none"> - Respiratory failure - Heart disorders - Dizziness (following gas exposure) - Increased possibilities of cancer - Nickel sulfide, Nickel oxide, and soluble Nickel compounds are all carcinogenic. - Workers in the nickel industry who are exposed to inhalation of the metal are at a greater risk of acquiring lung and nasal cancer 	<ul style="list-style-type: none"> - Whole wheat and grains - Millet - Oat & Rye - Tea & Soy - Cocoa and chocolate products - Baking powder - Chickpeas - Nuts & Peas - Lentils
Lead (Pb)	<ul style="list-style-type: none"> Density: 11.3 g/cm³ - 37th most abundant metal - Found in a mineral ore known as galena, which is made up of lead sulfide and can be combined with Silver, Zinc, and Copper - Dull silver-grey metal - Soft - Easily worked 	<ul style="list-style-type: none"> - Used in the past for hair dyes, pottery lead glazes, insecticides - Lead-acid batteries in cars - Computer screen sheets to safeguard from radiation - Ammunition and projectiles - Lead crystal glass - Cable sheeting - Sports equipment - Weight belts for divers - Canister for corrosive liquids - In buildings for roofing - Stained glass windows, Lead piping 	<ul style="list-style-type: none"> - Hypertension - Miscarriages - Premature and low births - Stillbirths - Renal impairment - Brain injury - Abdominal pain - Pica - Peripheral nerve damage - Sperm damage - Encephalopathic signs - Iron deficiency due to disruption of hemoglobin synthesis - Cognitive impairment - In children: brain and central nervous system development altered, reduced intelligence, a decline in educational achievement, a reduction in the attention span, increase in anti-social behavior 	<ul style="list-style-type: none"> - Fruit and vegetables - Grains - Seafood - Red meat - Wine - Soft drinks
Copper (Cu)	<ul style="list-style-type: none"> Density: 8.96 g/cm³ - 26th most abundant metal 	<ul style="list-style-type: none"> - Copper alloys such as bronze and brass - Copper wires 	<ul style="list-style-type: none"> - Metal fever as flu-like symptoms, diarrhea, vomiting, irritation of the eyes, dizziness, irritation caused in the mouth cavity 	<ul style="list-style-type: none"> - Liver - Oyster - Spirulina

Heavy metals	Properties	Uses	Effects on human	Food sources
	<ul style="list-style-type: none"> - Reddish-gold color - Found in minerals such as chalcopyrite containing Copper, Iron and Sulfur; bornite also containing Copper, Iron and Sulfur and known as the peacock ore - Easily worked - Good conductor of heat and electricity - Essential element 	<ul style="list-style-type: none"> -Plating - Coins - Pipes - Fertilizer - Preservation of wood - Preservation of fabric - Barrier cream, - Chemical tests for sugar detection in Fehling's solution - Copper sulfate is used as an algicide in water purification - Copper sulfate to cure mildew in agriculture 	<ul style="list-style-type: none"> - An acute dose of copper salts causes acute gastroenteritis due to necrosis - In excess: hepatocellular degeneration, necrosis, cytotoxic to erythrocytes leading to hemolysis - Oral intake will cause hepatic and kidney disease and death - Insomnia, anxiety, agitation, Restlessness - Wilson's disease (copper accumulated in organs instead of being excreted by bile): lack of appetite, fatigue, jaundice, Kayser-Fleischer rings, speech impairment, difficulty in swallowing, uncontrolled poisoning, brain damage, demyelination, hepatic cirrhosis, 	<ul style="list-style-type: none"> - Shiitake mushrooms - Nuts and seeds - Lobster - Leafy greens - Dark chocolate
Zinc (Zn)	<ul style="list-style-type: none"> Density: 7.134 g/cm³ - 24th most abundant metal - Silvery-white metal with a blue tinge - Two of the most common ores are Zinc blende, made up of Zinc sulfide; and calamine made up of Zinc silicate - Tarnishes in the air - Essential element 	<ul style="list-style-type: none"> - Galvanization to prevent metals from rusting - Die-casting - Zinc oxide is used in the production of paints, cosmetics, soaps, deodorants, anti-dandruff shampoo, weapons, electrical equipment, batteries, plastic, ink, pharmaceuticals, textiles, rubber - Zinc sulfide is used in X-ray screens, luminous paint, fluorescent lights 	<ul style="list-style-type: none"> - Nausea and vomiting - Stomach cramps - Anemia - Decrease in high-density lipoprotein (HDL) cholesterol - Pancreatic complications - Fatigue - Epigastric pain - Copper deficiency - Anemia - Impaired immune function - Neutropenia 	<ul style="list-style-type: none"> - Lamb - Beef - Cheese - Herring - Sunflower seeds

Source: Author's construction from Briffa et al., 2020

1.3. Heavy metals availability and accumulation in plants

1.3.1. Bioavailability

The primary sources of heavy metals for plants are their growth media: nutrient solutions or soils. Heavy metals in the soil are of great concern because of their toxicity (Khan et al., 2015; Khan et al., 2016). Metals such as Cd, Cr, Cu, Ni, Pb, and Zn, like organic pollutants, are non-biodegradable and accumulate in the environment (Kim et al., 2015; Selvi et al., 2019). Many authors have reported that an organism is adversely affected only by the bioavailable fraction of the total metal content in the soil but not by the non-available fraction that is sequestered in the soil matrix (Kim et al., 2007; Rieuwerts et al., 1998). The total metal content refers to the saturation level of metals in the soil matrix that can be used as a primary measure of soil contamination (Kim et al., 2015).

Bioavailability is the fraction of contaminants potentially available for uptake or taken up from the environment (Blasco et al., 2016). The bioavailability process was defined in the past by the National Research Council Committee as “the individual physical, chemical, and biological processes that determine the exposure of organisms to chemicals present in soils and sediments” (Ehlers & Luthy, 2003). The bioavailability can be described in three conceptual steps, which are: (1) availability of heavy metals in the soil (environmental availability), (2) uptake of heavy metals by the organism (environmental bioavailability), and (3) accumulation and toxic effects of heavy metals in an organism, including toxicological bioavailability (Harmsen, 2007).

The environmental availability of metals is influenced by factors such as soil pH, the total metal content, cation exchange capacity (CEC) of the soil, organic matter (OM) content, redox potential (Eh), electrical conductivity (EC), and the chemical form of elements (Kabata-Pendias & Pendias, 2001; Kim et al., 2015; Mirecki et al., 2015). For instance, when OM content increases, pH, CEC, clay, and the percentage and availability of metals decrease (Kabata-Pendias & Pendias, 2001). Depending on the soil pH and the type of OM, the latter can modify metal ions' adsorption and/or solubility (Wong et al., 2007). For a typical soil pH range, the stability of metal and organic complexes can be outlined as Cd, Zn, and Ni as having low stability and Pb, Cu, and Cr as having high stability (Smith, 2009). At moderately acidic up to slightly alkaline soils, pH, Cu, Cr, and Pb solubility can be significantly enhanced by dissolved OM, associated with an increase of their environmental availability (Kim et al., 2015). High pH is an important factor contributing to Pb retention in soil by forming Pb precipitates (Levonmäki & Hartikainen, 2007; Romero-Freire et al., 2015). Kabata-Pendias (2011) reported Pb fixation by soil OM as an important factor and that Pb accumulates in soils' upper horizon where OM contents are highest (Sipos et al., 2005). In addition, CEC and OM harm the mobility and bioavailability of heavy metals like Pb (Khan et al., 2015).

Previous work suggested that the bioavailability of metals in soil was a dynamic process that depended on specific combinations of chemical, biological, and environmental parameters (Guala et al., 2010; Caporale et al., 2018). The bioavailability of metals is also controlled by plants' requests for micronutrients and their capacity to absorb and eliminate toxic elements (Mirecki et al., 2015; Selvi et al., 2019). Climatic conditions influence the rate of trace metal uptake, which

may be partly an indirect effect due to the water flow phenomenon. Generally, a higher ambient temperature influences plants' greater uptake of trace elements (Kabata-Pendias & Pendias, 2001; Kabata-pendias, 2011). According to Miyasaka and Grunes (1997), increasing the soil temperature from 8°C to 16°C doubles the contents of Cu, Zn, and Mn in winter wheat leaves. For this reason, during a cold season, plants may have low levels of some metals that may affect their growth.

Some elements are more susceptible to phyto-availability than others, and their behaviors depend on soil properties (Table 5) and soil texture. Khan et al. (2015) reported that soil texture could significantly influence the soil-to-plant transfer of heavy metals. They reported that higher bioaccumulation in plants was linked to higher mobility of metals in sandy than in clay soil. Thus, plants cultivated on sandy soil would have higher concentrations of heavy metals than those grown on clay-loamy soil (Treder & Cieslinski, 2005). Furthermore, plants' mean heavy metal uptake increases with these metals' contents in the soil environment (Chaves et al., 2011). Galal and Shehata (2015) emphasized that heavy metals bioavailability and bioaccumulation depend on the distance from heavy traffic highways. The authors reported a higher bioavailability and accumulation rate in roadside plants near heavy traffics. Smith (2009) demonstrated that sludge- and compost-amended soils reduced metals availability to plants reducing contaminations along the food chain.

Table 5 Heavy metal bioavailability and behavior in soil

Metals	Behavior in soil	Bioavailability
Cr, Ag, Sn	Very slightly soluble in soil	Not easily taken up by plants
Pb, Hg, As, F	Relatively strongly absorbed by soil particles	Not readily transported to above-ground parts of plants
Cu, Ni, Mn, Co, Mo	Mobile in soil	Readily taken up by plants.
Cd, Zn, Se	Very mobile in soil	Easily bioaccumulated by plants

Source: Author's construction from Kabata-Pendias (2011)

Many of these factors are interrelated, temporal, and seasonal. Consequently, changing one factor may affect several others (Danjuma & Abdulkadir, 2019). In addition, according to Clemens (2006), other obscure biological factors appear to strongly influence the bioaccumulation of metals and severely inhibit the prediction of metal bioavailability. Bioavailability and bioaccumulation are two separate concepts that are considered jointly. Bioaccumulation assesses the changes of contaminants concentrations in the organisms, whereas bioavailability describes the portion of contaminants in the environment that is potentially available for bioaccumulation (Wang, 2016).

1.3.2. Bioaccumulation

Bioaccumulation is defined as the “net result of uptake, transformation and elimination of a substance in an organism due to all routes of exposures: air, water, sediment/soil, and food” (UNGHS, 2015). The bioaccumulation of heavy metals varies in plant species and affects their growth, occurrence, reproduction, and survival in contaminated soils (Khan et al., 2015). Guala et al. (2010) reported that heavy metals' dynamics in plant-soil interactions depend mainly on the

levels of soil contamination and plant species (Guala et al., 2010). Specific plant species can absorb, and hyper accumulate metal contaminants and/or excess nutrients in harvestable root and shoot tissue from the growth substrate through the phytoextraction process (Tangahu et al., 2011). Thus, heavy metal accumulation may vary from one plant species to another (Kumari & Mishra, 2020; Onakpa et al., 2018). For example, leafy vegetables metal uptake rates are higher and more contaminated than those of nonleafy vegetables (Khan et al., 2015; Li et al., 2006).

Plants absorb and adsorb heavy metals from the ground and parts exposed to air from polluted environments (Oti, 2015). Root absorption is the main pathway plants use to uptake heavy metal through an ionic exchange, redox reactions, or dissolution-precipitation (USDE, 1994; Tangahu et al., 2011), though other tissues can also readily absorb some nutrients, including heavy metals (Kabata-pendias, 2011). Heavy metal concentration in a plant can indicate the degree of its contamination (Cai et al., 2015; Kowalska et al., 2018; Hołtra & Zamorska, 2020). Therefore, the crop pollution index can be a good measure of the pollution level of heavy metals, for instance, in vegetables (Fouladi et al., 2020; Hu et al., 2017). The crop pollution index is a powerful index tool for processing, analyzing, and conveying raw environmental information for decision-makers and the public, including technicians and managers (Qingjie et al., 2008). It is used to determine the toxic level of heavy metals and thus the pollution class of, among others, vegetables (Oti, 2015).

The capacity of plants to uptake heavy metals differs for different heavy metals. Moreover, the same heavy metal can be accumulated at different ratios in different plant species (Singh et al., 2010). A ratio of plants' element concentration to soil elements indicates plants' ability to absorb chemical elements from the growth media. This ratio is called Biological Absorption Coefficient (BAF) (Cunha et al., 2014), Bioaccumulation factor (Blasco et al., 2016; Hu et al., 2017; Fouladi et al., 2020), Bioconcentration factor (Sahay et al., 2020), or transfer factors (Liao et al., 2016; Chaoua et al., 2019; Rehman et al., 2019; Edogbo et al., 2020; Prabasiwi et al., 2020). Although all these parameters can be used to assess a substance's bioaccumulation potential, the BAF is suitable when the measurements are conducted using field populations involving the uptake from water, air, and food sources (Blasco et al., 2016). BAF is an important concept in environmental risk assessment since it provides quantitative information regarding the ability of a contaminant to be taken up by organisms, especially plants, from the medium (Blasco et al., 2016). BAF values help quantify the concentration of an element taken by plants (Kulkarni et al., 2014; Mishra & Pandey, 2019; Sulaiman & Hamzah, 2018). The metal bioaccumulation varies greatly for different heavy metals in different vegetables (Singh et al., 2010; Onakpa et al., 2018; Rehman et al., 2018).

BAF is not constant and can be modified by soils' physical and chemical characteristics, the behavior of heavy metals in soils and plants, and environmental changes (Prabasiwi et al., 2020). Based on their BAFs, plants can be classified as accumulators, hyperaccumulators, and excluders. Metals are more easily transferred from soil to the edible parts of vegetables with a high BAF than ones with low BAFs. The BAF of specific metals can vary significantly based on the ambient concentration (DeForest et al., 2007). Moreover, soil characteristics, environmental changes, and metal behavior in soil and plants are among the parameters that modify the BAF (Li

et al., 2019). Studies on the heavy metal transfer from soil to plants and their bioaccumulation have been conducted worldwide (Table 6).

Table 6 Bioaccumulation of heavy metals in some edible leafy vegetable species.

Vegetables species	Cd	Pb	Cr	Cu	Ni	Zn	References
<i>L. sativa</i>	0.00	0.00	0.02	0.02	1.24	0.12	Oti (2015)
	6.52E-5	0.23	3.96	-	-	0.26	Edogbo et al. (2020)
<i>A. caudatus</i>	1.16	0.07	0.02	0.09	-	0.57	Jolly et al. (2013)
<i>S. oleracea</i>	-	0.07	-	0.11	-	1.15	
<i>B. vulgaris</i>	0.32	0.03	0.19	0.16	0.35	0.62	Mahmood & Malik
	0.05	0.06	0.07	0.12	0.28	0.16	(2014)
<i>A. viridis</i>	0.07	0.05	0.11	-	-	0.11	Olayinka et al. (2011)
<i>C. olerarius</i>	0.09	0.03	0.03	-	-	0.07	
<i>C. argentea</i>	0.07	0.03	0.07	-	-	0.10	
<i>T. triangulare</i>	-	2.11	0.001	0.22	0.01	2.19	Olusola et al. (2020)
<i>A. spinosus</i>	-	1.36	0.002	0.83	0.02	2.29	
<i>S. nigrum</i>	5.18	0.53	-	-	-	2.71	Akenga et al. (2019)
	5.26	0.38	-	-	-	1.81	
	0.04	0.02	0.33	-	0.11	0.21	Shahnaz et al. (2021)
<i>B. oleracea</i>	0.27	0.01	-	0.12	1.30	0.07	Rehman et al. (2019)
	0.67	0.62	-	0.51	1.81	0.07	

Source: Author's construction

Although the soil-to-plant interface is the major source of heavy metal accumulation in a plant, the contamination may also occur through the water-to-plant and air-to-plant interfaces (Khan et al., 2015). Many authors reported a significant correlation between heavy metals in soil and food crops (Khan et al., 2015; Emurotu & Onianwa, 2017; Gupta et al., 2019; Kumar et al., 2019). Heavy metals, transferred from soil media to growing vegetables, are critical components of health risk assessment (Prabasiwi et al., 2020). Bioaccumulation and transportation of heavy metals in vegetables are the main human exposure route to soil contamination through the food chain (Emurotu & Onianwa, 2017; Ali et al., 2019; Kumar Rai et al., 2019; Huang et al., 2021). Even at low concentrations, heavy metals may be toxic to plants, animals, and humans (Khan et al., 2016).

1.4. Heavy metals exposure through food consumption

Some heavy metals are essential micronutrients at a lower dose, but they can threaten human health at higher doses (Alloway & Trevors, 2013; Briffa et al., 2020; Jyothi, 2020). Chronic low-level intakes of heavy metals affect humans and other animals since there is no suitable

mechanism for their elimination. Heavy metals might enter the human body via inhalation of dust, consumption of contaminated foods and water, or soil ingestion (Engwa et al., 2019; Rai et al., 2019; Jyothi, 2020). Metal ingestion is the main route of exposure to the human population (Engwa et al., 2019). Various factors, including chemical forms of heavy metals, dose, time, frequency, age, nutritional source, and biological species, influence humans' toxicological effects by consuming heavy metal-contaminated food (Khan et al., 2015; Tchounwou et al., 2012). Metals such as Pb, Ni, Cr, As, Cu, and Zn are cumulative poisons (USEPA, 2004; Jaishankar et al., 2014). Some metals, such as As, Cd, Pb, are toxic even at trace concentrations (Khan et al., 2010; Gebrekidan et al., 2013) and are associated with carcinogenic health hazards (Abbasi et al., 2013; Fouladi et al., 2020; Sanaei et al., 2021). The US Department of Health and Human Services (USDHHS, 2011), California health and hazard assessment (USEPA, 2021), WHO International Agency for Cancer Research (WHO/IARC, 2012; WHO, 2015), and USEPA /IRIS (USEPA, 2014) classified Cd, As, Cr, Pb, and Ni, as human carcinogens.

Metals accumulation in the edible parts of vegetables can directly affect farmers and nearby inhabitants because vegetables produced from fields are mostly consumed locally (Antisari et al., 2015; Naser et al., 2018; Zafarzadeh et al., 2018). Thus, the concentration of the metals in vegetables could be a health concern to the residents. Then, resulting health problems can be mild to severe, including nausea, lung diseases, diarrhea, anemia, stomach problems, kidney disorders, skin diseases, neurological disorders, and cancers (ATSDR, 2020). Metal accumulated in human bodies produces toxic, neurotoxic, carcinogenic, mutagenic, or teratogenic illnesses (Engwa et al., 2019; Duruibe & Egwurugwu, 2016). In addition, heavy metals have been shown to cause acute, chronic poisoning in humans (Balali-Mood et al., 2021). The toxic hazard to humans via food depends on the metal concentration and the amount of food consumed. Food consumption is the major pathway of human exposure to heavy metals, accounting for > 90%, compared to other routes of exposure such as inhalation and dermal contact (Loutfy et al., 2006). Exposure to multiple heavy metals has been reported to potentially result in additive and interactive effects on health problems (Harrison & Chirgawi, 1989; Zheng et al., 2007; Zakaria et al., 2021). Whether or not a particular chemical mixture poses an additive risk to human health depends on its targets (tissues, organs, or organ systems) and the mechanisms of action of the individual chemical (USEPA, 1999).

Health risk assessment (HRA) is a process to evaluate nature and potential health effects to the target population, currently or in the future, exposed to contaminated environmental media. It is the key procedure for hazardous substance management, designing remediation policies, and taking control measures (USEPA, 2011; Khan et al., 2015; USEPA, 2021). Human health risk assessment evaluates the likelihood that a given or series of exposures may have damaged or will damage the health of individuals (USEPA, 2021b). An HRA of heavy metals is estimated by using the health risk index (HRI), which is based on the daily intake of metal (DI), the reference dose (RfD), and the children and adults body weight (Chaoua et al., 2018; Praveena et al., 2018; Rehman et al., 2019; Aboubakar et al., 2021). Other methods used for assessing health hazards include carcinogenic and non-carcinogenic risks associated with heavy metals using target cancer risk

(TCR), hazard index (HI), and target hazard quotient (THQ) (Alimohammadi et al., 2020; Antoine et al., 2017; Ashraf, Ahmad et al., 2021; Taghizadeh et al., 2020; USEPA, 2010). Because of their physiological and behavioral characteristics, children are more susceptible to heavy metal contamination than adults (ACHHRA, 2012; Kamunda et al., 2016; Hu et al., 2017; Baltas et al., 2020).

1.5. Health effects

Some heavy metals are essential to humans, but their dosage intakes can lead to unexpected hazardous health and physiological effects (Jyothi, 2020). Despite some health benefits, almost all heavy metals act as carcinogenic agents and can cause various diseases (Kim et al., 2019). Epidemiological studies revealed a higher prevalence of cancers in regions where heavy metals, radioactive elements, and derivatives were ubiquitous due to environmental pollution with industrial and agricultural wastes (Boulanger et al., 2017; Dhananjayan & Ravichandran, 2018). Dissolved forms of these metals, entering the food chain and ending in humans, lead to severe damage to the cellular system and cancer (Kim et al., 2019). Various reports have found that exposure to these compounds disrupts tumor suppressor gene expression, damage repair processes, and enzymatic activities in metabolism via oxidative damage (Bánfalvi, 2011; Jan et al., 2015; Kumar et al., 2019). According to the International Agency for Research (IARC), heavy metals such as As, Cd, Cr, Ni are category class 1 major cancer-causing agents (IARC, 2010; 2014; WHO, 2015). WHO classified heavy metals into four carcinogenic classes based on the overall weight of evidence for human carcinogenicity (Table 7).

Table 7 Carcinogen class of heavy metals

Group	Carcinogenicity level in humans	Evidence	Heavy metal classification
Group 1	Carcinogenic	Sufficient evidence in humans	-Cadmium and Cadmium compounds -Chromium VI compounds -Nickel compounds -Nickel refining
Group 2A	Probably carcinogenic	Limited evidence in humans, enough evidence in animals	Lead compounds inorganic
Group 2B	Possibly carcinogenic	Limited evidence in humans, not enough evidence in animals	-Nickel metallic and alloys -Lead
Group 3	Carcinogenicity not classifiable	Insufficient evidence in humans and animals	-Chromium III compounds -Chromium metallic compounds -Copper
Group 4	Probably not carcinogenic	Evidence suggests no carcinogenic properties in humans or animals	-Zinc

Source: Author's construction from WHO, (2015)

Dietary intake of heavy metal contaminated food can seriously deplete some essential nutrients in the human body and decrease immunological defenses (Arora et al., 2008; Guerra et al., 2012; Kumar et al., 2019; Onakpa et al., 2018). Many studies reported a strong link between human health hazards and metal-contaminated food crops (Table 9). Heavy metals in human bodies are responsible for intra-uterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer rates (Rather et al., 2017). Some heavy metals (Al, Cd, Mn, and Pb) might cause intra-uterine growth retardation (Khan et al., 2015; Kumar et al., 2019). Heavy metal-induced toxicity and carcinogenicity involve many mechanistic aspects that are not all elucidated or understood. However, each metal is known to have unique features and physico-chemical properties conferring specific toxicological mechanisms of action. Some harmful effects of heavy metals are summarized in Table 8.

Table 8 Clinical aspects of heavy metals chronic toxicities

Elements	Target Organs	Primary Sources	Clinical effects
Cadmium	Renal, Skeletal, Pulmonary	Industrial dust and fumes and polluted water and contaminated food	Proteinuria, Glucosuria, Osteomalacia, Aminoaciduria, Emphysema, Cancer
Chromium	Pulmonary	Industrial dust and fumes and contaminated food	Ulcer, Perforation of Nasal Septum, Respiratory Cancer
Lead	Nervous System, Hematopoietic System, Renal	Industrial dust and fumes and contaminated food	Encephalopathy, Peripheral Neuropathy, Central Nervous Disorders, Anemia, Cancer
Nickel	Pulmonary, Skin	Industrial dust, aerosols	Cancer, Dramatis
Copper	Liver, Kidney	Industrial activities and polluted water and soil	Nausea, Anemia, Osteoporosis, Hepatorenal Syndrome
Zinc	Prostate, Liver, Kidney,	Industrial dust and fumes, polluted water, and contaminated food	Abdominal pain, Nausea, Lethargy, Anemia, and Dizziness

Source: Author's modification of Mahurpawar, (2015)

Cadmium (Cd)

Foods are the main source of Cadmium exposure in the non-smoking general population, especially in vegetarians (EU, 2021; IARC, 2012). WHO has established a provisional tolerable weekly intake (PTWI) for Cd at 7 µg/kg of body weight for 70 kg (WHO, 2007a). Intake of highly Cd-contaminated food causes symptoms such as burning sensation, abdominal pain, nausea, salivation, vomiting, muscle cramps, shock, vertigo, convulsions, and loss of consciousness, usually within 15 to 30 min (Tchounwou et al. 2012). Moreover, literature support Cd's role in inducing hypertension (Gallagher & Meliker, 2010) and diabetes

(Edwards & Prozialeck, 2009), as well as its apparent direct toxic impact on gene transcription in the vascular endothelium (Bernhard et al., 2006). Epidemiological evidence links Cd to sudden cardiac death (Menke et al., 2009), peripheral arterial disease (Navas-Acien et al., 2005), increased vascular intima-media thickness (Messner et al., 2009), and myocardial infarction (Everett & Frithsen, 2008).

Several regulatory agencies classified Cd compounds as human carcinogens based on adequate evidence (IARC, 1993). Thus, the lung is the most well-established organ of human carcinogenesis from Cd exposure (Sorahan et al., 1995). Kazantzis (1992) reported a significant increase in the mortality rate from lung cancer for Cd-processing workers from 17 plants in the United Kingdom (standardized mortality ratio [SMR], 1.12; 95%CI: 1.00–1.24), with apparent rising trends associated with a longer duration of employment and the higher intensity of exposure. Although not associated with cumulative exposure to Cd, a significant increase in the SMR for pharynx cancers and a non-significantly increased SMR for lung cancer were observed (Sorahan & Esmen, 2004). Slightly increased odds ratios for prostate cancer were also reported from a case-control study nested within occupational cohorts (Armstrong & Kazantzis, 1985). For kidney cancer, small numbers were reported in two cohort studies without any evidence of an association with Cd exposure (Sorahan & Esmen, 2004).

Lead (Pb)

Young children are particularly vulnerable to lead poisoning because their bodies could absorb Pb 4–5 times more than adults once ingested (WHO, 2007a, 2013). This can be concerning since the cumulative exposure from all pollutants' sources should not exceed the PTWI of 25 µg/kg body weight/week (JECFA, 1993; 2011). The greatest percentage of Pb in the human body is taken into the kidney, followed by the liver and other soft tissues such as the heart and brain. Pb in the skeleton represents the major body fraction (Flora et al., 2006). Thus, the nervous system is the most vulnerable target of Pb poisoning. Headache, poor attention span, irritability, loss of memory, and dullness are early symptoms of Pb effects on the central nervous system (CDC, 2002; ATSDR, 2020). Aside from its acute toxicity, the most important effect of exposure is chronic neurotoxicity, which is particularly severe during the first two to three years of life when the central nervous system is in its early development. Exposure to Pb during this period increases the risk of mild mental retardation, attention deficit hyperactivity disorder, and other developmental disabilities (Lidsky & Schneider, 2003; WHO, 2007a).

Many studies have reported adverse effects of Pb in children and adult populations (Flora et al., 2006; WHO, 2010; Wani et al., 2015; ATSDR, 2020). These studies associated Pb blood level poisoning and diminished intelligence, lower quotient-IQ, delayed or impaired neurobehavioral development, underperformance in school, decreased hearing acuity, speech and language handicaps, growth retardation, decreased ability to pay attention, and anti-social and dynamic behaviors (CDC, 2002; WHO, 2010, 2013;

Wani et al., 2015). In adults, reproductive effects, such as decreased sperm count in men and spontaneous abortions in women, have been associated with high Pb exposure (Apostoli et al., 1998; Hertz-Picciotto, 2000). Acute exposure to Pb induces brain damage, kidney damage, and gastrointestinal diseases. In contrast, chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (ATSDR, 2020; Wani et al., 2015; CDC, 2002; Hertz-Picciotto, 2000; Apostoli et al., 1998).

Pb and Pb compounds have been reported as probable human carcinogens (USDHHS, 2016) with sufficient animal evidence (IARC, 2006; IRIS/USEPA, 2004). Numerous epidemiological and experimental studies indicated Pb as potentially carcinogenic, inducing renal tumors in rats and mice (Goyer, 1993; Waalkes et al., 1995). Researchers have also reported associations between blood Pb concentrations and all cancers (ATSDR, 2020; McElvenny et al., 2015), cancers of the bronchus, trachea, and lung (Kim et al., 2015; McElvenny et al., 2015; Steenland et al., 2019), cancer of the larynx (Barry & Steenland, 2019; Chowdhury et al., 2014), esophageal cancer (Steenland et al., 2019), stomach cancer (Steenland et al., 2017, 2019), intestinal or rectal cancer (Kim et al., 2015; Steenland et al., 2019), and bladder cancer (Steenland et al., 2017).

Chromium (Cr)

The oxidation state and solubility are the main factors influencing the toxicity of Chromium (Tchounwou et al., 2012). Although both most common forms of Cr (trivalent and hexavalent) are toxic at high concentrations (above the PTWI), Cr (VI) compounds are likely to be much more toxic systemically than Cr (III) (De Flora et al., 1990). Toxic forms of Cr can be absorbed by the lung and the gastrointestinal tract, and even to a certain extent by intact skin. Solubility and other characteristics of Cr, such as size, crystal modification, surface charge, and the ability to be phagocytized, might be important in determining cancer risk (Cohen et al., 1993). The Department of Health and Human Services (DHHS) and the IARC have classified Cr (VI) as group 1 occupational carcinogens (ATSDR, 2010; WHO/IARC, 2012; Tulasi & Rao, 2014; Loomis et al., 2018).

Epidemiological investigations have reported respiratory cancers in workers occupationally exposed to Cr (VI) containing compounds (Costa, 1997; Dayan & Paine, 2001). A meta-analysis of 973,697 workers involving 17 standardized incidence ratios from seven countries and four kinds of occupations reported that 11,564 of them had had cancer (Deng et al., 2019).

Table 9 Health risks from dietary intake of foodstuff contaminated with heavy metals.

Heavy metals	Sources of metallic contamination	Route/ medium of exposure	Dose-response/ toxicity limits	Health risks (Acute, chronic, critical)	References	
Cd	Soil amendments with fertilizer and sewage sludge, Ni-Cd batteries, alloys, smoking	Food crops in non-smoking population; smoking; Fe status also affects gastrointestinal absorption	NOAEL (food): 0.01 mg/ kg/day; RfD (mg/kg/day): 0.01×10^{-2}	Adversely affects kidney functioning through increased secretion of low molecular weight proteins ($\beta 2$ - macroglobulin & $\alpha 1$ - macroglobulin), enzymes (N-acetyl- β -D- glycosaminidase), pneumonitis (oxide fumes), inhibition of sex hormones	Proteinuria in humans, kidney damage, human carcinogen (group I) causing lung and breast cancer, long-term exposure can result in itai-itai due to conjunction of osteomalacia and osteoporosis as evidenced in Japan	Brama et al. (2007); Hough et al. (2004); Järup (2003); Peralta-Videa et al. (2009); WHO (2002); Zhang et al. (2008)
Pb	Mining & smelting, paint, thermal power plants, crude petrol	Air/particulate deposition in food crops, occupational exposure	NOAEL:25 $\mu\text{g}/\text{dL}$; RfD (mg/kg/day): 0.35×10^{-3} [toxic limit] $\text{Pb} \geq 70$	Encephalopathy, nausea and vomiting, adverse impact on CNS, circulatory, cardiovascular systems, children are vulnerable to problems with learning and concentration	Accumulation of erythrocyte protoporphyrin through inhibition of ferrochelatase, anemia, abdominal pain, nephropathy, possible human carcinogen	Ma et al. (1996); Järup (2003); Hough et al. (2004); Peralta-Videa et al. (2009); El-Kady & Abdel-Wahhab (2018)
Cu	Irrigation with contaminated wastewater	Intake of contaminated food	LOAEL: 10 mg/kg/day	Can affect renal and metabolic functions	Excess protein droplets in epithelial cells of the proximal convoluted tubules in rats	Dong et al. (2007); Sipter et al., (2008); Peralta-Videa et al. (2009)
Cr	Electroplating/ chrome plating industries, dye industry, sewage wastewater/ sludge	Intake of food contaminated by wastewater and soil amendment with industrial sludge	Toxic limits in humans not specified clearly	Kidney/renal dysfunction/failure, Cr (VI) is more health hazardous than Cr (III) due to rapid absorption, hemolysis and	Collapse/dysfunction of the respiratory system through lung cancer and pulmonary fibrosis	Hough et al. (2004)

Ni	Ni-Cd batteries, wastewater	Intake contaminated food	NOAEL: 5 mg/kg/day; RfD: 0.05×10^{-1} mg/kg/day	gastrointestinal hemorrhage Can affect renal functioning, an integral component of urease enzyme in the kidney	Remarkable decrease in body and organ weights	Järup (2003); Peralta-Videa et al. (2009); El-Kady & Abdel-Wahhab (2018); Islam et al. (2017)
Zn	Irrigation with contaminated wastewater	Contaminated foodstuffs	LOAEL: 59.3 mg/kg/day; RfD: 1.00×10 mg/kg/day	Respiratory problems	A significant decrease (47%) in erythrocyte superoxide dismutase concentration in adult females	Hough et al. (2004)

NOAEL: No observed adverse effect level; LOAEL: lowest observed adverse effects level; RfD: reference dose defined as the maximum tolerable daily intake of a specific metal that does not have deleterious health effects. Source: Rai et al. (2019)

The results of a recent meta-analysis study showed exposure to Cr (VI) may cause increased mortality and incidence of some cancers, including lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer in humans (Deng et al., 2019). Moreover, an ecological study in 2011 in Greece found high levels of Cr (IV) (41–156 µg/L) in drinking water and a significantly increased incidences of liver ($p < 0.001$), lung ($p < 0.047$), and genitourinary cancers ($p < 0.025$) among women (Linos et al., 2011). Another study in 2012 in India showed an increased incidence of gastrointestinal and dermatological complications among populations exposed to Cr (VI) contaminated groundwater (Sharma et al., 2012).

Cr carcinogenicity is followed by DNA damage due to disruption of transcription regulation (Balali-Mood et al., 2021). DNA strand breaks in peripheral lymphocytes and lipid peroxidation products in urine observed in Cr-exposed workers also support the evidence of Cr (VI) induced toxicity in humans (Gambelunghe et al., 2003; Goulart et al., 2005). Oxidative damage is the underlying cause of these genotoxic effects, including chromosomal abnormalities (Wise et al., 2002; Wise et al., 2004) and DNA strand breaks (Xie et al., 2005). Recent studies indicate a biological relevance of non-oxidative mechanisms in Cr (VI) carcinogenesis (Zhitkovich et al., 2001).

Nickel (Ni)

Nickel is an essential element in humans. According to Montgomery (2010) and Nkwunonwo et al. (2020), Ni deficiency decreases plasma cholesterol, increases liver cholesterol, leads to ultrastructural changes in the liver cells, rough hair, impaired reproduction, and poor growth of the offspring. Excessive exposure to Ni leads to health risks, including fibrosis, chronic bronchitis, impaired pulmonary function, and emphysema (WHO, 2017). In the general population, allergic contact dermatitis is the most prevalent effect of Ni toxicity (WHO, 2006). Trombetta et al. (2005) reported that oral exposure to Ni compounds induces skin and oral epithelium damage. Apart from increasing prenatal and natal mortality, Ni may cause a different type of malformation in the embryos.

Experimental studies have established that Ni compounds have potent effects on carcinogenicity and teratogenicity (Leonard et al., 1981; 1984). IARC classified soluble, and insoluble Ni compounds as carcinogens to humans (Group 1) and Ni and alloys as possibly carcinogenic to humans (Group 2B). Although the carcinogenic molecular mechanisms of Ni toxicity are not precise, several studies suggest that Ni exposure induces oxidative stress via a reduction in expression of antioxidant enzymes and DNA single and double-strand breaking (Lynn et al., 1997; Kim & Seo, 2012). Many authors reported that breathing in Ni-contaminated dust from Ni smelting, mining, and tobacco smoking resulted in significant damage to lungs and nasal cavities, leading to occupational diseases such as lung and nasal cancer in Ni refinery workers (Grimsrud et al., 2003; Kasprzak et al., 2003; Küpper et al., 2015).

Copper (Cu)

Copper is an important essential element, but excess consumption leads to toxicity (McDowell et al., 2013). Limited data are available to identify a recommended threshold for long-

term effects of Cu on sensitive populations, such as those who carry genes for Wilson disease (see below) and other metabolic disorders of Cu homeostasis (WHO, 2004, 2017). Neurologically, Cu deficiency can manifest as myelopathy and peripheral neuropathy simulating subacute combined degeneration. Moreover, it results in kinky and steely hair syndrome in humans (Kumar et al., 2004; Wazir & Ghobrial, 2017). Excessive Cu intake leads to hepatolenticular degeneration with progressive impairment of Cu-laden tissues until death results (WHO, 2017). Cu toxicity is manifest in Wilson disease, in which excessive Cu deposition in tissues leads to cardiac dysfunction, liver cirrhosis, and pancreatic dysfunction (Nkwunonwo et al., 2020). Hypothetically, Zinc-Cu interaction is linked to ischemic heart disease as decreased Cu intake with excessive Zn may play an etiologic role in cardiac deaths in animals, including humans (Choi et al., 2018; Davies et al., 2013). Nkwunonwo et al. (2020) reported a toxic syndrome in an infant called pink disease caused by excessive Cu in drinking water near smelters.

Zinc (Zn)

Zinc essentiality involves in catalytic activities of many enzymes, protein synthesis, T-cell maturation and differentiation, cell division, DNA synthesis. Its deficiency is associated with increased lipid peroxidation of mitochondrial and microsomal membranes and osmotic fragility of the erythrocyte membrane. In a study, Jamieson et al. (2006) reported that Zn deficiency enhanced Pb accumulation in bone, while supplementation reduced its absorption and accumulation in rats. Zn supplementation protects against oxidative damage of iron in the instance of iron supplementation. Long-term or higher dosage treatment of Zn has been associated with the depletion of Cu (Yoneda & Suzuki, 1997; Maret & Sandstead, 2006). Moreover, Zn deficiency is associated with growth retardation, loss of appetite, and impaired immune function.

Zn deficiency can cause weight loss, taste abnormalities, delayed healing of wounds, and mental lethargy (Maret & Sandstead, 2006; Ross et al., 2014). Severe Zn shortage results in hair loss, delayed sexual maturation, impotence, diarrhea, hypogonadism in males, and eye and skin lesions (IMSFH, 2002; Wang et al., 2005; Maret & Sandstead, 2006). Zn toxicity can be both acute and chronic. The acute adverse symptoms of high Zn intake (above the tolerable upper intake level) include nausea, appetite loss, vomiting, abdominal cramps, headaches, and diarrhea (IMSFH, 2002). Long-term intakes above the tolerable upper intake level increase the risk of adverse health effects (IMSFH, 2002).

2 Study design and methodology

2.1 Study design

The schematic overview (Figure 3) shows different research steps, starting from the study area selection, data collection, heavy metals determination to the health risk assessment. Each box of the schematic design requires specific methods depending on specific elements of the study. This representation helps to bring individual activities of this study into perspective and promote the interdisciplinary focus of this research.

Urban agriculture forms key characteristics of the study areas. Urban agriculture in land use stands for the interaction between human behaviors and components of the physical environment. Urban agriculture is an informal activity found in all neighborhoods of Yaoundé, Cameroon. The development of this activity reflects people's attachment to their rural origins and their unemployment, and the need for food and income. However, with the city's development, most of Yaoundé's land is being converted to real estate. Urban agriculture has been a move to improve undeveloped plots in the interstices of the town, mainly the lowland along waterways. This study focuses on urban agriculture in the lowlands of Yaoundé. The underlying processes leading to the contamination of the environmental components and pollution levels are assessed in this study as the primary and secondary objectives. Specifically, the study has been designed to assess the heavy metals contamination in urban agriculture and its health outcomes, especially cancer risks. The outcomes in terms of health risks are estimated and quantified.

Understanding the factors leading to heavy metals contamination in vegetables, especially the most important type of plants produced in Yaoundé's urban agriculture, is necessary to estimate the health risks later that consuming contaminated vegetables may pose to humans. Multiple methods are employed in the research design to achieve this purpose. The first objective of this study is to characterize the farming practices, especially agricultural inputs, and the types of irrigation water used in Yaoundé lowlands. A cross-sectional study design was implemented since the farming methods were not expected to vary throughout the research period. For the second phase, heavy metals were analyzed in water, soil, and vegetables. Assuming the concentration of heavy metals in the environmental component to vary throughout the climatic seasons, a longitudinal study design was selected. Since human exposure to heavy metals was quantified via their bioaccumulation in the food chain, a health risk assessment was conducted with the contaminated vegetables as exposure and the cancer risk as outcomes.

Six main cultivated lowland areas in Yaoundé were selected for the study's first phase. The inclusion criteria were that the locations were in the urban areas of Yaoundé (i.e., within the seven subdivisions of the town) and were used for agriculture. Farmers were followed up from April to June 2019. Cross-sectional surveys and a spot-check approach were conducted to gain further insights into potential confounding factors. These two surveys aim to collect information on farming practices in the selected lowlands, emphasizing confounding factors such as the use of fertilizers, pesticides, and irrigation water. During these preliminary investigations, households located in the six selected lowland areas in Yaoundé were visited twice. During the first visit, informal interviews were conducted to identify the farmers engaged in urban agriculture and set up the farm surveys. The questions were about the principal activities of the household's head, types of plants grown, and their farm locations. A total of 130 household heads whose main activity was urban agriculture were identified during this baseline study (farmer's identification) out of 431 households visited. From May to June 2019, the cross-sectional study (farm surveys) followed in which more detailed surveys were conducted among the selected farmers (for inclusion factors, see next section; for more information, see appendix I).

The spot check approach by Ruel & Arimond (Ruel & Arimond, 2002) was used to validate the farmer's survey regarding farming practices, especially occupational health and safety (OHS), pesticides, and fertilizers. This observational approach allows cross-verification of the farm survey and the farmer's management practices. The spot-check approach was used to confirm some variables recorded during the farmer's survey. For instance, hygienic variables such as “the use of personal protective equipment (PPE)” and “handwashing,” respectively, during and after pesticides and fertilizers applications were verified using the spot-check approach. The use of protective clothing and adequate work behaviors may reduce the exposure to heavy metal content in the irrigation water and alternative farm inputs. In addition, proper management of pesticide containers reduces the environmental exposure to heavy metals. On the other hand, certain post-harvest behaviors like washing vegetables with wastewater or storing vegetables in fertilizers containers might enhance heavy metal contamination in food crops. Controlling these factors is important as they influence the exposure to contaminants and increase human health risks. During each farm visit (five times in total), particular attention was paid to farmers' hygiene behaviors toward their environment and themselves. The completed surveys and the observational approach were quality checked and analyzed for similarities and differences.

For the second phase of this study, four lowland areas corresponding to 103 farmers were chosen. The inclusion criteria were areas where farming was conducted during the dry and wet seasons, and the main cultivated vegetable was *S. nigrum*, which during the cross-sectional study turned out to be the most frequently cultivated vegetable species across the study region of the Yaoundé lowlands. Out of the farmers who passed the screening criteria, 30 farmers were selected randomly for further water, soil, and plants analyses. For these selected farmers, the main purpose of growing *S. nigrum* was for own consumption. From July to August (short dry season) and September to October (short wet season), 180 samples were collected for heavy metal analysis and physico-chemical parameters (pH, EC, and Cation-exchange capacity [CEC]). These samples consisted of 60 samples of irrigation water, 60 samples of farmed soils, and 60 samples of *S. nigrum* (most abundant and consumed vegetables). All the samples were collected twice at the beginning of each short season: during the short dry season (July) and secondly during the short-wet season (September). All 30 farmers were visited four times (each twice during the wet and dry seasons). Irrigation water and soils were sampled the same day when farmers were intensively engaged in irrigation and during the harvest when the vegetables were mature (mainly three weeks after planting).

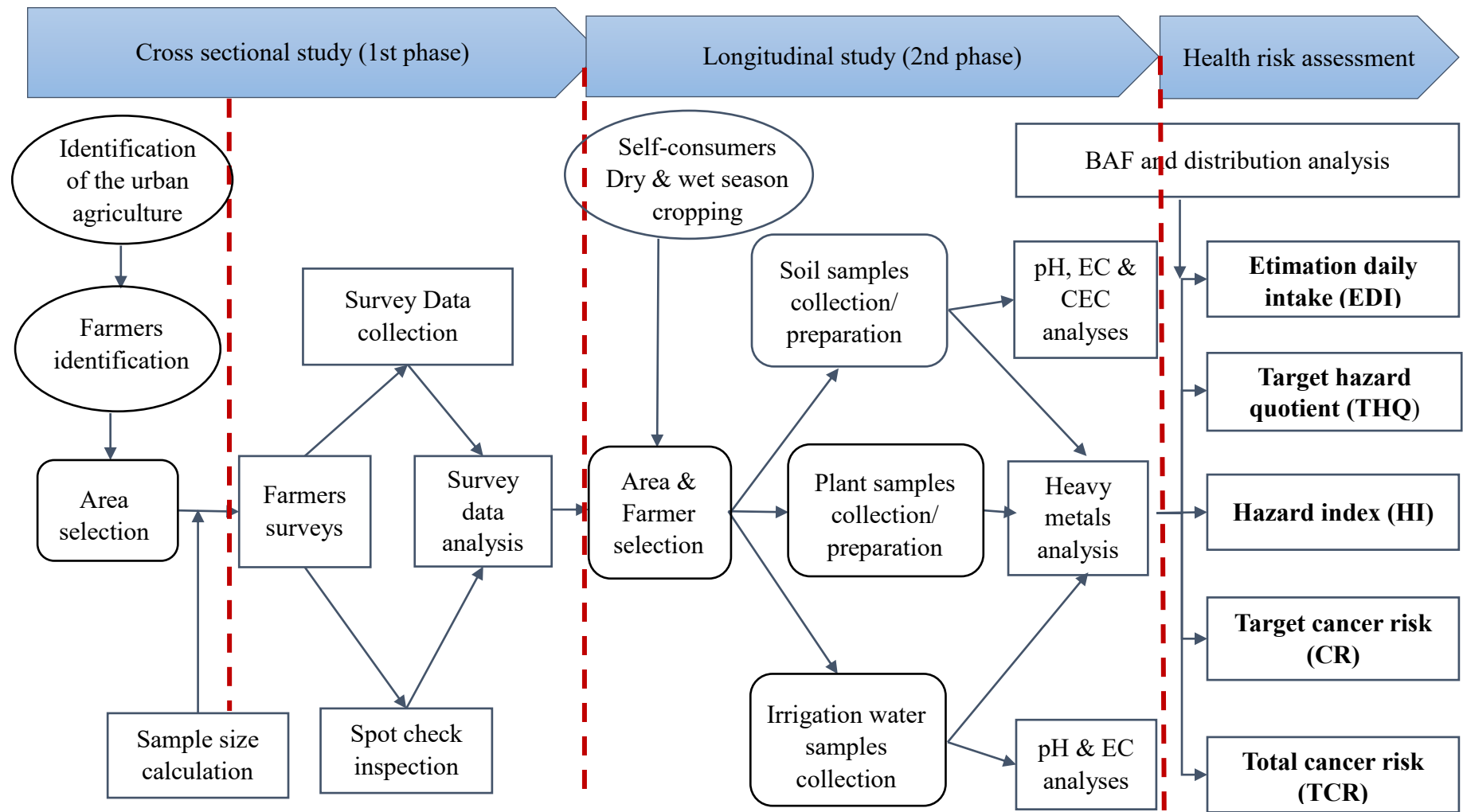


Figure 3 Overview of the experimental program, from the study design, location and sample collection, preparation, and heavy metals determinations to the health risk assessment. Shapes meaning: (circle= inclusion criteria; rectangle= methods; hexagon= sampling selection); bold= outcomes; line dashes = different steps of the studies; pH= Potential of Hydrogen; EC= Electrical conductivity; CEC= Cation Exchange Capacity; BAF= Bioaccumulation factor.

Source: Author's construction

Physical parameters such as the pH and EC were analyzed at the Waste Water Research Unit (WWRU) laboratory of the University of Yaoundé using the standard methods for examining Water and Wastewater (APHA/AWWA/WEF, 2017). CEC and heavy metals were analyzed at the International Institute of Tropical Agriculture (IITA) laboratory. The heavy metals concentration was assessed by measuring six metals (Cu, Cd, Cr, Ni, Pb, and Zn) classified as priority control pollutants (USEPA, 2014). These metals were analyzed using the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) technique (Benton et al., 1990; Chen & Ma, 2001). The composition of elements in samples was determined using plasma and spectrometers.

In general, a wide range of sample types can be analyzed for multi-elements, but the liquid is the most typical sample form. Heavy metals are ready to form complexes with organic constituents; therefore, they can be destroyed by strong acid digestion. Digestion destroys the organic matter, removes interfering ions, and brings metallic compounds suspension to the solution (Singh & Chandel, 2006). All steps followed in carrying out the entire selection, sampling, collection, preparation, and analytical procedures in this study are detailed in the following paragraphs.

2.2. Sampling

2.2.1. Farmers sampling

Before this study, there was no recent number or list of farmers engaged in urban agriculture in the Yaoundé lowlands. The only available data, in 2011, reported 121 crop producers in 11 lowlands both in the central and the peripheral areas of Yaoundé (Bopda et al., 2011). Only six lowlands identified completed the inclusion criteria mentioned above during the baseline study. The remaining five lowlands were used either for floriculture (Bois Saint Anastasie, Etetak) or city buildings (Usine Bastos, Warda, Etoug-Ebe). Thus, a random sampling technique could not be employed for farmer selection. Given the unknown population size, the equation developed by Morse (2000) for qualitative health research was used. The necessary sample size formula is as follows:

$$\text{Necessary Sample Size} = (Z\text{-score})^2 * \text{StdDev} * (1 - \text{StdDev}) / (\text{margin of error})^2 \text{ (Morse, 2000)}$$

where Z is the level of confidence of the results from the survey findings, which was accurately set at 95% (0.05: a Z value equal to 1.96) and corresponded to the level required in management research (Taherdoost, 2017); 0.5 StdDev is the standard deviation, set to ensure the sample size was large enough (Morse, 2000); the marginal error was set to 8% and the sample size for this study obtained from the calculation using the above formula is 150.

Due to the unavailability of information, a snowball sampling approach (Falkenberg, 2016) was applied to the six selected areas to identify farmers. This method is cost-effective because it takes a shorter time to identify farmers. Snowball sampling involved asking the households to list six nearby households who were also engaged in urban crop production. We randomly chose two farmers from each of the mentioned six farmers to be part of our sample. In the end, the interviewer asked for the name of nearby farmers, and the process was repeated. To avoid clustering and ensure

good coverage, attention was paid during the sampling processes to the spatial distribution of the selected farmers. The same procedure was conducted in all six selected lowland areas. In the end, 130 questionnaires were retained after cleaning and removing incomplete answers. Thus, depending on the population size of farmers engaged in crop production in a given locality, 11 farmers were enrolled in Emana, 20 in Mokolo, 16 in Minkoameyos, 24 in Nkolbisson, 26 in Ekounou, and 33 in Ekoumdoum. Each farmer's consent was obtained before participating in the study (see consent form in Appendix 2).

Selection of sub-sample of farmers

During the cross-sectional study, agreements were made with the farmers to collect water, plants, and soil samples from their farms. Four out of six areas were chosen based on criteria, such as presence and year-round cultivation of *S. nigrum*. Thirty (30) farmers were randomly selected out of the pool of those who had passed the selection criteria, 11 in Ekoumdoum, 8 in Ekounou, 5 in Mokolo, and 6 in Nkolbisson lowland areas. The selection was made to avoid overfitting and the disproportionality of the areas. In addition, some farmers were unwilling to continue participating in the second phase of the study, i.e, the longitudinal study.

2.2.2. Water, soil, and vegetable sampling

Sixty (60) samples of each sample type were collected twice during the dry and wet seasons, respectively, in the four selected lowland areas. Water was sampled where the farmers regularly drew water for irrigation. In the case of multiple irrigation water sources, farmers' main water collection points were selected. This selection was considered representative of the different water sources identified during the survey. Groundwater was the only source of irrigation water in one area.

The collection methods for soil and vegetables were adapted from the ISO 18400-202 standard (ISO 18400-202, 2018) and the methods developed by AYNEKULU et al. (2016; 2011). These methods were adapted to the soil condition of the selected lowlands and the farmers' availability and acceptability. The soil and vegetable samples were taken based on the irrigation water sampling points. Table 10 below summarizes the number of samples chosen according to the cultivated size of the lowland areas.

Table 10 Summary of the size of areas, sampling approach, and number of sample points

Sites	Superficies	Number of samples	Sampling method	Distance between sample	Collection radius
Ekoumdoum	6 ha	11			
Ekounou	4 ha	8			
Nkolbisson	2 ha	6	Composite	12.2 m	5.64 m
Mokolo	1 ha	5			

Source: Author's construction

Description of plant species

Solanum is the largest and most complex genus of the family Solanaceae with more than 1,500 species, comprising of many essential vegetables and fruits, many of them economically significant (Mohy-Ud-Din et al., 2010). *S. nigrum* L. is commonly known as black garden or nightshade. It is more usually referred to as the genus *Morella* (Maurella (Dun.) discovered by Dumort and Morelle noire in French (Edmonds & Chweya, 1997; Schippers, 1998). *S. nigrum* plants are sub-glabrous or pubescent and usually have appressed, glandular-headed multicellular hairs, e.g., black nightshade (Mohy-Ud-Din et al., 2010). The crop can grow up to 60 cm in height and is often found growing like a weed on fertile soils with ovate leaves (Akenga et al., 2020). It is cultivated in humid highlands and lowlands in many African countries, including Kenya and Nigeria. It is an important subsistence crop and is also used for medical purposes (Akenga et al., 2020; Akubugwo & Ugbogu, 2007; Klocke et al., 2016). In Cameroon, *S. nigrum* is one of the most popular cash crops and is commonly grown as a traditional vegetable in almost all regions of the country (Asongwe et al., 2014; CGIAR, 2013).

S. nigrum has a short life cycle and can be harvested 4-5 weeks after transplanting (Figure 4a). The germination usually takes 5-7 days after sowing the seeds (Schippers, 1998). Harvest can occur at 7-14 days intervals and, depending on the soil fertility, 3-10 times per plant (PROTA, 2015). At an immature stage (Figure 4b), *S. nigrum* has green to dark purple berries and yellow anthers white flowers, which consumers remove to avoid their bitter taste. Green berries contain some toxic alkaloids like solanine and solanidine (Schippers, 1998). Their consumption can lead to adverse effects such as dizziness, mental confusion, vomiting, loss of speech, and sometimes blindness. However, its leaves contain low levels of alkaloids, and heating during cooking eliminates the toxic effects (Schippers, 1998). Nutritional contents depend on many factors, including the age of the crops, growing seasons, soil fertility, etc. About 89% of the vitamins and other micro-nutrients are lost during the cooking process (Akubugwo, Obasi, & Ginika, 2007).

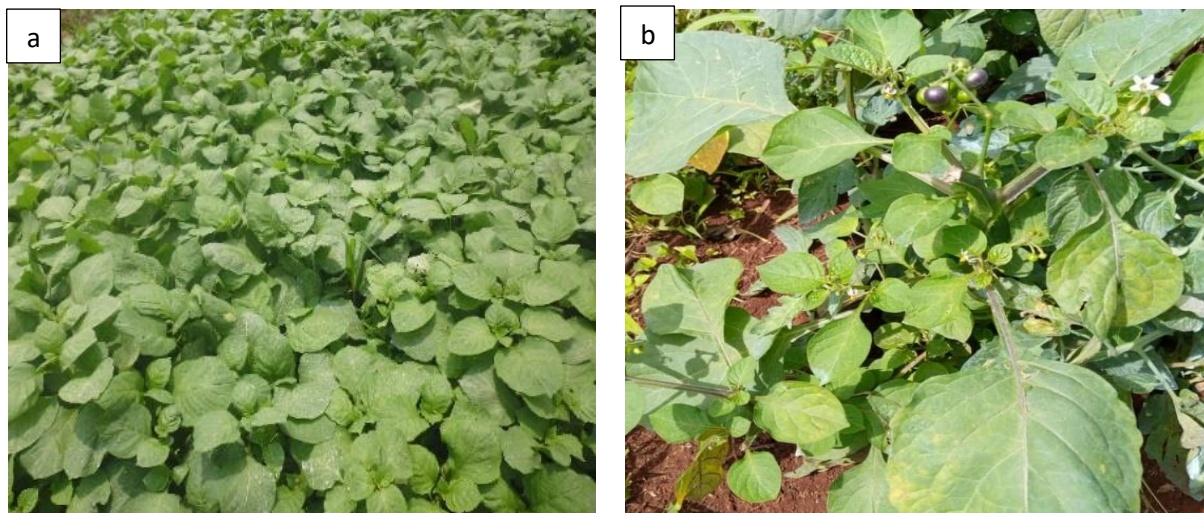


Figure 4 *Solanum nigrum* L. (Solanaceae); a) Three weeks after transplanting; b) weeks after harvest. Vernacular name black nightshade; local name Zoom/Jamajama/ Morelle noire. Source: Author's construction

2.3. Methods

2.3.1. Farmer surveys

A cross-sectional survey was used to interview farmers. Farm survey enables observations and cross-verification during the interview process. It also allows confirming the type of water used for irrigation, the predominance of cultivated vegetables, and the hygiene behaviors while applying agricultural inputs. Additionally, some key factors of the farming system, such as the farm size, crop varieties, fertilizers, and pesticides, could be cross-checked during the interviews. The quantitative survey of farmers provided information on the demographic characteristics, farming, diet and foods, agricultural inputs, irrigation water, sanitation, and health. The information was collected using the open-source software Kobo collect (UNHCR, 2016). The language used for the interview was French. The questionnaire, divided into six main sections (Table 9), was a series of closed-ended questions with multiple-choice answers, open-ended, and contingency questions (i.e., questions asked only when the respondent gives a particular response to a previously specified question).

The demographics section included questions concerning gender, marital status, level of education, age, region of origin, number of people involved in lowland agriculture, membership in an association, and their primary occupation and motivation to conduct urban agriculture. The frequency of farming activities and the principal funding for these were assessed. The areas/crops sections include two groups. The first group, known as "area," aimed to characterize the land, the ownership, the number of years of farming on that plot. Meanwhile, the second one, termed "crops," was about the cropping phase, type of vegetables grown in a particular season, the planting period, fertilization period, irrigation period and frequency, the harvest period, and output in kilograms, and the purpose of cultivating these particular crops (i.e., own consumption, market sale, or bartering).

Table 11 Key information and different components of farmer questionnaires

Demographics Information	Area/crops	Diet & Food	Agricultural inputs	Water, Sanitation, and Hygiene	Health
Gender	Farm size	Type	Seeds	Origin of water	Main diseases
Marital status	Ownership	Mode ate	Amendment	Other use of water	Symptoms
Education level	Operating years	Daily intake	Fertilizers	Drinking water source	chronic disease
Region	Types of crops	Origin	Pesticides	Toilet facility	Type of medication
Main occupation	Irrigation			OHS	
Motivation	Machinery			Contact water/ soil	Farming constraints
Weight	Purpose of the growing			Post-work behaviors	
				Occurrence of disease	

Source: Author's construction. OHS: Occupational Health and Safety

The food and diet section focused on the type of foods consumed in the last 24 hours, the proportion of *S. nigrum* consumed, consumption mode, average daily intake, the origin of the product, and storage place. The agricultural inputs section included the type and origin of seeds, the type of soil amendment used and pesticides, their characteristics, and purchases price. The water, sanitation, and hygiene section gave an overview of the primary type of water used for irrigation and its application method, the perceived issues with this water source, the primary sources of drinking water, the kind of toilet facility and related problems, and the use of occupational health and safety (OHS) during watering and pesticides applications.

The health section quantified health-related problems experienced by farmers during the last seven days and over the past month. If any sickness was noted, the symptoms and their perceived causes were inquired. Long-term health problems/chronic diseases and any family history of cancer were recorded. Additionally, the utilized treatment options during sickness were noted. The importance of urban agriculture was queried, especially whether the farmer had ever received training in farming practices such as the safe application of fertilizers and pesticides. Finally, the main constraints faced during farming in lowland areas and the possible ways of improving their activities were examined.

2.3.2. Irrigation water

2.3.2.1. Samples collection

Irrigation water (350 ml) was sampled in the mid-cropping season when crop demand was highest. The irrigation water samples were collected using the standard method described by WHO (2006). The samples were collected two times, once in July (short dry season) and once in September (short rainy season). Water samples were collected in two pre-cleaned containers for duplicate measurement at each sampling location. The water sample (100 ml) was poured into labeled plastic bottles previously washed with double distilled water and dried in a clean metal-free area (Singh et al., 2010). The bottles were rinsed three times with the water sample of the particular location before each sample collection to avoid contamination. All collected irrigation water samples were transported in a cooler box at about 4 °C by using a thermo-coal box with ice packs to the IITA laboratory. The samples were stored in a refrigerator before heavy metal analysis. The remaining 250 ml samples were transported to the WWRU laboratory at the University of Yaoundé I, where the pH and EC were determined.

2.3.2.2. Physico-chemical analyses

The pH of water is known to affect the processes of disinfection and solubility of metals. Therefore, the pH of the irrigation water was determined using a portable HACH pH meter (HQ11d). After calibrating the pH meter using buffer values 7.00 and 4.00, the electrode was introduced into a 100 ml volume of sample, and the values were read on the digital display screen.

Electrical conductivity (EC) of water is the conductance of a column of water between two metal electrodes of 1 cm² of surface and separated from each other by 1 cm. Measurements of the

electrical conductivity in Micro Siemens per Centimetre ($\mu\text{S}/\text{cm}$) were performed using a HACH brand conductivity meter (HQ14d). This apparatus was equipped with a standard probe dipped vertically into the solution, of which its concentration was determined. The value of the conductivity was read on a digital display screen.

2.3.2.3. Heavy metals in water

A Perkin Elmer Optima ICP-OES 8000 Mass Spectrometer was used to analyze the heavy metal content of the samples. ICP-OES is a trace-level, elemental analysis technique that uses the emission spectra to identify and quantify the elements in the samples. After the ICP-OES calibration of Cd, Cr, Pb, Ni, Zn, and Cu heavy metals prepared from a certified mixed element with standards, water samples were introduced into the ICP detection. The procedure consisted of introducing 50 ml of a digested sample water into the plasma in a dissolve and ionizing process. A high-frequency argon plasma at 6,000-8,000 K flame temperature excites the metal atoms in the sample. In general, only fine aerosols are transferred into a plasma, generating high-frequency alternate current flows. A grating spectrometer was used to disperse the emitted light since the constituent elements could be identified by their characteristic emission lines and quantified by the intensity of the same lines. The results obtained were reported in milligrams per liter (mg/l) at the respective wavelengths mentioned on the results sheet.

2.3.3. Agricultural soils

2.3.3.1. Sampling collection technique

The composite soil sampling and water collection were carried out simultaneously, assuming the soil surface was heterogeneous. The methodology consisted of using a pre-marked chain, measuring out the distance (12.2 m) from the plot center-point "A" to the center of the up-slope sub-plot. Mark this sub-plot (2) center point. Sub-plots 3 and 4 should be offset 120 and 240 degrees from the up-slope point, respectively. The angles can be measured using a compass, or a sampling plate can be marked and used to locate Sub-plots 3 and 4 (Figure 5). From center point A, four cores (sub-samples) were collected. In the end, the "composite sample A" corresponds to a mixture of soil from the four sub-plots ($A = a_1 + a_2 + a_3 + a_4$) associated with core A.

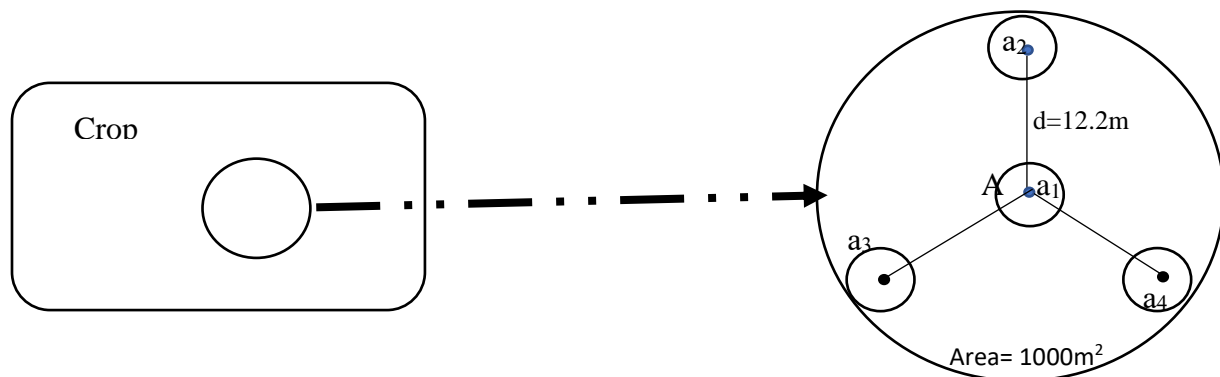


Figure 5 Sampling scheme for a composite sample of soil. Each sub-plot has a radius of 5.64 m (area = 100 m^2), and the distance along the radial arms between subplot centers is 12.2 m. The whole plot has a radius of 17.84 m (area = 1,000 m^2). Source: Author's construction adapted from Aynekulu et al. (2011)

The sampling depth varied between 0-20 cm layer for arable soil as recommended for the soil investigation depths in agricultural use (Aynekulu et al., 2016). This depth range usually represents the depth of the plow layer or hoe work.

Soil samples, like water samples, were collected twice over four months. Sampling was done with a hand auger, and 500 g of composite soil samples were packaged into transparent zipper-lock plastic bags and transported to the IITA laboratory in a cooler box.

2.3.3.2. Sample preparation

Composite soil samples were collected, labeled, and transported to the IITA soil laboratory of Yaoundé. The samples were air-dried for two weeks in the soil laboratory, sieved through a 2 mm sieve, and further finely grounded with a rolling pin through a 0.25 mm sieve (Figure 6). The operating mode respected the five steps of pre-treatment of samples: drying, grinding, sieving, separation and spraying, according to NF ISO 1146 standard (ISO 11464, 2006). Then, 500 mg of <0.5 mm soil sample were packed and preserved for future analysis.

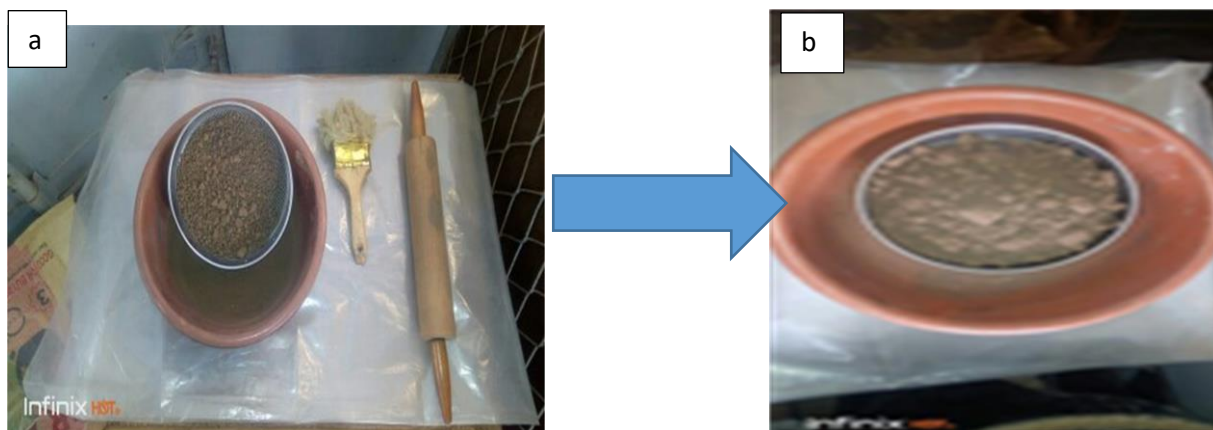


Figure 6 Soil sample preparation: grind and sift through 2 mm (a) and 0.25 mm (b) sieve
Source: Author's construction

2.3.3.3. Physico-chemical analyses

The samples were prepared following the standard method of pH determination ISO 10390:2005. This method is based on the 5:1 principle, which includes preparing a soil sample suspension in a five-time volume of water, shaking it for about 5 minutes, and resting for at least 30 minutes. Twenty (20) g of the prepared sample was later diluted in a beaker with distilled water to achieve the 100 ml solution. The "water" pH of each sample obtained was then determined using a calibrated portable HACH pH meter (HQ11d). The electrode responds to the hydrogen ion concentration by developing an electrical potential at the samples' glass-liquid interface. The apparatus displays the value of pH on the digital screen.

The analysis of the EC was also carried out following the 5:1 principle. Twenty (20) g of soil were diluted in 100 ml of distilled water. The solution was stirred for 30 minutes and then

filtered. The conductivity of the purified extract was measured in Micro Siemens per Centimeter ($\mu\text{S}/\text{cm}$) unit using a HACH brand multifunctional conductivity meter (HQ14d).

Cation exchange capacity (CEC) is the maximum number of cations that 100 g of dry soil can absorb. CEC was determined using the ammonium acetate 1M method at pH 7 and quantified in flame atomic absorption spectrophotometric analysis as described by Anderson and Ingram (1993). CEC is determined by the sum of exchangeable cations determined at pH 7. The exchangeable soil CEC value is in $\text{cmol (+)}/\text{kg}$, similar to $\text{meq}/100\text{g}$.

2.3.3.4. Heavy metals analysis

Digestion and measurement

The conventional aqua regia digestion methods (Chen & Ma, 2001) were performed in 250 ml glass beakers. Solid samples were digested in an aqueous acidic solution to determine the heavy metal contents. Five hundred (500) mg of soil sample was digested in 12 ml of aqua regia on a hotplate at 110°C under a fume hood for 3 h. After evaporation to near dryness, the sample was soaked up with 20 ml of 2% nitric acids to convert metal ions into their highly soluble nitrate salts. After filtration through a Whatman no. 0.42 paper, the sample was diluted to a 100 ml volumetric flask with deionized distilled water. Total Cd, Cr, Cu, Ni, Pb, and Zn in the digest samples were analyzed inductively via coupled plasma-optical emission spectroscopy (ICP-OES; Perkin Elmer Optima 8000) after calibrating the instrument with certified standards.

Quality control included soaking glassware and plastic containers for ICP's operations in 5% HNO_3 and drying before use. Deionized water was used in all analyses, and all reagents were of analytical grade. The analytical reagent blanks and soil reference materials were prepared and inserted in each batch of 15 soil samples for analysis to monitor the accuracy and precision of the analytical methods used. Reference soil materials included four external references samples and one certified sample from the International Soil Exchange Program were used in this study. Triplicate analysis was performed.

The results of the analyses were accepted when the measured concentrations of studied heavy metals in the reference material were within two standard deviations on both sides of the average certified values in the lab control chart. All results obtained were expressed in micrograms per grams ($\mu\text{g}/\text{g}$) at the respective wavelengths mentioned on the result sheet.

2.3.3.5. Vegetable analysis

Samples collection

Solanum nigrum, commonly called black nightshade, was chosen for the metal analysis. The vegetable was selected based on the classification made by Chagomoka et al. (2014). It has several properties and high nutritional value compared to exotic crops. It is among Cameroon's top five most consumed vegetable plants (Kamga et al., 2013). Since leafy vegetables have a higher tendency to accumulate metals from agricultural soils (Gupta et al., 2019), the chosen species was collected for heavy metal analysis at its maturity (six to eight weeks after planting)

during the harvest period. The sample was a combination of five sub-samples collected from five locations described in detail in the soil sample collection section. The vegetable samples were placed in labeled paper bags and transported to IITA for samples preparation and heavy metal analysis.

Samples preparation

The pre-treatment of leaf vegetable samples started from washing with tap water and rinsing with deionized water to remove surface contamination (Figure 7a). These samples were then put in labeled paper bags and oven-dried at 60°C for two days until reaching a constant weight to stop enzymatic reactions and remove moisture (Figure 7b). The dried herbs were finally ground to a fine powder using a commercial blender/mixer, passed through a 2 mm sieve, and further finely grounded to pass through a 0.5 mm sieve. The grinding process aims to reduce the particle size to homogenate and obtain a suitable laboratory sample. The powder was stored at room temperature in clean and dry labeled plastic bags (Figure 7c).

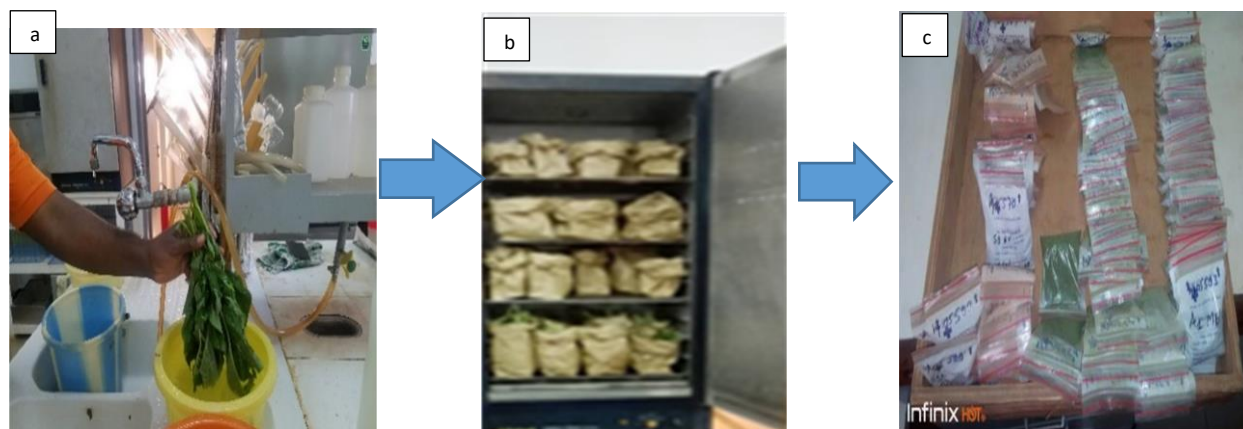


Figure 7 Vegetable samples preparation: a) washing, b) drying, c) packaging and ready for analysis
Source: Author's construction

Digestion and measurement

The digestion process started from weighing 500 mg of finely grounded dried plant tissue powder into a digestion tube and adding 5 mL of concentrated HNO_3 . The funnel was placed into the mouth of the digestion tube and left overnight. The digestion tube was placed into a port of the digestion block to digest at 125°C for 4 h. The digestion tube was removed from the digestion block and let cool. Carefully, the supernatant was transferred into centrifuge tubes, and pure water was added to bring the level to 10 mL (Benton & Vernon, 1990).

Cr, Cu, Zn, Cd, Ni, and Pb were determined by the ICP OES method outlined above for soils. The Quality Control Measures were done via instrument calibration standards prepared from certified standards. Four external reference samples were included in every batch analyzed. In addition, one standard reference sample from the National Institute of Standards and Technology was included in every set. All chemicals used were of analytical reagent grade with high purity. All solutions were prepared with deionized water.

2.4. Assessment of vegetable-heavy metal pollution

2.4.1. Crops pollution index (Pi)

The degree of heavy metal contamination in vegetables for a single pollutant was assessed using the crop pollution index (Pi). The Pi is the ratio of an element concentration in the vegetable samples to the standard value of the corresponding element (Hu et al., 2017). Table 12 gives an overview of the classification and interpretation of the Pi.

The Pi was calculated using equation (1):

$$Pi = \frac{CCi}{CSI} \quad (1)$$

Pi is the crop pollution index, CCi (mg/kg) is the measured concentration of element i , and CSI (mg/kg) is the standard value of element i according to the WHO/FAO.

The NCPI or integrated pollution index is a comprehensive index used to classify the plant according to heavy metal contamination (Hu et al., 2017). It highlights the importance of the most contaminated elements ($Pimax$) while considering all the individual evaluation factors (Zhang et al., 2019).

It was calculated using Equation (2).

$$NCPI = \sqrt{\frac{(Pimax)^2 + (Pim)^2}{2}} \quad (2)$$

Where $Pimax$ is the maximum Pi value, and Pim is the mean Pi of all the studied heavy metals (Dey et al., 2021; Li et al., 2018). Pi and NCPI classifications are defined in Table 12.

Table 12 Classification and interpretation of the crop pollution index

Class	Pi	Grade	Description
I	$Pi \leq 1$	Safety	Clean
II	$1 < Pi \leq 2$	Slight pollution	Slightly clean
III	$2 < Pi \leq 3$	Mild pollution	Crops start to be polluted
IV	$3 < Pi < 5$	Moderate pollution	Crops have been polluted moderately
V	$Pi > 5$	Strong pollution	Crops have been polluted severely

Source: Author's construction from Hu et al. (2017)

2.4.2. Heavy metal bioaccumulation

The accumulation of heavy metals from agricultural soils and irrigation water in plants is the major pathway of human exposure to heavy metal contamination (Jolly et al., 2013; Satpathy et al., 2014). Many authors have reported that BAF is one of the crucial parameters of human exposure to toxic heavy metals through the food chain (Satpathy et al., 2014). The BAF can be used to assess the bioaccumulation potential of a substance. In this study, BAF was calculated as a ratio of the concentration of test substance in the test organism (*S. nigrum*) to the concentration of the substance in the surrounding medium, such as soil (Zhou et al., 2016).

BAF >1 indicates high metal absorption from the soil and high suitability of the vegetable for phytoextraction/phytoremediation (Letshwenyo & Mokokwe, 2020; Mishra & Pandey, 2019). These high BAF plants can also be referred to as hyperaccumulators (Yashim et al., 2014). On the contrary, a BAF <1 indicates the inadequate response of plants towards metal absorption (Mirecki et al., 2015), and the plants show phytostabilization potential. These plants are excluders because they possess mechanisms that maintain low uptake of soil-metal contents (Gordana et al., 2020) and can be used for human consumption. For accumulator plants, the ratios of BAF = 1 demonstrate that the plants are not influenced by the heavy metal elements (Rangnekar et al., 2013). It also implies that the same concentration is found in soil and plant.

The BAF equation is as follows:

$$BAF = \frac{c_c}{c_s} \quad (2)$$

c_c and c_s are the concentration of heavy metals in vegetable and corresponding soil samples, respectively (Zhou et al., 2016). When calculating the overall BAF, c_c indicates the mean content of heavy metals in all crop samples. c_s represents the mean heavy metal content of the corresponding soil samples based on their dry weights (Mirecki et al., 2015).

2.5. Human health risk assessment

The risk evaluation started with identifying the potential sources to introduce risk agents into the environment, estimating the number of risk agents that come into contact with the human-environment boundaries, and quantifying the health consequences of the exposure. The human health risk assessment of heavy metals in foods includes non-carcinogenic and carcinogenic effects according to the United States Environmental Protection Agency (USEPA) method. The USEPA method assesses three exposure pathways, i.e., ingestion, dermal contact, and inhalation, and has been widely studied worldwide (Liu et al., 2013; Hu et al., 2017; Li et al., 2018; Yang et al., 2018). Such risk assessment is essential to identify the health risks of heavy metal contaminated foods and human (agricultural) activities to provide evidence for decision-makers (Liu & Ma, 2020).

This study used the USEPA methodology and its threshold values to assess the potential risk of contaminated vegetables with heavy metals (USEPA, 1989, 2001). The parameters include the average or estimated daily intake of metals (EDI), exposure frequency, exposure duration, body weight, and oral reference dose (Table 13). The EDI, as proposed by the USEPA, was used to quantify the amount of heavy metal intake through vegetables per kg of body weight per day. The EDI of metals was calculated based on the WHO standard daily intake of vegetables using equation 3 (USEPA, 2000; USEPA, 2002a, 2004).

$$EDI_v = \frac{C_v \times IR_v \times EF \times ED}{BW \times AT} \quad (3)$$

}

- Estimated daily intake (EDI in mg/kg/day)
- Concentration of the metal in the vegetables (C_v in mg/kg)
- Ingestion rate (IR_v in kg/meal)
- Exposure frequency (EF in meals/years)
- Exposure duration (ED in years)
- Average time (AT in days) and Bodyweight BW (Kg)

Table 13 Input parameters of non-carcinogenic and carcinogenic effects

Parameter/ definition	Unit	Adult (default value)	References
Body weight (BW)	Kg	70	DEASA, 2010; USEPA, 1991
Exposure frequency (EF)	days/year	350	DEASA, 2010; US EPA, 2011
Exposure duration (ED)	years	30	DEASA, 2010; US EPA, 2011
Ingestion rate (IRv)	Kg/d	0.4	WHO and FAO, 2003
Average time (AT)			
For carcinogens	days	365 x 70	DEASA, 2010; USEPA, 2002b
For non-carcinogens		365 x ED	DEASA, 2010; US EPA, 2011

Source: Author's construction

2.5.1. Non-carcinogenic risk assessment

The non-carcinogenic risk from consumption of heavy metal contaminated vegetables was calculated using the hazard quotient (USEPA, 1989). The hazard quotient (HQ) represents the ratio between the exposure to toxicity (EDI) and the reference dose (RfD), as in equation 4. RfD assumes the level of exposure that is unlikely for even sensitive populations to experience adverse health effects (Table 12) (USEPA, 1989, 2010). An HQ < 1 signifies very low risk; a daily exposure at this level is believed not to cause any adverse effects during a person's lifetime. Equation 4 is used for estimating HQ:

$$HQ = \frac{EDI}{RfD} \quad (4)$$

With HQ being the ratio of exposure to hazardous substances, EDI is the average daily intake (mg/kg/day); the RfD is expressed in milligrams per kilogram of body weight per day (mg/kg/day). Table 14 shows the reference dose for the selected heavy metals in the food according to USEPA (2002b).

Table 14 Reference doses (RfD) and slope factors (SF) of the studied metals over oral pathways

Elements	RfD	References	SF	References	Carc. Class (USEPA, 2018)	PMTDI (ug/kg.bw/day)
Cadmium	0.001	USEPA, 1989	15	USEPA, 2017	B1	0.8
Chromium	0.003	USEPA, 1998, 2017	0.50	USEPA, 2017	A	FAO/WHO, 2011 0.05–0.2 Wang et al., 2011
Lead	0.025	DEASA, 2010	0.085	DEASA, 2010; USEPA, 2017	B2	3 FAO/WHO, 2011
Nickel	0.02	DEASA, 2010; USEPA, 1991	1.7	USEPA, 2017	A	12 WHO, 2006
Zinc	0.3	USEPA, 1989, 2004	n.a	-	D	300 WHO, 1982
Copper	0.04	USEPA, 2017	n.a	-	D	0.5 WHO/FAO, 2011

PMTDI: provisional maximum tolerable daily intake. Source: Author's construction

Exposure to two or more pollutants may result in additive and interactive effects (Amirah et al., 2013). Assuming that the individual health risks of metals within the same vegetable are cumulative, the hazard index (HI) is treated as the arithmetic sum of the individual risk described by USEPA (1989). Therefore, the HI is calculated the following (equation 5):

$$HI = \sum_{x=1}^6 \frac{EDI_x}{RfD_x} = \left[\frac{EDI_{Pb}}{RfD_{Pb}} + \frac{EDI_{Cd}}{RfD_{Cd}} + \frac{EDI_{Ni}}{RfD_{Ni}} + \frac{EDI_{Cr}}{RfD_{Cr}} + \frac{EDI_{Cu}}{RfD_{Cu}} + \frac{EDI_{Zn}}{RfD_{Zn}} \right] \quad (5)$$

where the EDI is the average chronic daily intake of the x^{th} heavy metal in mg/kg-day, and RfD is the oral reference dose of the x^{th} metal in mg/kg-day. HI is the sum of more than one hazard quotient for multiple substances. Values of HI >1 represent a chronic health risk, and HI <1 indicates the reverse (a protective effect). The accepted standard for HI is =1, at which there is no health effect for the exposed population (Enitan et al., 2018; Manea et al., 2020).

2.5.2. Carcinogenic risk assessment

The carcinogenic risk is the incremental probability of an individual developing any cancer type over a lifetime due to exposure to a potentially carcinogenic element (USEPA, 1989; Luo et al., 2012). Equation 6 aims to calculate the probability of cancer risk (Hu et al., 2017). Since Cu and Zn are classified as non-carcinogenic (class D), only the cancer hazard indices for Cr, Pb, Cd, and Ni were estimated (see SF in Table 9).

$$TCR = EDI \times SF \quad (6)$$

Where TCR is the target cancer risk (dimensionless); SF is the cancer slope factor of hazardous pollutants (mg/kg/day)⁻¹, and EDI is the chronic daily intake of carcinogens (mg/kg/day).

The cumulative cancer risk for simultaneous exposure to several carcinogens was estimated based on the following equation (7).

$$CCR = \sum_{x=1}^4 EDI_x \times SF_x = [EDI_{Pb} \times SF_{Pb} + EDI_{Cd} \times SF_{Cd} + EDI_{Cr} \times SF_{Cr} + EDI_{Ni} \times SF_{Ni}] \quad (7)$$

where EDI_x is the chronic daily intake (mg/kg/d) of metal x, SF_x is the slope factor of substance x (kg/d/mg). The acceptable risk for regulatory purposes set by the USEPA is within the range of 10⁻⁶, 10⁻⁵ or 10⁻⁴, meaning one additional case in a population of one million, 100,000 or 10,000 (USEPA, 2001, 2018c). The WHO has set the low carcinogenic risk (LCR) in the range of 10⁻⁵ to 10⁻⁶ (Sadeghi-Yarandi et al., 2020). Metals with an LCR >10⁻⁴ are considered as a "definite risk",

LCR $10^{-5} - 10^{-4}$ as a "probable risk," LCR $10^{-6} - 10^{-5}$ as a "possible risk," and LCR $< 10^{-6}$ as a "negligible risk" (Zhang et al., 2018).

2.6. Data analysis

2.6.1. Survey data

All survey data were uploaded from the Kobo collect app into Microsoft Excel 2016. The data were cleaned and cross-checked manually with related questions to their accuracy and then coded for further analyses using Stata version 15.1 (STATA, 2020). Descriptive statistics using frequencies and the Chi-square test were used to explore associations between categorical variables. Logistic regression analyses was used to test the difference between the mean values and all the model was checked for homoscedasticity and corrected using robust when necessary (Rothman, 2012). To correct the normality, the models were bootstrapped (Cameron & Trivedi, 2010). Box plots were used to illustrate the fertilizer use as they are useful when the variables are not normally distributed as the box shows the quartiles of the data, while the whiskers extend to show the rest of the distribution (Tukey, 1974).

Pairwise comparison of marginal effects was then used to assess the difference between areas at 95% confidence interval.

Logistic regression analysis using the model in Equation 8 was used to examine the relationship between socio-economic characteristics and the use of agrochemical inputs. Previous research in Cameroon implies that various individual characteristics affect adoption behavior (Nkamleu & Adesina, 2000; Sotamenou & Parrot, 2013). Thus, these factors were hypothesized to be important determinants of an individual's ability to process new information and possibly use them in changing behavior. Therefore, this model was employed to investigate the pesticide and fertilizer adoption decision:

$$Y_i = \gamma_0 + \gamma_1 \text{gender}_i + \gamma_2 \text{education}_i + \gamma_3 \text{age}_i + \gamma_4 \text{landown}_i + \gamma_5 \text{farm size}_i + \gamma_6 \text{GIC}_{it} + \gamma_7 \text{people}_i + \gamma_8 \text{Area type}_i + \gamma_9 \text{house distance}_i + \epsilon_i \quad (8)$$

where Y_i is whether farmers i used both mineral and organic fertilizer or not (in other estimations, Y_1 is a dummy variable of whether the farmers used only organic fertilizers or did not only use organic fertilizers); Y_2 is a dummy variable of whether the farmers used only mineral fertilizer or did not only use mineral fertilizer; Y_3 is a dummy variable of whether the farmers used both mineral and organic fertilizers or did not use both mineral and organic fertilizers; and Y_4 is a dummy variable of whether the farmers used pesticides or not; gender_i is the gender of the farmers (female farmers are believed to adopt organic fertilizers with a higher likelihood because they can use kitchen residues and home livestock waste); age_i is the age of the farmers in years (younger farmers may be more inclined towards experimenting or trying out the use of old and new agricultural inputs); education_i is the education level of the farmers more educated farmers being more likely to comprehend better the agronomic and environmental advantages related to the use of organic fertilizer (educated farmers are therefore believed to adopt organic fertilizers with a greater likelihood); landown_i is the land ownership; GIC_{it} is the membership of the farmer to an

association; *farm size* is the farm's surface (likely to affect the use of mineral farm inputs negatively because they are costly); *people_i* is the number of family households involved in farming activities (a large number of people are a source of labor and will act positively with the use of any agricultural inputs to increase farm productivity); *Area type_i* whether the farm is permanently or temporarily flooded (the rationale being that farmers whose farms are frequently flooded possibly refrain from using farm inputs like fertilizers as the flood water will transport nutrients from nearby waste depots to their plots); *house distance_i* is the distance between the house of the farmer and his land use for cultivation [with according to Nkamleu and Adesina (2000) cultivated land far away from the household may be given less application of agriculture inputs]; and finally ε_i : is the error term that explains other unobserved confounding factors.

2.6.2. Laboratory data analysis

Laboratory data were explored using Stata version 15.1 and R statistic version 4.0.4. The following analyses and descriptive statistics of the analyzed parameters, including minimum, maximum, mean, standard errors, standard deviation, and variances, were carried out to estimate and describe the heavy metal contents in water, soil, and plants.

Regression analysis

The influence of season and division on the distribution of heavy metals was tested and estimated using a (robust in case of heteroscedasticity) two factorial linear model with interaction. This model was chosen because season and division were fixed factors while farmers were random factors. Pairwise comparison of the adjusted prediction margin was used to compare the heavy metal concentration between seasons and areas. Model fit was determined using an alpha value of 0.05, with a p-value < 0.05 indicating the level of statistical significance. The complexity in the model was reduced by eliminating less relevant inputs and avoid overfitting. Thus, "Overfitting" is not expected as the model contains four terms on the predictor side (right side) and knowing that ten to 15 "events" per term are needed (i.e. 40-60 "events") to avoid "overfitting" (Rothman, 2012); the here chosen model has >40 events.

Geospatial analysis of heavy metal distribution in the soil

Mapping the spatial distribution of heavy metal concentration in soils in a given area is an important process towards understanding the spatial variability and levels of chemical contamination to inform decision-making. The ordinary kriging method was adopted for geostatistical analysis of heavy metal distribution in soils, using the spatial analyst tool in ArcGIS (10.8). Ordinary kriging is used to predict values for unsampled locations. The ordinary kriging is frequently used due to its robustness, unbiased predictions, and capability of smoothening out local variations in the field. It was employed with the normal distribution semi-variogram model embedded in ArcGIS. The model outputs were classified into ten classes using equal intervals. The same methodological steps were repeated to obtain the distribution of heavy metal concentrations (mg/kg) in the soils for Yaoundé's dry and wet seasons.

Pearson correlation

Pearson's correlations analysis defines the relationship between heavy metals and their major contribution to the environment (Godson et al., 2018; Perumal et al., 2021). Pearson's correlation coefficients for Cr, Cu, Ni, Cd, Pb, and Zn components in water, soil, and plants were used to investigate their potential associations and the strength of their relationship.

Before the analysis, the issue of missing values in the data was fixed by case-wise deletion, and the normality was checked. Pearson's correlation produces a sample correlation coefficient r , which measures the strength and direction of linear relationships between pairs of continuous variables and correlations within and between sets of variables. A correlation can take on any value in the range of $[-1, 1]$. The sign of the correlation coefficient indicates the direction of the relationship, while the magnitude of the correlation (how close it is to -1 or $+1$) indicates the strength of the relationship (Miller & Miller, 2005).

Pearson's correlation and cluster analysis are commonly used multivariate statistical methods for identifying the relationship between heavy metals in the environment and their potential sources (Lu et al., 2010; Liu et al., 2013).

Multivariate analysis

Multivariate analyses were applied to evaluate the variability of irrigation water, soil, and plant metal concentration and their potential sources. These analyses included Principal Component Analysis (PCA) to aggregate the effects of multiple variables into a small subset of factors termed principal components (PCs) (Jolliffe & Cadima, 2016). Hierarchical Cluster Analysis (HCA) was used to classify the samples and assign them to similar subgroups or clusters (Zhang et al., 2017). HCA has the advantage of not requiring any prior knowledge of the cluster number that the non-hierarchical methods like PCA do (Reghunath et al., 2002; Řezanková, 2014). PCA and HCA are increasingly used in environmental studies, including measuring and monitoring heavy metals in environmental media (Usman et al., 2017; Mishra et al., 2018; Ahmed et al., 2019; Sulaiman et al., 2020; Shaheen et al., 2021). PCA was used to determine the origin of the heavy metals in the environment, while HCA was utilized to show the similitudes between the samples.

PCA and HCA techniques were applied to analyze the dataset of 60 irrigation water, 60 soil, and 60 vegetables samples of the dry and wet seasons. Data consisted of six heavy metals, i.e., Cu, Cd, Cr, Pb, Ni, and Zn, for each environmental component analyzed. Cd parameter was excluded from the soil analyses because the value was below the detection limit during both seasons and, for this reason, was deemed not suitable for multivariate analysis (Güler et al. (2002)). Before running the multivariate analyses, data were first screened for homoscedasticity (homogeneity of variance) and normality via histograms and Q-Q plots. The distributions of most parameters were highly skewed to the right. Hence, all data were log-transformed to correspond to a normal distribution and to remove heteroscedasticity. Data were also standardized using the median and the mean absolute deviation to ensure that each variable was weighed equally using median values minimizes errors created by outliers (Güler et al., 2002; Nyenje et al., 2014).

Principal component analysis (PCA)

PCA techniques extract the eigenvalues and eigenvectors from the covariance matrix of original variables. The PCs are the uncorrelated (orthogonal) variables, obtained by multiplying the original correlated variables with the eigenvector, a list of coefficients (loadings or weightings). Thus, the PCs are weighted linear combinations of the original variables. PCs provide information on the most meaningful parameters, which describe the whole dataset, with data reduction at the minimum loss of original information (Liu et al., 2015). PCA is a powerful technique for pattern recognition that attempts to explain the variance of a large set of inter-correlated variables and transform them into a smaller set of independent (uncorrelated) variables (PCs). The main element can be expressed as:

$$z_{ij} = a_{i1}x_{1j} + a_{i2}x_{2j} + a_{i3}x_{3j} + \dots + a_{im}x_{mj} \quad (9)$$

where z is the component score, a is the component loading, x is the measured value of a variable, i is the component number, j is the variable, and m is the total number of variables.

PCAs of the water, soil, and vegetable dataset were performed to extract significant PCs and reduce the contribution of variables with minor significance; these PCs were subjected to Varimax rotation (raw) generating vari-factors (Gupta et al., 2018; Lu et al., 2010). Thus, a small number of factors will usually account for approximately the same amount of information as the much larger original observation set. Kaiser's (1958) eigenvalue criterion of retaining only those components/factors whose eigenvalues are >1 was adopted for the initial factor extraction. Other criteria used to determine the appropriate number of components to retain include scree plot, variance, and residuals. Kaiser's varimax rotation, an orthogonal rotation procedure that produces a set of component loadings having the maximum variance of the squares of the loadings, was used in conducting the PCA to make the factor solutions more interpretable without altering the underlying mathematical structure (Mertler & Vannatta, 2016).

Hierarchical cluster analysis (HCA)

HCA is an unsupervised pattern recognition technique that uncovers the dataset's intrinsic structures or underlying behaviors without prior assumptions about the data. It classifies the system's objects in categories or clusters based on their nearness or similarity (Anderberg, 2014). Hierarchical clustering is the most common approach in which clusters are formed sequentially by starting with the most similar pair of objects and forming higher clusters step by step (Bu et al., 2020). The Euclidean distance is usually used to unravel the similarities or dissimilarities between two samples, and a distance can be represented by the difference between analytical values from both samples (Patras et al., 2011). HCA was performed in this study using Ward's method, i.e., the Euclidean distances between centroids objects of classes were used to measure the similarities between classes of samples (Bu et al., 2020; Ghasemi et al., 2015). This method uses the analysis of variance to evaluate the distances between clusters, attempting to minimize the sum of squares of any two clusters that can be formed at each step (Singh et al., 2013). The linkage distance between classes (Height) is represented on the y -axis. HCA results are presented as dendrograms

using the Euclidean distance for similarity measures and Ward's method for linkage (Güler et al., 2002).

3. Study area

3.1. Description of Yaoundé

Yaoundé is the capital of Cameroon and the second-largest city in the country after the port city Douala. It is the chief town of the Mfoundi division located in the central region of Cameroon and has an elevation of about 760 meters above sea level (m.a.s.l.). Yaoundé has an estimated 2.7 million inhabitants and covers 304 km² (UNDESA, 2019). About 60% of Yaoundé's population lives in temporary housing. The whole city is divided into seven districts, and there is less than 1,500 km of roads, of which 30% are paved. Administratively, each of Yaoundé's districts has various numbers of neighborhoods under the traditional authority of a neighborhood chief.

Yaoundé has an equatorial climate with a classical bimodal structure comprising an alternate dry and wet season. This climatic structure defines the first and second cropping seasons, separated by a four-month dry season. The long dry season occurs from December to February and the short dry season from July to August. The two rainfall regimes extend from September to November and from March to June. The full length of these seasons varies increasingly due to global warming (Abossolo et al., 2015).

The mean annual rainfall is about 1564.7 mm, with an average temperature of 23.5 °C (Abossolo et al., 2015). The humidity is very high and shows an annual mean of 83% with full deviations (L'Hote, 2000). The exceptionally dense hydrologic network in and around Yaoundé is comprised mainly of the Mfoundi river and its perennial tributaries: Olezoa, Ewoué, Abiergué, Mingoua, Tongolo, Aké, Biyeme, Djoungolo, Ekozoa, Ntem, Ebogo, Nkié, Anga'a (Figure 8).

The soil in the lowlands is hydromorphic with a mixture of fine sand and organic material in decomposition. It is constituted of from 0 to 20 cm, a dark brown horizon rich in materials organic; from 20 to 40 cm, a sandy to white sandy loam horizon; from 40 to 42 cm, a very thin sandy loam horizon with clayey resulting from weathering in the water of embrechite. While other parts (uplands) are primarily ferralitic with excellent physical properties and retention capacity, their cation exchange capacity is low, and gneisses are the essential component of the lithological bedrock (Ambassa-Kiki & Nill, 1999; Mfopou et al., 2017).

Yaoundé is known as the "seven-hill city" because of its hilly relief. The city hosts major industries, including tobacco, dairy products, beer, clay, glass goods, and timber. Like many other metropolitan cities in developing countries, Yaoundé faces overpopulation, with a high population density of 14,000 inhabitants/km². Parrot et al. (2009) mentioned that more than half (51%) of the capital consists of slums without piped water supply, centralized sanitation, or waste disposal infrastructure.

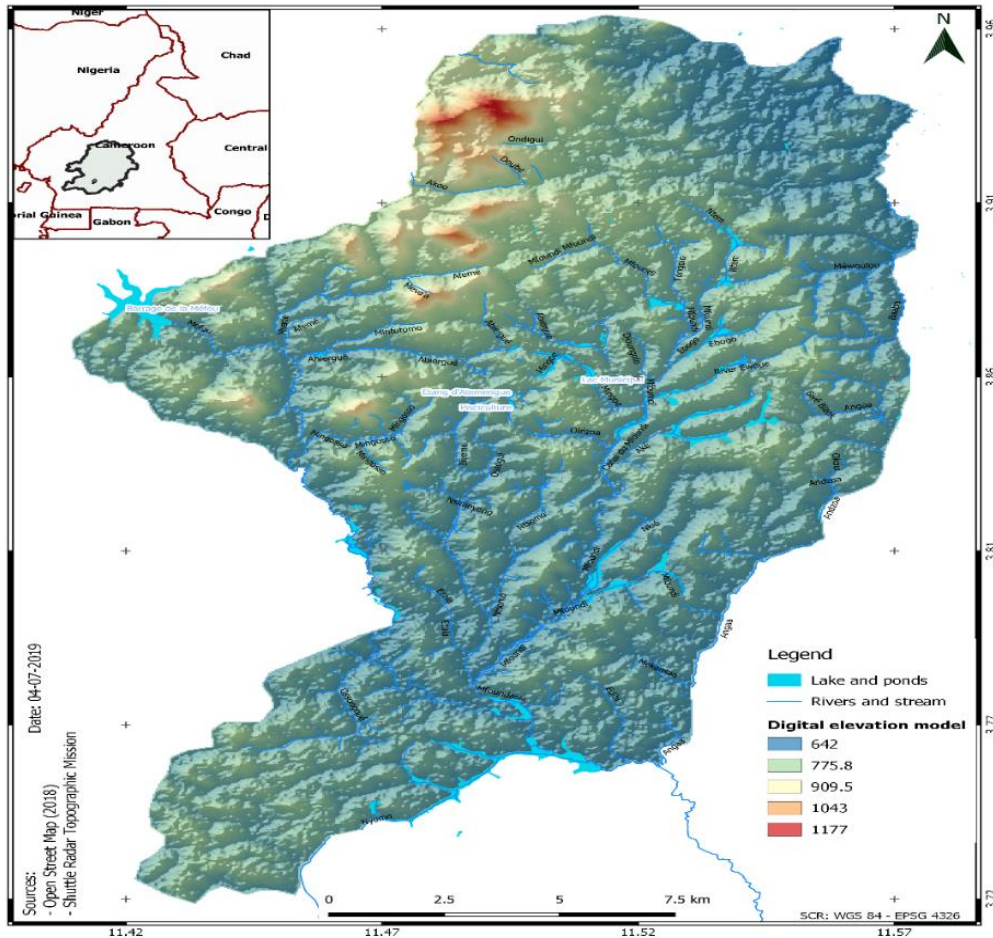


Figure 8 Map of Yaoundé in Cameroon and its topographic and hydrologic networks
 Source: Author's construction

The population has to rely mainly on shallow dug wells and springs for drinking water. Only 26% of households in Yaoundé are directly connected to the Cameroon Water Utilities Corporation (CAMWATER) network (INS, 2011). In addition, the city has no fecal sludge treatment station, and about 700 to 1,300 m³ of fecal sludge are discharged weekly into the environment of the peri-urban areas surrounding Yaoundé (Letah, 2018). The average domestic waste production in Yaoundé is 81 tons per day, and the waste collection company collects only about 32 tons leaving the remaining wastes dispatched into the natural environment without treatment (Ndongo et al., 2016).

Non-constructible areas, such as urban lowlands, are the most frequent dwelling options for the urban poor, especially for newcomers and migrants, due to the difficulty accessing real estate (Parrot et al., 2009). Since the 1990s, rapid urbanization and rural-urban migration have led to a disorderly occupation of non-constructible areas, formerly hillsides and lowlands (Djatcheu, 2018). The paradoxical coexistence of urban life and agricultural production in non-constructible areas has not yet been resolved in Yaoundé, except for the expropriation of settlers, which only transfers the problems from one location to another.

Informal farming accounts for 10% of employment in Cameroon and at least 3% in Yaoundé (INS, 2011). Urban farmers utilize the swampy lowlands for crop production to meet the unmet high urban market demand for perishable vegetables supplied by the countryside production (FAO, 2017). Lowlands in Yaoundé can be defined as areas that are lower than surrounding areas. Yaoundé’s lowlands are considered non-constructible by the municipality because of the steep hills or rivers, which pose a flood risk during the wet season. However, the urban poor keep on constructing informally (Parrot et al., 2009). The lowland zones of the city then increased, stretching out along the various rivers crossing the city from 12 zones in 2005 (Nguengang, 2008) to 11 zones in 2011 (Bopda et al., 2011), and 10 zones in 2014 (Nguendo, 2014).

With the growth of urbanization, lowland areas in Yaoundé have been colonized for industrial purposes, habitation, or city gardens (Nguendo, 2014). More people recover lowlands by planting eucalyptus to dry them and turn them into building areas (Tchindjang, 2017). Consequently, only six lowlands are nowadays used for urban farming. According to the National Institute of Statistics in Cameroon, no official census nor any statistical information about the populations living in these areas are available.

3.2. Description of study population and areas

The first phase of the research was conducted in six urban lowlands of Yaoundé, the city's main cultivated areas. Figure 9 shows the location of the six selected sites in Yaoundé, Cameroon.

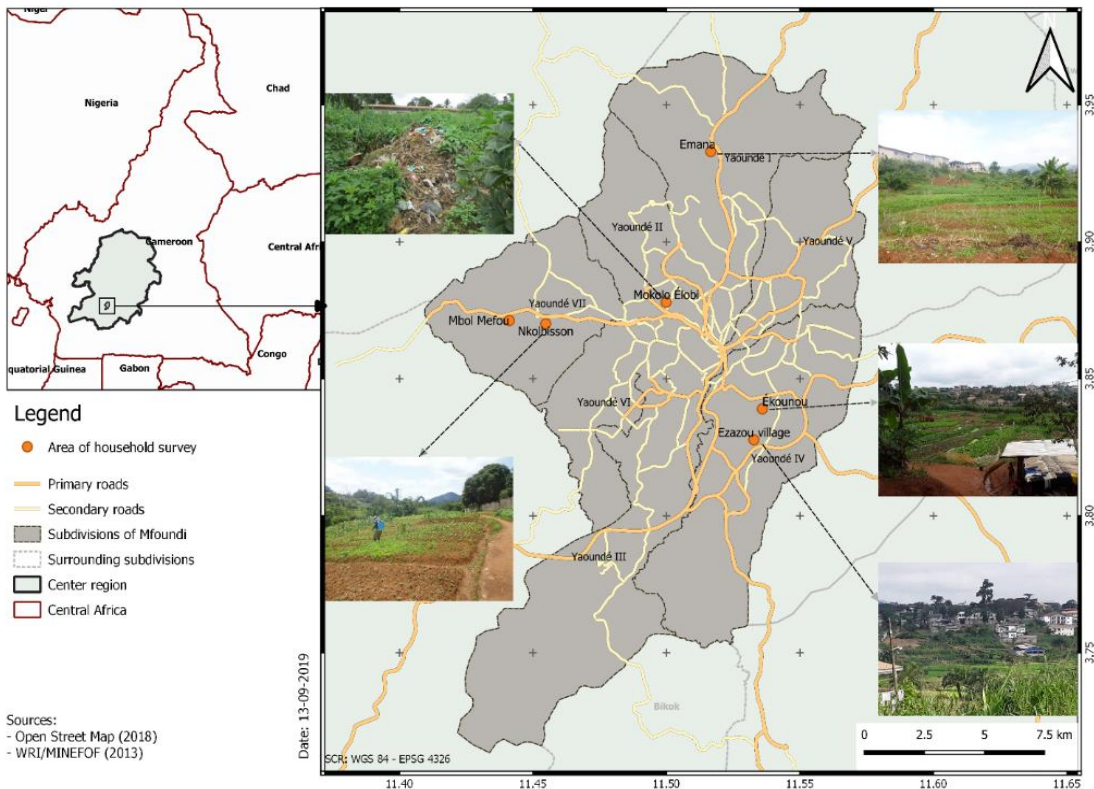


Figure 9 Location of Yaoundé and the selected lowlands in Cameroon
Source: Author’s construction

3.3. Demographic characteristics of farmers

The description of each lowland revealed some similarities and differences among the farmers in each lowland. These characteristics are summarized in Table 15. Men in Emana and Ekounou lowlands conduct farming activities, while women take the lead in other areas. The age of the farmers ranged from 18 to 76 years, with an average of 44 (± 15 SD) years of age. Four household members, on average, were involved in the farm works. The education level was high among farmers, with about 47% of farmers achieving at least a secondary level of education. A high percentage of illiterate farmers was recorded in the Ekounou lowlands. In contrast, more than half of Mokolo and Nkolbisson farmers have completed at least a secondary level of education. Although farmers' motivation to conduct urban agriculture varies, income enhancement, food supply, and unemployment are the main purposes.

Table 15 Farmer's socio-demographic profile (N=130).

Variables	Lowlands (%)						Average (%)
	Emana	Minkoameyos	Mokolo	Nkolbisson	Ekounou	Ekoumdoum	
Gender							
Male	82	25	35	34	70	36	45
Female	18	75	65	66	30	64	55
Age (years)	50 \pm 12	43 \pm 11	42 \pm 15	43 \pm 16	46 \pm 15	44 \pm 16	44 \pm 15
Status							
Single	36	19	40	33	15	42	36
Married	46	56	35	58	62	42	46
Widow(er)	18	25	25	9	23	15	18
Household size (mean \pm SD)	5 \pm 4	4 \pm 2	3 \pm 2	3 \pm 3	3 \pm 2	4 \pm 4	4 \pm 3
Education							
None	-	6	-	13	18	4	8
Primary	64	50	15	33	30	58	39
Secondary	27	44	80	54	46	27	47
Higher	9	-	5	-	6	11	5
Motivations							
Unemployment	54	25	10	25	65	33	35
Incomes	45	69	20	58	73	45	62
Family habits	27	25	12	25	31	33	27
Food supply	-	-	60	8	8	18	9

Source: Author's construction

3.4. Characteristic of lowlands and plots

Land ownership varies between farmers and within lowlands. Instead of owning the lands, most farmers in Yaoundé's lowlands rent the lands from private individuals. Some of them installed and do farming activities freely without any prior agreement with the government or the private owner. The farmers who owned the land purchased it mostly without the respective land title or have received it by donation. However, it is worth mentioning that the government considers lowland areas in Yaoundé as green space for preserving vegetation in the city. These areas are not intended for urban agriculture nor residential use; thus, building premises and performing urban activities are officially forbidden. Controversially, urban agriculture has been there for more than half a century (Table 16).

The farms in some areas are often permanently flooded, and farming activity can happen only in the dry season (in the case of Emana lowlands) when the water level in the soil is low. On the contrary, the farms located in the dry areas are used for urban agriculture only during the wet season (e.g., in Minkoameyos).

The farm size varies from small (50 m²) to large (2,000 m²), with an average farm size of 298m² for all six areas. Lowlands with the largest farm sizes are Ekounou and Ekoumdom while the smallest are found in the Mokolo area. High labor and capital costs and increasing urbanization are among the barriers preventing farmers from cultivating larger surface areas. The studied lowlands are drained by one watercourse (Emana, Minkoameyos, and Ekounou), two (Mokolo and Nkolbisson), or three (Ekoumdom). Surface and groundwater (shallow dug wells built near the plots) are mainly used for irrigation in five out of the six areas. Although living between two rivers (Abiegué and Ekozoa), farmers in the Mokolo area use only groundwater to meet their irrigation water needs. Their reasons are the unpleasant smell of the river water and its contaminated appearance as water from these rivers is considered wastewater from receiving fecal sludge from nearby households.

Table 16 Summary of farm characteristics, N=130.

Variables	Lowlands					
	Emana	Minkoameyos	Mokolo	Nkolbisson	Ekounou	Ekoumdoum
Location	Yaoundé I	Yaoundé VII	Yaoundé II	Yaoundé VII	Yaoundé IV	Yaoundé IV
Urban settings	Informal settlements	peri-urban interfaces	Informal settlements	Informal settlements	Peri-urban interfaces	Peri-urban interfaces
Population level	Highly populated	Less populated	Highly populated	Middle populated	Highly populated	Less populated
Land size (ha)	2	4	1	2	4	6
Farm size (m ² ± <i>SD</i>)	245±233	186±155	86±45	275±223	478±496	375±381
Operational years (± <i>SD</i>)	7±6	6±7	14±11	11±8	11±6	11±11
Watercourse drained	Ngonlo	Mefou	Abiegué & Ekozoa	Abiegué & Mefou	Aké	Nkié, Aké, and Anga'a
Irrigation water	Surface & Groundwater	Surface & Groundwater	Groundwater	Surface & Groundwater	Surface & Groundwater	Surface & Groundwater
Farming period	Wet season	Wet season	Dry and wet seasons	dry and wet season.	Dry and wet season.	Dry and wet season.
Pollution sources	Waste dumpsite and waste oil from open car repair	Road and surrounding sanitation	Craft activities: tannery, waste oil from open cars repairs, wheels burned, and irons incinerated	Discharge of solid waste and discharge of raw sewage; leachate from the dumpsite	Waste dumpsite, road, and surrounding sanitation	Road and surrounding sanitation

Source: Author's construction

The farming system is labor-intensive. The land preparation is similar in all the investigated areas, e.g., the method of plowing (Figure 10). Machinery is not common. All farmers rely on traditional tools such as hoes, machetes, pickaxes, and rakes for farm preparation, and cutlass and wheelbarrow are used during harvest. Similarly, almost all farmers practice manual irrigation (watering cans and pumps) to distribute water across their respective lands. Farmers usually walk in the irrigation water during the irrigation process, exposing their clothing and themselves to the water. Wealthy farmers with farms far from the rivers rely on diesel pumps to draw water and pump it via plastic hoses onto their farms.



Figure 10 Urban farming: field preparation (a) and vegetable planting (b).

Source: Author's construction

3.5. Farming systems

All farmers practiced polyculture, with leafy vegetables being the dominant crop, followed by herbs and vegetable fruits (Table 17). Vegetable species such as *Amaranthus hybridus* (89%), *Solanum nigrum* (77%), *Corchorus olitorius* (66%), *Lactuca sativa* (49%), and *Vernonia amygdalina* (39%) constitute the primary species cultivated; whilst *Abelmoschus esculentus* (34%) and *Solanum melangera* (28%) are among the most grown vegetable fruits and *Apium graveolens* (31%), *Ocimum basilicum* (22%), and *Petroselinum crispum* (21%) are the species of the commonly grown herb. The choice of crops is based on the farmer's consumption preferences, the short vegetable cycle, and the local market demands. The vast majority of farmers in all the six lowlands grow *A. hybridus* and *S. nigrum* primarily for self-consumption. Only the surplus is sold at the nearby market. On the contrary, *L. sativa* is primarily grown for market sale.

Table 17 Percentages of land use for vegetable crops grown in six lowland study sites

Land use	Areas						Average (%)
	Emana	Minkoameyos	Mokolo	Nkolbisson	Ekounou	Ekoumdoum	
<i>A. hybridus</i>	91	85	94	92	88	88	89
<i>S. nigrum</i>	91	69	70	71	77	85	77
<i>C. olitorius</i>	64	25	60	38	57	56	66
<i>L. sativa</i>	5	43	5	37	67	62	49
<i>V. amygdalina</i>	36	37	90	37	23	24	39
<i>A. esculentus</i>	55	31	80	25	15	24	34
<i>A. graveolens</i>	55	44	0	42	27	30	31
<i>S. melangera</i>	45	25	60	21	18	15	28
<i>O. basilicum</i>	55	50	0	29	12	15	22
<i>P. crispum</i>	45	25	0	21	24	19	21

Source: Author's construction

Vegetable production is intensive in all studied areas, using fertilizers to enhance their soil fertility and pesticides to control pests, weeds, and insects that affect yields. The percentage of users, the type of agricultural inputs, and the quantity vary depending on the farmers. In addition, all farmers use their hands to apply fertilizers as they are unaware of occupational safety and safety measures. Thus, most of them do not wear protective clothing (gloves, safety raincoats, boots, etc.) during agricultural activities. Handwashing with clean water after pesticide or fertilizer applications is not very common. The farmers rely on tube well/borewell, springs (Figure 11a), and tap water for drinking. The level of sanitation facilities is very low. The majority of farmers in all studied areas use mainly pits latrine, and some practice open defecation. Poor sanitation practices are among the contributors that increase environmental pollutants such as chemicals, including heavy metals. The farms are generally flooded (Figure 11b) after heavy rain, promoting the contamination of vegetables with a mixture of liquid and solid wastes from the nearby anthropogenic activities and households.



Figure 11 Irrigation water: groundwater (a) and vegetable farms after a rainfall event (b)
Source: Author's construction

Farming practices such as fertilizers, pesticides, and irrigation water sources are essential to consider in this study as they are critical variables that can lead to food contamination. Results from the first phase of this study provide some statistical analyses that highlight the considerable differences among the farming practices in these lowlands.

4. Results and discussions

This chapter presenting the study results is divided into four sub-chapters: 1) agricultural practices, 2) irrigation water, agricultural soil and vegetable quality, 3) the level of vegetable contamination, and the bioaccumulation factor of heavy metal and 4) human health risks. Each sub-chapter is discussed, and a summary is given at the end of each chapter.

The first sub-chapter highlighting the agricultural practices has four sections. The first section includes sources of irrigation water and percentages of users. The second section presents application rates, types, and quantity of fertilizers applied per area. The third section outlines the common types of pesticides applied and percentages of users in the study areas. The last section explains the factors influencing the choice of agrochemicals applied.

The second sub-chapter is divided into five sections: the first and second sections describe the physicochemical and heavy metal characteristics of irrigation water and agricultural soils. The heavy metal contamination of the selected vegetable is discussed in the third section. The correlation analysis of heavy metals present in water, soil, and plants is discussed in the fourth section. In the fifth section, multivariate analysis concerning the origin of heavy metal contamination in the environment is discussed.

The third sub-chapter focuses on the level of heavy metal contamination in *S. nigrum* and the capacity of the vegetable to uptake heavy metals from the agricultural soil. This sub-chapter aims to test the hypothesis of whether *S. nigrum* cultivated in these lowlands contains heavy metals to the level that is not safe for human consumption. The first section presents the status of the vegetables, and the second shows the transfer factor of each heavy metal from the soil to the plant.

The final subchapter is divided into three sections to answer the main research question: How do heavy metals in contaminated vegetables contribute to health risks among lowlands farmers in Yaoundé? The first section presents the estimated daily intake of heavy metals via *S. nigrum* consumption. The second section outlines each heavy metal's hazard quotient and the hazard index (non-carcinogenic health risk) of heavy metals in each area. Finally, the third part gives the target carcinogenic hazards of heavy metals through the consumption of contaminated vegetables and the cumulative cancer risk of all the studied heavy metals in each study area.

4.1. Agricultural practices in Yaoundé lowlands

4.1.1. Irrigation water

More than half of the farmers (56%) used surface water sources (rivers) for their irrigation needs. Among the participants, 44% relied on groundwater (GW) for irrigation, mainly obtained from shallow dug wells built near the plots. Figure 12 shows the percentages of irrigation water sources in all study areas. Farmers from five out of six studied lowland areas utilized water from different origins. Farmers from Mokolo used only GW to irrigate their fields, while those in Nkolbisson and Minkoameyos mainly utilized surface water (SW) (61%). Ekounou farmers equally used GW and SW. With the irrigation water sector accounting for only 7.25% of water use

in Cameroon, farmers rely on potentially polluted water for irrigation because of the general water scarcity. The type of irrigation water sources is one of the most critical parameters for the potential contamination of vegetables. Untreated water used for irrigation is the primary source of pathogens and heavy metal contamination in farms (M. Mfopou et al., 2014; Muhammad et al., 2020). This may lead to the deterioration of GW quality, soil structure, and changes in lands' physicochemical properties that significantly reduce their fertility (Mfopou et al., 2017).

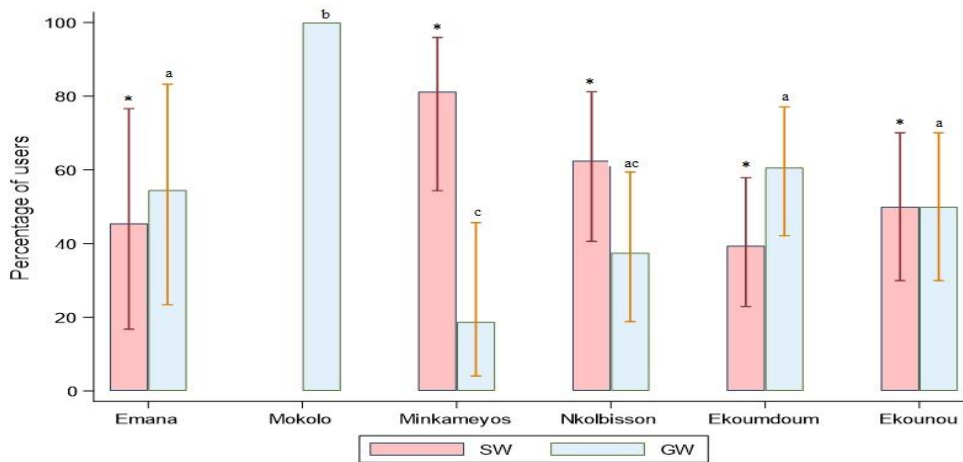


Figure 12. Percentages and types of irrigation water used in the studied areas of Yaoundé lowlands (SW: surface water; GW: groundwater; Same letter for the respective irrigation water type are not significantly different at $p < 0.05$; * not significantly different at 95% exact confidence intervals).

The percentage of Mokolo farmers using GW for irrigation was significantly higher than in the other studied areas of Yaoundé. On the contrary, except for the Nkolbisson areas, the percentage of farmers from Minkoameyos using GW for irrigation was significantly lower than those from the Emana, Ekounou, and Ekoumdoum sites (Appendix 3).

4.1.2. Fertilizers

Vegetable production in urban environments requires several external inputs, including mineral or organic fertilizers. Of the surveyed farmers, 84.6% used fertilizers to enhance soil fertility, with the highest use in Nkolbisson (88%) and Emana (73%), and no significant differences in fertilizer use among the six studied lowland areas of Yaoundé (Figure 13). Most farms in Nkolbisson belong to the neighboring Institute of Agricultural Research for Development or are owned by former workers of the institute, possibly explaining the relatively higher fertilizer use than in the other studied lowland areas. Improper farm fertilizers management can lead to nutrient accumulation and other chemical pollutants into water bodies through runoff and soil erosion (Mfopou et al., 2017), and such contaminants can negatively affect plants, animals, and humans (Soro et al., 2018).

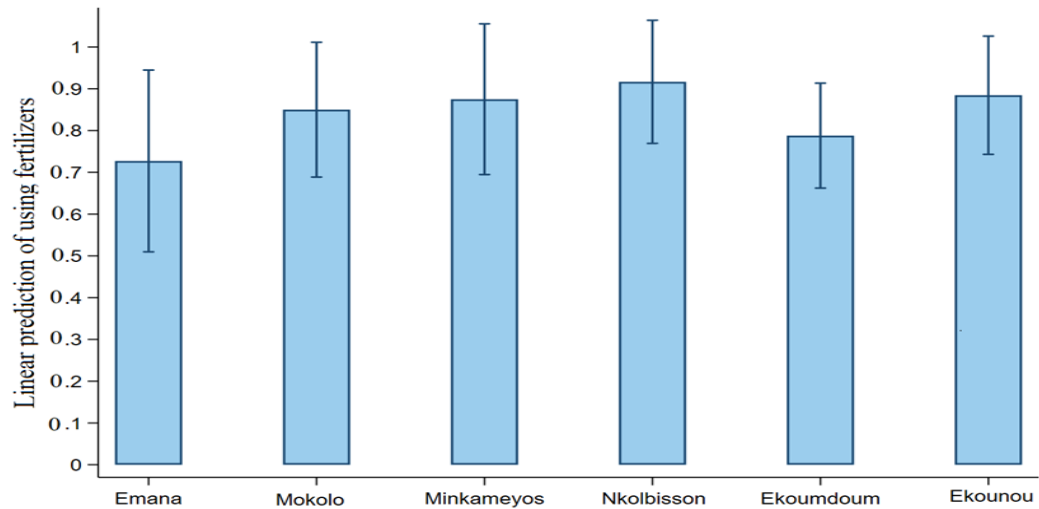


Figure 13 Probability of using fertilizers in the studied lowlands areas (at 95% confidence 278 intervals).

The type of fertilizers applied varied among farmers based on their availability and affordability. Of the interviewed farmers, 50% used a combination of mineral and organic fertilizers, while 28.5% utilized only organic and 6.1% only mineral fertilizers in their fields. The three most common organic fertilizers used were chicken manure (65.4%), pig manure (4.6%), and compost (8.5%). Comparison of organic fertilizers shows that the proportion of farmers using chicken manure was significantly higher. Among the mineral fertilizers, urea was the most used type (27.7%), followed by NPK (22.3%) and ammonium sulfate (6.2%) (Figure 14).

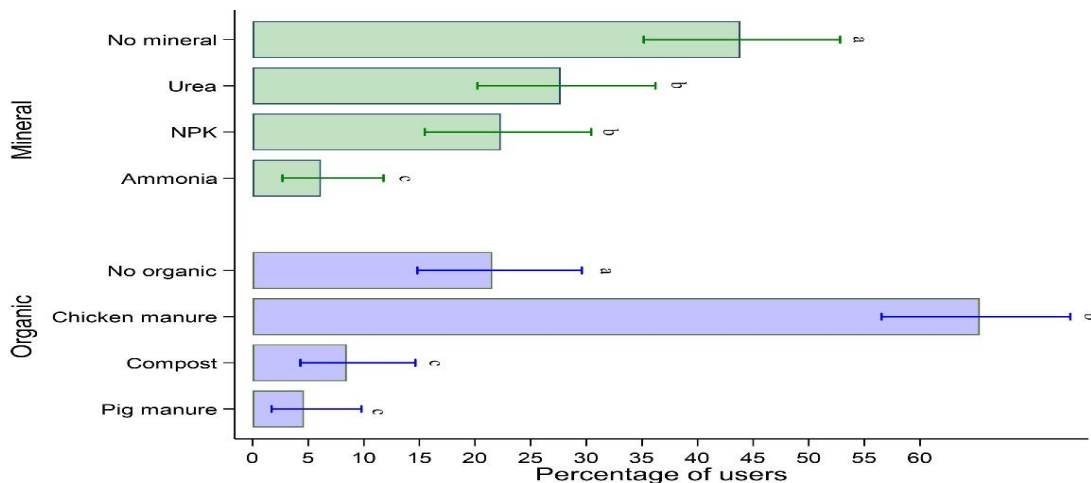


Figure 14 Types of fertilizers used in Yaoundé lowlands (No mineral: farmers use only organic fertilizers; No organic: farmers use only mineral fertilizers; Same letter for the respective fertilizer types are not significantly different at $p < 0.05$ at 95% exact confidence intervals).

Analysis revealed that the proportion of farmers using ammonia was statistically lower. The high popularity of chicken manure, especially compared to mineral fertilizers like urea or NPK, can be explained by its better availability and greater affordability. For instance, a 50 kg bag

of chicken manure costs approximately 1,200 to 2,000 CFA francs (€ 2-3), while a 50 kg bag of mineral fertilizers costs around 18,000 to 25,000 CFA francs (€ 27-38) which is often not affordable for many smallholder farmers.

Previous studies reported that prices of mineral fertilizer in different parts of Africa were not affordable for smallholder farmers (Giller et al., 2009; Palm et al., 2001), and often such farmers instead revert to using poultry manure to replenish soil fertility (Saidou & Pritchard, 2019). Because of its low moisture content, chicken manure is easy to dry, thus requiring less labor, and its use can substantially improve the nutrient availability in soil. It also contains less toxic and nonessential elements compared to mineral fertilizers (Saidou & Pritchard, 2019). In addition, chicken manure has a higher concentration of N, P, and K per unit than other animal manure types (Hass et al., 2012; Z. Yang & Han, 2013). However, chicken manure may lead to undesirable effects, such as phosphorus (P) reduction and an extended stockpiling period (Hoover et al., 2019). In some of the study areas, e.g., Nkolbisson, Emana, and Mokolo, farmers grow maize intercropped with leguminous vegetables. This practice provides additional nitrogen as an environmentally friendly and more sustainable way of improving soil fertility. Nonetheless, intercropping was absent in the other three study areas, leading to higher (mostly mineral) fertilizer inputs into the environment.

4.1.2.1. Organic fertilizers used per square meter

The on-farm quantity of fertilizer used per m^2 varied widely among farmers and areas studied. On average, farmers applied $2.14 (\pm 2.16 \text{ SD}) \text{ kg/m}^2$ organic fertilizers, ranging from 0.05 to 12.5 kg/m^2 . Figure 15 summarizes the amount of organic fertilizers (in kg) used per m^2 . In the study area, the average amounts of fertilizers applied were 1.61 kg/m^2 , 3.60 kg/m^2 , 1.05 kg/m^2 , 1.42 kg/m^2 , 2.98 kg/m^2 , and 1.99 kg/m^2 for Emana, Mokolo, Minkoameyos, Nkolbisson, Ekoumdoum and Ekounou, respectively.

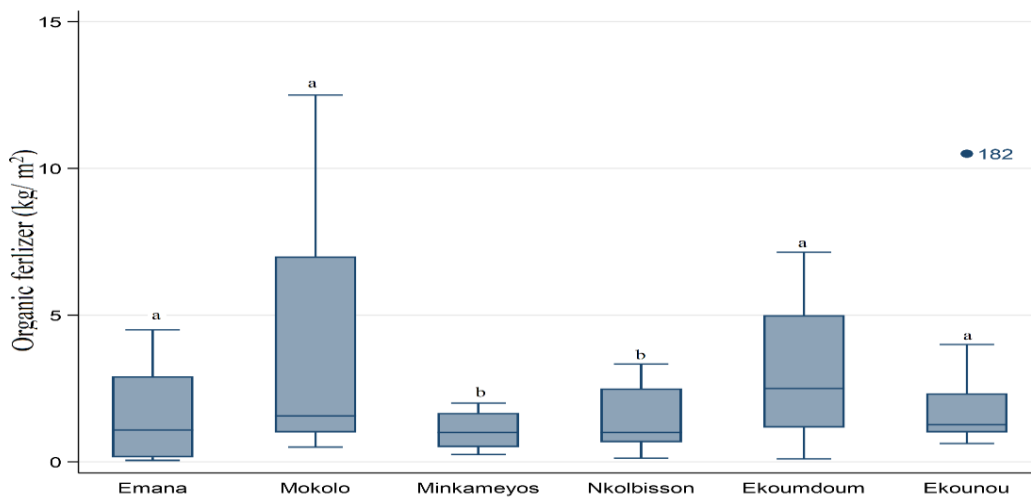


Figure 15 Amount of organic fertilizers used per m^2 in the studied areas. (Same letters indicate no significant differences at $p < 0.05$; data are shown using box whisker-plot to reveal the heteroscedasticity; the

significance indicator refers to a robust generalized linear model).

The on-farm use of organic fertilizers in each area was evaluated using regression analysis and pairwise comparison. The results show that the amount of organic fertilizers used per m² in farms in the Mokolo, Emanas, and Ekoumdoum areas was significantly higher than in Minkoameyos, Nkolbisson, and Ekounou (Appendix 3).

4.1.2.2. Mineral fertilizers use per square meter

The use of mineral fertilizers varies from 0.01 kg/m² to 0.50 kg/m², with an average of 0.08 ± 0.09 kg/m² (Figure 16). In the different locations, the average quantity of fertilizers applied were 0.07 kg/m², 0.17 kg/m², 0.10 kg/m², 0.05 kg/m², 0.07 kg/m² and 0.05 kg/m² for Emanas, Mokolo, Minkoameyos, Nkolbisson, Ekoumdoum and Ekounou, respectively.

The regression analysis results revealed that the amounts of mineral fertilizers were significantly higher in the farms of Mokolo compared to those in the Ekounou, Nkolbisson, Emanas, and Ekoumdoum areas. However, there was no difference in mineral fertilizer use between farms in the Mokolo and Minkoameyos areas (Appendix 3).

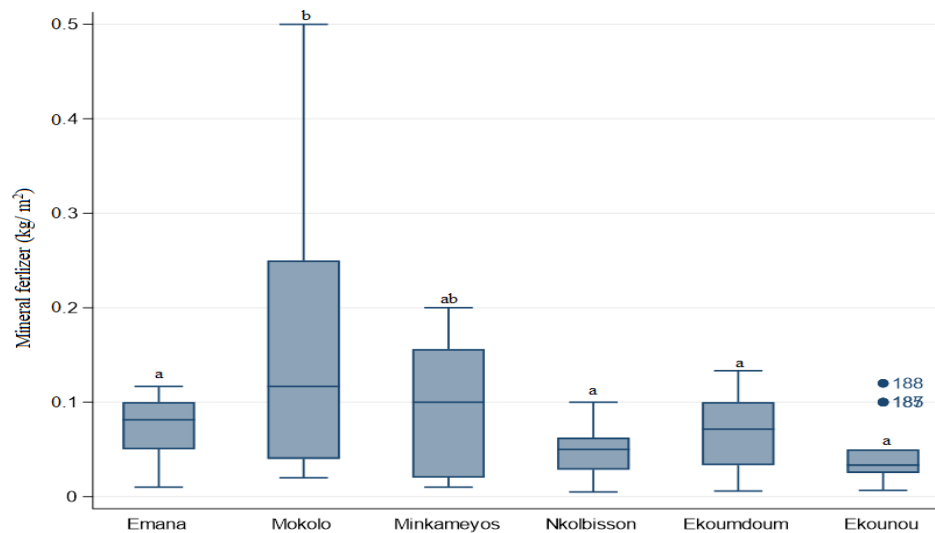


Figure 16 Amount of mineral fertilizers used per m² in the study sites (same letters indicate no significant differences at p < 0.05; data are shown using box whisker-plot to reveal the heteroscedasticity; the significance indicator refers to a robust generalized linear model).

The differences in fertilizer use between the different areas studied might have been influenced by the variability in the price of fertilizer in the respective surrounding markets of these areas. Farmers involved in animal husbandry have greater access to comparatively less expensive organic fertilizer and are thus more likely to use larger quantities of such fertilizer on their fields. A recent study in Yaoundé reported that poultry manure was the cheapest source of N to purchase, although a larger volume was required than with, e.g., urea or NPK (Saidou & Pritchard, 2019). The authors highlighted that the application of 200 kg chicken manure would supply 7.8 kg

N/ha/pa, which is lower than the Kenya Agricultural Research Institute (KARI) recommended rate of 50 kg of DAP (diammonium phosphate) and 60 kg of calcium ammonium nitrate (CAN) in sub-Saharan Africa (Olwande, Sikei, & Mathenge, 2009; Vanlauwe et al., 2011). In comparison, 150 kg/ha of NPK and 50 kg/ha of urea (46% N) provide a total N loading of 53 kg/ha/pa (Saidou & Pritchard, 2019). This study found the highest amount of organic and mineral fertilizers applied on farms in the Mokolo area, even though these farms tended to be smaller than those in the other study areas. This comparatively higher fertilizer use in Mokolo was probably due to the longer duration of cultivation and the soil typology there. On smaller farms, farmers often tend to overuse soil amendments to increase their productivity with little awareness of potential health and environmental effects. In addition, because of a lack of information and training on occupational health and safety, farmers often apply fertilizers with their bare hands, a practice that potentially increases long-term health hazards.

4.1.3. Pesticides

Pesticides like fertilizer applications are a common in urban agriculture in the Yaoundé lowlands. Sixty-four percent (64%) of farmers apply pesticides to control pests, weeds, and diseases that reduce yields, and only 36 % do not utilize chemical pest control. The proportions of pesticides users are 91%, 70%, 19%, 67%, 72%, and 62% for the Emaná, Mokolo, Minkoameyos, Nkolbisson, Ekoumdoum, and Ekounou sites, respectively (Figure 17). The highest proportion of farmers using pesticides in their farms was found in the Emaná and the significantly lowest in the Minkoameyos area (Appendix 3).

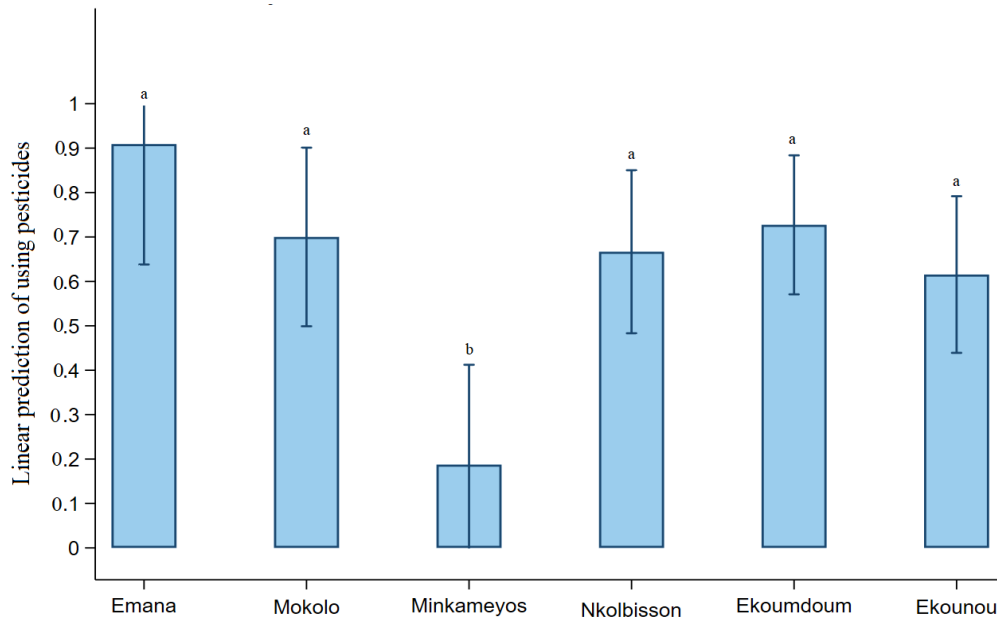


Figure 17 Probability of using pesticides in Yaoundé lowlands (error bars representing standard error of the mean; same letter indicates no significant differences at $p < 0.05$ at 95% confidence intervals).

Farmers involved in urban lowland agriculture in Yaoundé apply different types of pesticides. The most commonly used include six fungicides, two insecticides, one nematicide, and

one herbicide, which were classified according to the type and commercial brand name, active ingredient(s), and WHO classification (Table 18). Fungicides were most frequently used (76%), followed by insecticides (66%) and nematicides (14%). Only 9% of farmers applied herbicides in their fields.

Table 18 Types of pesticides used in Yaoundé lowland farms

Active substances and classification	Formulations	WHO classification	Proportion (%)
Cypermethrin (I)	Cypercal 12 EC	II	48
	Cypercot 25EC	II	
	Cyperplant 50 EC	II	
	Cigone 12 EC	II	
Lambda-cyhalothrin (I)	Lamida Gold 90 EC	II	18
Chlorothalonil 550 g/l +Carbendazime 100g/l (F)	Banko Plus	III	27
Maneb 80% (F)	Plantineb 80 WP	III	4
Metalaxyl 80g/kg + Mancozeb 640g/Kg (F)	Mancoxyl plus 720 wp	III	10
Mancozeb 800 g/kg (F)	Penncozeb 80 WP	III	4
Metalaxyl-M 6%+ Copper Oxide 60% (F)	Rodomil gold plus 66 wp	III	25
	Callomil plus 66 WP	III	
Glyphosate 360g/l (H)	Herbi-star 360 SL	III	9
	Roundup 360 SL ^a	III	
Chlorothalonil 720 g/l (F)	Balear 720 Sc SL	III	6
-	Beauchamps ^b	-	12
Oxamyl 50g/kg (N)	Bastion Super	I a	14

Notes: Ia = Extremely hazardous; II = moderately hazardous; III = slightly hazardous (WHO, 2020) a = obsolete, b = unclassified; I = insecticide, F = fungicide, H = herbicide and N = nematicide.

The low proportions of farmers applying herbicides in their farms can be explained by the high cost of these products, their only partial availability, and the low labor costs, with the vast majority of farmers thus preferring to weed their farms manually. A high proportion of farmers using fungicides was also reported by Tambe et al. (2019) in their study on pesticide use by smallholders tomato farmers in Cameroon. They reported that fungicides were the most common pesticides used among urban farmers and that most farmers apply these pesticides rather indiscriminately without using any protective gear. Moreover, Cameroon's study revealed that pesticide usage is responsible for 78% of accidental poisoning cases, 12% of suicide attempts, and 4% of criminals (thieves arrested are injected with Gramoxone or Paraquat) (Pouokam et al., 2017). Furthermore, the potential human health risk from pesticides residues in drinking water in many countries has been reported by El-Nahhal and El-Nahhal (El-nahhal & El-nahhal, 2021).

Most farmers in this study lacked training in farming practices and knowledge on safe occupational practices. Thus, only 34% of the farmers wear personal protective clothing during irrigation, fertilizer, and pesticide applications. Even though the farmers are aware of the

occupational health risks, none wear complete protective clothing such as boots, gloves, and masks. Most farmers use only one or two pieces of personal protective gear, like boots, raincoats, or gloves. In addition, most farmers are exposed to wastewater and often ingest some soil particles during farm work, leading to health risks (Baltas et al., 2020; Keshavarzi & Kumar, 2020).

More than half of the farmers generally walk in the irrigation water or get their clothes wet during the irrigation process. Although 60% of farmers reported no feeling of any inconvenience related to the untreated water, 40% noticed skin itching, burning, and the wretched water smell. These findings can be explained by the lack of awareness of occupational health risks and the low level of education of most of the encountered farmers. Yet, appropriate education is key to understanding safe working procedures and health risks related to certain behaviors. Asongwe et al. (2014), working with vegetable farmers in the Bamenda wetlands of Cameroon, reported that wearing personal protective gear could significantly reduce work-related threats.

4.1.4. Farming practices and explanatory variables

A logistic model was used to understand the factors influencing agricultural inputs usage. Four independent variables, i.e., education, land ownership, farm size, and the number of years the farm has been cultivated, significantly affected the use of fertilizers and pesticides (Table 19).

Education is significant in negatively affecting the use of organic fertilizers. Possibly even (better) educated farmers are not sufficiently aware of the benefits of nutrient management on their farms or lack training on environmentally friendly farming techniques. Thus, there is a need to better inform and train farmers via agricultural extension agents. Cameroon's Ministry of Agriculture often grants expenditures for organic fertilizers through NGOs or farmers' associations. Therefore, educated farmers opt to use both types of fertilizers instead of only mineral fertilizers. Using only the latter might cause harm to environmental and human health. Yet, integrated use of both organic and mineral fertilizers requires higher managerial skills, for instance, for combining the two inputs in the correct proportions. Sotamenou and Parrot (2013), in their study on sustainable urban agriculture and the adoption of composts in Cameroon, found that education favors combined fertilizer use on farms. The use of pesticides is significantly negatively associated with land ownership (Table 19). Farmers that own the land are more interested in long-term investments such as sustainable farming techniques and are thus less likely to use pesticides. The use of pesticides has been widely regarded as an unsustainable practice in Yaoundé because of its effect on soil, water, and human health (Branchet et al., 2018; Pouokam et al., 2017). Those who do not own the land, such as those living in the lowlands of Yaoundé, who often face eviction from the land, are more interested in short-term profits rather than the sustainable maintenance of soil health and thus more prone to use pesticides. There is also an issue of awareness whereby the majority of these farmers, irrespective of land ownership, are aware that pesticides usage is harmful to soil health because of consumer organizations that sensitize them on the health risks linked to improper management of pesticides. These consumer organizations frequently hold informal

debates with communities about human and environmental health issues from pesticide and other agrochemical use in farming systems.

Table 19 Factors influencing the use of various agrochemicals (fertilizers and pesticides) in smallholder farms in Yaoundé lowlands.

Variables	No fertilizers	Organic fertilizers only	Organic and mineral fertilizers	Mineral fertilizers only	Pesticides
Gender	0.0004 (0.0475)	-0.0564 (0.0848)	0.0363 (0.0916)	-0.0390 (0.0470)	0.0867 (0.0875)
Education	0.0156 (0.0507)	-0.2120** (0.0719)	0.1510 (0.0838)	0.1090 (0.0610)	-0.0216 (0.0823)
Age (years)	0.0005 (0.0021)	-0.0038 (0.0027)	0.0045 (0.0030)	-0.0018 (0.0021)	-0.0022 (0.0028)
Land ownership	-0.2110** (0.0572)	0.0856 (0.0852)	0.1330 (0.0920)	0.0050 (0.0451)	-0.2910** (0.0766)
Years of cultivation	0.0026 (0.0040)	0.0010 (0.0043)	0.0028 (0.0049)	-0.0070* (0.0033)	0.0096* (0.0047)
Farm size (m ²)	-0.0018** (0.0005)	0.0000 (0.0001)	0.0004* (0.0001)	0.0000 (0.0000)	-0.0002 (0.0001)
CIG ¹ membership	-0.0413 (0.0656)	-0.0853 (0.1190)	0.0771 (0.1190)	-0.0201 (0.0563)	-0.1470 (0.1100)
Number of people	-0.0030 (0.0073)	-0.0122 (0.0135)	0.0235 (0.0143)	-0.0131 (0.0132)	-0.0142 (0.0131)
Area type ²	-0.0699* (0.0300)	-0.0253 (0.0531)	0.0847 (0.0613)	0.0311 (0.0312)	-0.0923 (0.0598)
House distance (m)	-0.0001 (0.0001)	0.0001 (0.0001)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Number of observations	130	130	130	130	130

Notes: The dependent variable is the use of agrochemicals (fertilizers and pesticides). Coefficient estimates are reported with robust standard errors in parentheses. Other variables controlled for include: gender and level of education of farmers, age, land ownership and size, number of household members involved in farm activities, and distance between farm and house. ¹ Common initiative grouping (CIG) membership and ² Areas type (temporary flooded or permanently flooded) is also controlled. ** p<0.01, * p<0.05 represent statistical significance at 1%, and 5% respectively.

The number of years the farm has been under cultivation (years of cultivation) has mixed effects on externality use. It is significantly negatively and positively associated with mineral fertilizer and pesticide use, respectively (Table 19). Possibly farmers are aware that long-term use of mineral fertilizers leads to diminishing productivity and soil fertility, resulting in low crop production. Yet long-term cultivation of crops on the same piece of land leads to a build-up of pests and diseases, negatively affecting crop productivity and soil health. Therefore, farmers probably tend to address these problems through the increased use of pesticides.

There is a significant positive association between farm size and the use of a combination of organic and mineral fertilizers (Table 19). The findings suggest that the smaller the farm, the greater the likelihood of using higher fertilizers rates to increase crop yield and productivity.

Previous studies have also demonstrated that farm size influences the use of fertilizers. For instance, a study conducted on fertilizer adoption in Ghana revealed that farmers were more likely not to adopt fertilizer as the farm size increased (Martey et al., 2013). Nkamleu (2007) reported in Cameroon that farm size was significant and negatively related to adopting inorganic fertilizer, organic fertilizer, and integrated use of combined organic-inorganic fertilizers. In the present study, farmers with larger farms usually had ownership of this land and hence are interested in long-term farm management strategies, including applying mineral and organic fertilizers to maintain soil fertility. On the contrary, in smaller farms, where the farmers do not own the land, there is a focus on producing as much as possible in the short term to make money quickly and hence the tendency to use more inputs, in this case, fertilizers.

Summary

The use of agricultural inputs like fertilizers and pesticides is affected by the size of the farm, the level of farmers' education, the number of years the land has been under cultivation, and land ownership. Often associated with hygiene and sanitation, the study further found that farmers' general lack of appropriate protective gear for applying fertilizers and pesticides and their exposure to untreated wastewater used for irrigation purposes. Despite these health risks, vegetable farming provides crucial food for domestic consumption, employment, and income to farmers, contributing to the well-being of the farmers and their families. Importantly, the study shows that most health problems could possibly be avoided by stricter adherence of farmers to basic occupational health and safety measures. Effective extension services, which provide improved training on the safe application of agrochemicals and sound production techniques and regular monitoring of water, soil, and product quality, will also reduce health risks to farmers and consumers.

The next subchapters highlight the physicochemical and heavy metals characteristics of irrigation water, soil, and plant from the selected lowland areas.

4.2. Quality of irrigation water, agricultural soils, and vegetable

4.2.1. Physico-chemical characteristics of irrigation water

The pH of irrigation water samples is reported in Figure 18. Variations were observed both within samples from the same area and between samples from different sites. The pH values were 7.7, 6.9, 6.5, and 6.7 in the dry season and 6.3, 6.7, 6.8, and 6.7 in the wet season, respectively for Mokolo, Ekounou, Ekoumdoum, and Nkolbisson. The findings suggest that the irrigation water in the areas was almost neutral to slightly acid. Almost all the obtained pH values ranged between 6.5 and 8.4, which are within the FAO/WHO recommended range for irrigation water (FAO/WHO, 2011). The high fertilizer uses in Mokolo caused the highest pH values recorded. These high pH values can influence plant growth and the bioavailability of Cu and Zn (Brunton, 2011). The pH values obtained in the Nkolbisson irrigation water (4.4 to 6.9) were lower than the range of 7.1 to 7.7 reported in that area in a previous study by Mewouo et al. (2014). Similarly, almost neutral or slightly acidic irrigation water has also been reported by Ntangmo et al. (2012) in their study in Dschang, Cameroon. Many authors estimated that this pH range is typical for irrigation water in Africa (Ajon et al., 2018; Baï et al., 2019).

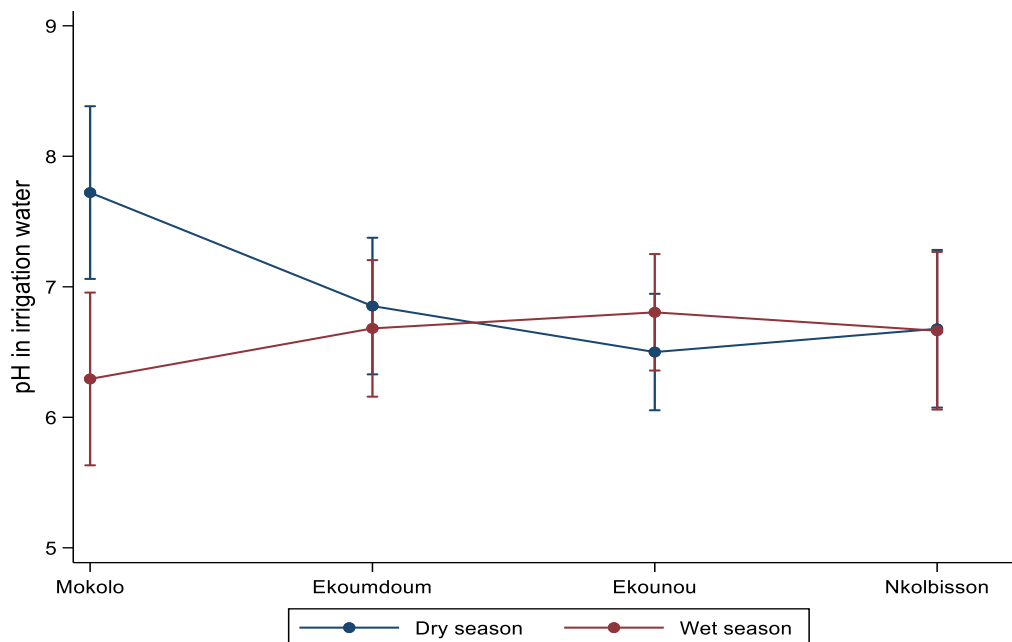


Figure 18 pH (mean value (dot) and 95% confidence interval (line)) in the irrigation water during the dry and wet seasons in Yaoundé lowlands.

The EC (a measure of water salinity) values were 489, 243, 193, and 158 $\mu\text{S}/\text{cm}$ in the dry season and 153, 252, 283, and 186 $\mu\text{S}/\text{cm}$ in the wet season for Mokolo, Ekounou Ekoumdoum, and Nkolbisson, respectively (Figure 19). The highest EC was recorded in the Mokolo sample during the dry season, indicating a high concentration of dissolved substances, chemicals, and minerals in the water. According to Fipps (1995), an EC of water $<250 \mu\text{S}/\text{cm}$ is excellent, while the range of 250 to 750 $\mu\text{S}/\text{cm}$ is suitable for irrigation purposes. All EC values of irrigation water samples recorded in the four study areas were within the FAO thresholds ($>3,000 \mu\text{S}/\text{cm}$), thus suitable for vegetable irrigation (WHO/FAO, 2011). These results are similar to those obtained by Ntangmo et al. (2012). In their study on the quality of water used for vegetable irrigation in Dschang, Cameroon, the authors reported that low values of EC meant water was slightly mineralized and unsuitable to be used for irrigation. The high EC value recorded in Mokolo was probably caused by the high use of mineral fertilizer in this area. The difference in altitude could also be one of the causes, as Mokolo (728 m.a.s.l.) was far higher than other areas (Ekounou (613 m.a.s.l.), Ekoumdoum (630 m.a.s.l.) and Nkolbisson (696 m.a.s.l.)). Thus, Mokolo may presumably have the lowest water table, which may likely result in more leaching.

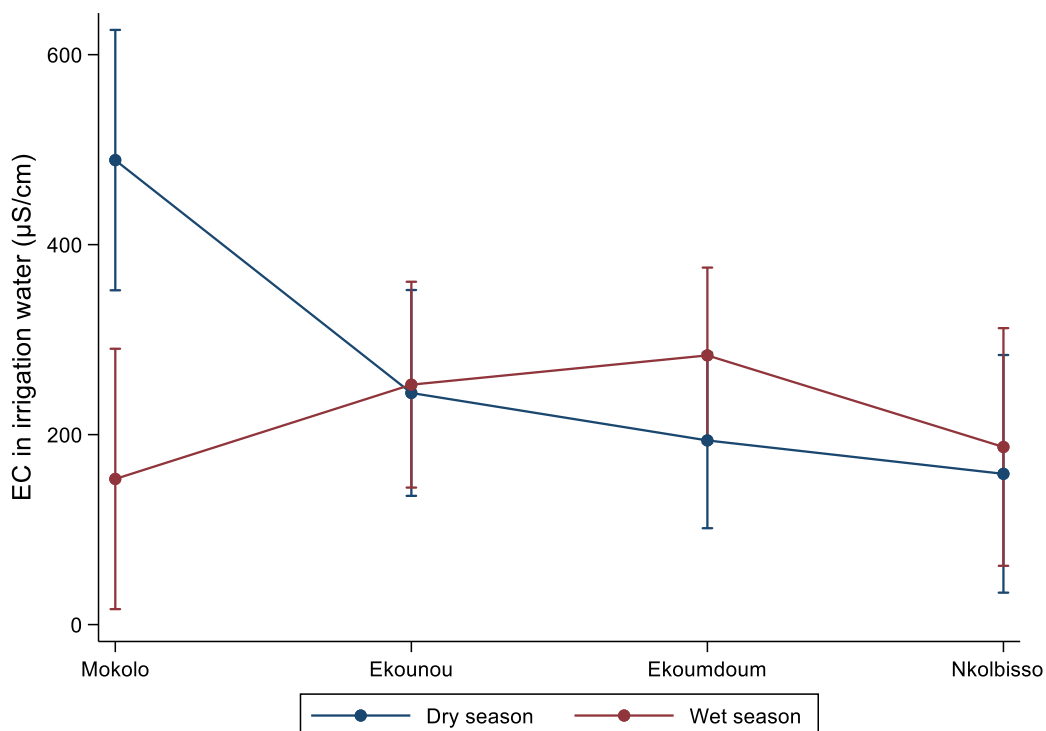


Figure 19 EC (mean value (dot) and 95% confidence interval (line)) in the irrigation water during the dry and wet seasons in the study areas.

According to Ayers and Westcot (1994), if the soil and climatic conditions and the cultural practices remain constant, higher total salinity of irrigation water means higher salinity hazard for crops. Higher values of EC were recorded in the wet season in three out of the four studied areas compared to the dry season. High values of EC in the wet season suggest more mineralization than in the dry season. The lower values of EC in the dry season were also recorded by Mewouo et al. (2014) in the Nkolbisson area and Kadyampakeni et al. (2017) in northern Ghana.

The results of the linear mixed-effect models revealed an overall significant difference in the interaction of areas and season for both season and areas (Table 20). Yet, the seasonal and areas variation in the distribution of pH and EC is inconclusive because the effects are different depending on the seasons and the areas. Therefore, further analysis may help highlight the contrast of pH and EC between each season and area.

Table 20 Contrasts of marginal linear predictions from mixed-effect linear regression.

Variables	df	pH		EC	
		F	P>F	F	P>F
Area	3	0.59	0.627	1.73	0.172
Season	1	2.71	0.106	1.61	0.210
Area#season	3	3.23	0.029	4.65	0.006

Note: Degrees of freedom (df) is the number of independent variables. Number in bold: statistically significantly different at $p < 0.05$.

The predictive margins for specific parameters were compared using pairwise comparison. The results indicate no significant differences in the irrigation water's pH and EC in most areas, except for irrigation water collected in the dry season from Mokolo vis-à-vis that from Ekounou, Ekoumdoum, and Nkolbisson, and about a comparison of irrigation collected in Mokolo in the dry and wet seasons (Table 21).

Table 21 Seasonal and areas variation of pH and EC irrigation water.

Areas	Season	pH		EC	
		Contrast	P>t	Contrast	P>t
Mokolo	Dry Vs Wet	-1.43	0.003	-335.66	0.001
Ekounou	Dry Vs Wet	-0.17	0.644	8.73	0.909
Ekoumdoum	Dry Vs Wet	0.30	0.337	89.55	0.175
Nkolbisson	Dry Vs Wet	-0.02	0.972	28.27	0.75
Season	Areas				
Dry	Ekounou vs Mokolo	-0.87	0.044	-245.13	0.007
Dry	Ekoumdoum vs Mokolo	-1.22	0.003	-295.11	0.001
Dry	Nkolbisson vs Mokolo	-1.04	0.023	-330.28	0.001
Dry	Ekoumdoum vs Ekounou	-0.35	0.308	-49.98	0.484
Dry	Nkolbisson vs Ekounou	-0.17	0.664	-85.16	0.307
Dry	Nkolbisson vs Ekoumdoum	0.18	0.636	-35.17	0.652
Wet	Ekounou vs Mokolo	0.39	0.361	99.26	0.259
Wet	Ekoumdoum vs Mokolo	0.51	0.205	130.10	0.12
Wet	Nkolbisson vs Mokolo	0.37	0.412	33.64	0.717
Wet	Ekoumdoum vs Ekounou	0.12	0.72	30.84	0.666
Wet	Nkolbisson vs Ekounou	-0.02	0.964	-65.62	0.43
Wet	Nkolbisson vs Ekoumdoum	-0.14	0.707	-96.45	0.219

Number in bold: statistically significant difference at $p < 0.05$ and $P < 0.01$.

4.2.1. Heavy metal characteristics

The heavy metals concentrations measured in the irrigation water samples varied within and between the studied areas. Table 22 and Figure 20 show the concentrations of Cu, Cd, Pb, Cr, Ni, and Zn in the irrigation water gathered in the dry and wet seasons. In general, the heavy metals concentrations in the irrigation water followed a general trend with Ekounou > Ekoumdoum > Mokolo > Nkolbisson, and with Zn > Cu > Ni > Cr > Pb > Cd during the dry and Zn > Cu > Cr > Pb > Ni > Cd during the wet seasons. Among the heavy metals tested, the concentration of Zn in the irrigation water was the highest, and Cd was the lowest in both dry and wet seasons.

The highest concentrations of Cu, Ni, and Zn were recorded in the irrigation water from Ekounou during the dry season, possibly resulting from wastes and run-off from surrounding human activities such as domestic wastewater/sewage and other discharge of contaminated municipal wastes and urban run-off, discarded into the environment without treatment.

Table 22 Means of heavy metal concentrations (mg/L) in irrigation water (\pm SD) and their threshold limits.

Countries	Cities/Areas	Season	Cu	Cd	Pb	Cr	Ni	Zn
Cameroon	Mokolo	Dry	0.007 \pm 0.002	0.001 \pm 0.0002	0.001 \pm 0.003	0.0003 \pm 0.0003	0.006 \pm 0.001	0.001 \pm 0.003
Yaoundé		Wet	0.004 \pm 0.004	0.0001 \pm 0.0001	0.003 \pm 0.002	0.000	0.0003 \pm 0.001	0.003 \pm 0.002
(this study)	Ekounou	Dry	0.012 \pm 0.008	0.001 \pm 0.0003	0.001 \pm 0.001	0.002 \pm 0.003	0.007 \pm 0.001	0.001 \pm 0.001
		Wet	0.003 \pm 0.002	0.000	0.002 \pm 0.002	0.002 \pm 0.002	0.001 \pm 0.001	0.002 \pm 0.002
	Ekoumdoum	Dry	0.010 \pm 0.006	0.001 \pm 0.0002	0.0001 \pm 0.001	0.003 \pm 0.003	0.006 \pm 0.002	0.0001 \pm 0.001
		Wet	0.002 \pm 0.002	0.000	0.001 \pm 0.002	0.003 \pm 0.003	0.002 \pm 0.002	0.001 \pm 0.002
	Nkolbisson	Dry	0.006 \pm 0.002	0.001 \pm 0.0001	0.003 \pm 0.003	0.003 \pm 0.002	0.006 \pm 0.002	0.003 \pm 0.003
		Wet	0.004 \pm 0.002	0.000	0.000	0.005 \pm 0.001	0.002 \pm 0.001	0.000
Cameroon Yaoundé (Djouaka et al., 2016)	Nkolomdom	-	0.001	0.001	0.422	-	-	-
DRC (Ngweme et al., 2020)	Kingshasa	-	0.43-3.80E-3	0.02-0.06E-3	0.06-0.90E-3	0.94-1.53E-3	-	1.57-30.85E-3
Nigeria (Muhammad et al., 2020)	Bauchi State	-	5.48- 22.32	0.05- 0.10	0.10-0.38	0.05-0.15	0.05-0.10	0.90-8.02
Maroc (Chaoua et al., 2019)	Marrakech	-	0.102 -0.197	0.076-0.086	1.417-1.417	-	-	1.59-1.41
Threshold limits (FAO, 1994)		-	0.20	0.01	5.00	0.10	0.20	2.00

Notes. -: not-applicable; DRC: Democratic Republic of Congo

Nkolbisson irrigation water contained the highest concentrations of Pb during the dry and Cr during the wet season. Pb in the irrigation water may have originated from nearby open car repair shops and from household pollutants such as paints, cosmetics, crystals, and ceramic containers that contaminate the surrounding SW and GW in either dissolved or waterborne particles. Another source could have been tannery and dyeing activities in the area, and geogenic processes such as weathering of ultramafic rocks. In addition, fertilizer may have also increased the level of Cr in the irrigation water via run-off. Cr is generally found in water in hexavalent form, which is highly toxic and has a potential human carcinogenic effect (Chrysochoou et al., 2016).

The highest concentration of Cd was reported in Mokolo irrigation water during the dry season, with fertilizer used on the farms as the most likely source. However, the heavy metal concentrations in the irrigation water were below the FAO/WHO recommended permissible limits. Similar results were found in the irrigation water from many urban areas in Cameroon and other African cities (Table 24). Compared to Djouaka et al. (2016) study in Nkolomdom, a peri-urban area of Yaoundé, the Pb values of this study were lower, while those of Cu were higher. Compared to this study, Ngweme et al. (2020) assessed the levels of heavy metals in irrigation water in peri-urban areas in Kinshasa found concentrations of Cr, Cu, Zn, Cd, and Pb lower than those obtained here.

Yet Muhammad et al. (2020) in the Bauchi State of Nigeria reported higher concentrations of Cr, Pb, Ni though still within the permissible limits of WHO, while those of Cu, Zn, and Cd were exceeding the said limits. Chaoua et al. (2019) reported in general higher concentrations of heavy metals in the wastewater used for irrigation in the region of Marrakech, though except for Cd, all were below the FAO/WHO threshold. Hence, the authors concluded that the wastewater was acceptable for crop irrigation.

The rainfall that diluted both SW and GW sources used can explain the lower heavy metal contamination of irrigation water during the wet season. During the dry season, the primary water source for irrigation comes from GW and is filled by often contaminated wastewater from nearby activities such as industry or domestics. In the rainy season, cultivated areas receive a significant amount of rain that fills up rivers and irrigation ponds. According to Ruben et al. (2013), irrigation ponds serve as reservoirs to contain rain and GW and receptacle of significant amounts of irrigation return flow and channel water. Thus, pollutants are more concentrated during the dry than the rainy season, which, among others, could explain the higher heavy metals contamination in irrigation water collected during the dry season at specific sampling locations. Yet, it should be stressed again that the found levels were most of the time below the FAO thresholds. Ahmed et al. (2019) reported lower heavy metals contamination in irrigation water during the wet compared to the dry season. However, agricultural residues, domestic and municipal wastes, and seasonal industries might directly affect the variations of heavy metals concentrations in the irrigation water sources. In addition, heavy metals can accumulate in sediments and soil and become bioavailable for crops.

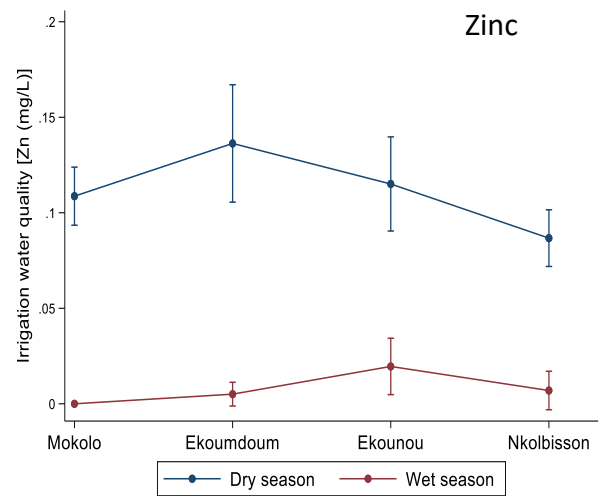
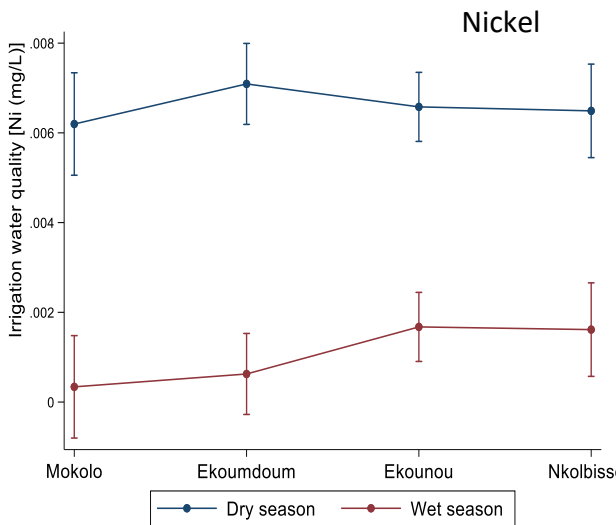
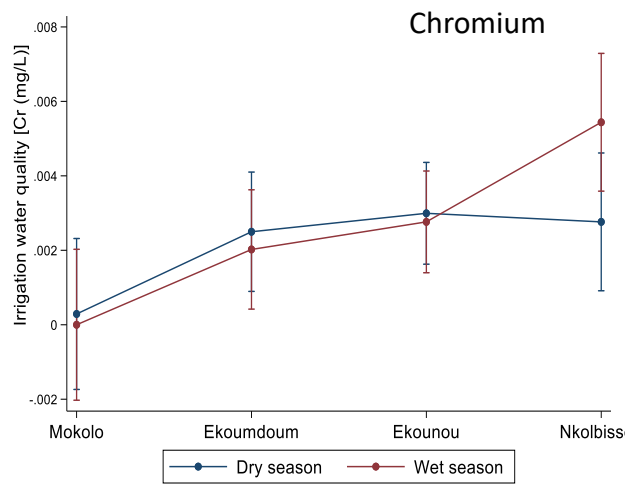
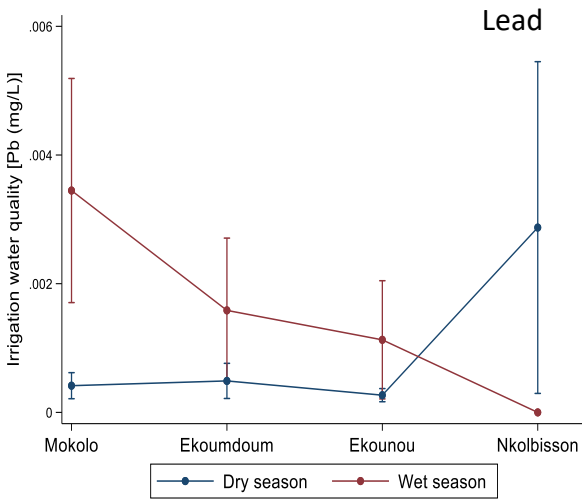
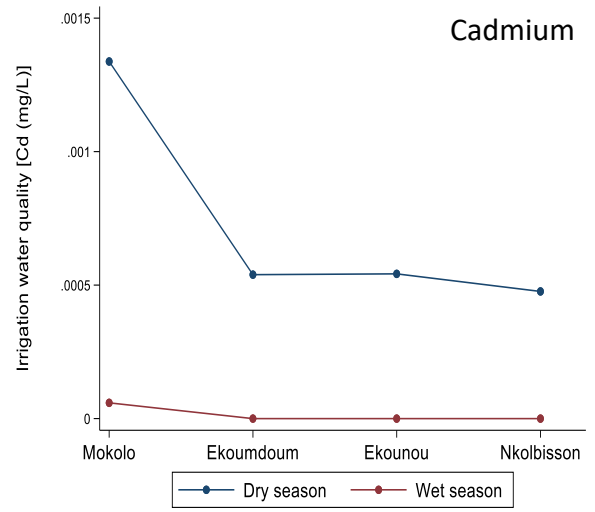
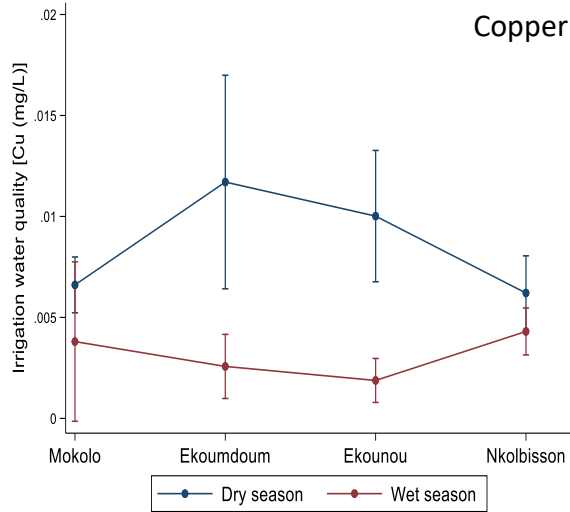


Figure 20 Seasonal variation of heavy metals in the irrigation water in the four study areas (mean value (dot) and 95% confidence interval (line)).

The results of the linear mixed-effect models are summarized in Table 23. The findings show an overall statistically significant difference in the interaction of areas and seasons for Cu, Cd, Pd, and Zn, implying that no conclusion on the factors involved can be made. Yet, the interaction season and areas were not significant for Ni and Cr, with a significantly different Cr distribution between areas and significantly different for Ni between seasons. The difference of Cu, Cd, Pb, Zn, Cr, and Ni between areas and seasons is summarized in Appendix 4.

Table 23 Contrasts of marginal linear predictions from robust mixed-effects linear regression.

Variables	df	Cu		Cd		Pb		Zn		Cr		Ni	
		chi ²	P>chi ²	chi ²	P>chi ²	chi ²	P>chi ²	chi ²	P>chi ²	chi ²	P>chi ²	chi ²	P>chi ²
Areas	3	2.3	0.506	45.1	0.000	6.1	0.105	8.5	0.037	4.9	0.010	0.9	0.435
Season	1	25.1	0.000	121	0.000	1.4	0.227	345.7	0.000	0.4	0.53	214	0.000
Areas #season	3	11.2	0.011	27.8	0.000	14.3	0.000	10.9	0.012	1.2	0.33	1.2	0.312

Note: Degrees of freedom (df) is the number of independent variables. Number in bold: statistically significantly different at p<0.05.

4.2.1.3. Multivariate analysis

Multivariate analyses (MA) are commonly used to distinguish factors such as the natural and anthropogenic contributions of the elements according to the various levels of the relationship (Huang et al., 2020; Perumal et al., 2021; Shaheen et al., 2021). In this study, MA was conducted using principal component analysis (PCA) to obtain information on the relationship between the metals and their sources of contamination (lithogenic background or anthropogenic activities) (Mishra et al., 2018). Principal components with eigenvalues greater than one were considered for the data set's variability (Mishra et al., 2018). The main components extracted with scores >0.55 were considered statistically significant and used for interpretation (Truong & McColl, 2011).

Hierarchical cluster analysis (HCA) is a statistical technique that is used to identify groups or clusters of similar sites based on similarities within a class and dissimilarities between different classes (Anbuselvan et al., 2018; Mishra et al., 2018). HCA aims to group the samples from different areas where heavy metals concentration followed a similar pattern. The result of HCA is a tree-based representation of the objects, named dendrogram. In the dendrogram, each leaf corresponds to one object. Then, objects similar to each other are combined into branches when moving up the tree, which are themselves fused with increasing height. The height of the fusion, the vertical axis, indicates the similarity, dissimilarity, or distance between two objects or clusters. The higher the height of the fusion, the less similar the objects are (Kassambara, 2015). Since performing HCA on variables rather than on cases is preferred in many research studies (Enitan et al., 2018; Ahmed et al., 2019; Shaheen et al., 2021), the dendrogram is used to assess and understand the distribution of the sampling sites regarding the heavy metals analyzed (Shirani et al., 2020). The Hierarchical Clustering on Principal Components or HCPC approach used in this study allows combining the three standard methods ACP, HCA, and Partitioning in k-means used in multivariate data analyses (Husson et al., 2010).

Principal Component and Cluster Analyses

Table 24 shows the factor loadings of the first two principal components in the PCA and their percentage contribution to the total variance for heavy metals in the irrigation water in the dry season. The eigenvalues of the first two extracted components for heavy metal concentrations have values >1 . Figure 21 shows the loading plots for PC1 and PC2 obtained from the PCA.

Table 24 Factor loadings of the first two PC and percentage contributions in the dry season.

Component matrix	Rotated component matrix	
	PC1	PC2
Cu	0.78	-0.44
Ni	0.47	0.63
Cr	0.61	0.67
Cd	-0.52	0.05
Zn	0.79	0.40
Pb	-0.05	0.56
Eigenvalue	2.1	1.52
Variability (%)	34.92	25.39
Cumulative (%)	34.94	60.31
Values in bold with factor loadings > 0.55		

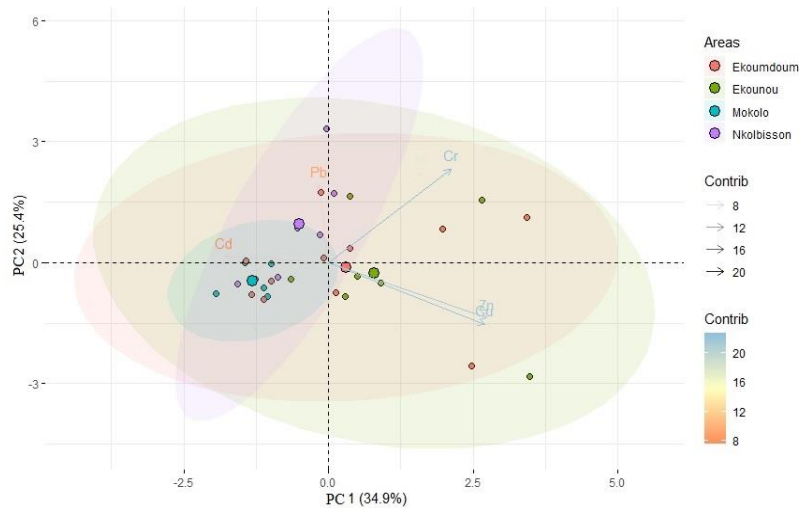


Figure 21 PCA loading plots for PC1 and PC2

This result suggests a two-component model that explains 60.31% of the total variance in the dry season. The first principal component, PC1, accounted for 34.92% of the total variance, with an eigenvalue of >2 , and included Cu, Cr, and Zn. PC2 explained 25.39% of the total variance, with an eigenvalue >1.52 , and contained Ni, Cr, Pb. The high positive loadings of Cu and Zn in the PC1 and Ni and Pb in PC2 suggest that the presence of these metals in the irrigation water may originate from the same source. Cu and Zn were found in high variability, and the high positive loading with PC1 may derive from anthropogenic sources. PC2 with low variability and a high positively loading may come from the natural environment such as local geology (for Ni) and atmospheric deposition for Pb. Cr derives come from both sources, i.e., natural and anthropogenic, due to their significant presence in both components (PC1 and PC2).

HCA was conducted after a PCA to cluster the areas and sampling points contaminated with specific metals that originated from the same sources of pollution. The characteristics of each cluster for the six parameters used in the HCA during the dry season are described in Table 25. The highest levels of Cu and Zn are reported in cluster IV, suggesting that this cluster may be associated with PC1. In contrast, clusters II and III might likely be correlated with PC2 due to their high levels of Cr and Pb.

Table 25 Description of each cluster by quantitative variables in the dry season

Variables	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Pb	0.0004	0.0009	0.0004	0.0018	0.0171
Zn	0.1009	0.1140	0.0251	0.0390	0.0010
Cr	0.0015	0.0024	0.0015	0.0025	0.0007
Cu	0.0071	0.0091	0.0022	0.0057	0.0005
Cluster II					
Pb	0.0070	0.0009	0.0019	0.0018	0.0000
Cluster III					
Cr	0.0078	0.0023	0.0017	0.0025	0.0001
Ni	0.0089	0.0066	0.0011	0.0015	0.0045
Zn	0.1733	0.1140	0.0182	0.0390	0.0064
Cluster IV					
Cu	0.0278	0.0091	0.0023	0.0057	0.0000
Zn	0.1947	0.1140	0.0169	0.0390	0.0029
Cd	0.00006	0.0007	0.0000	0.0004	0.0475

Four main clusters can be identified in the dendrogram shown in Figure 22. The heavy metals which show the higher and constant similarities were found in sites 16 and 15. Hence, the clusters with a lower distance between centroid classes are expected to contain water samples with similar heavy metal concentrations (Cf. Table 26).

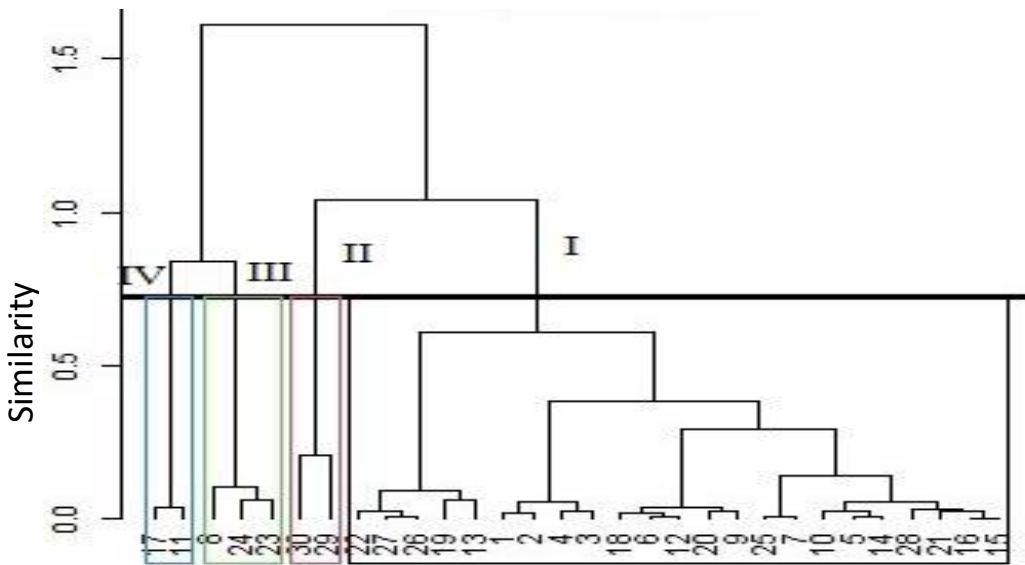


Figure 22 Dendrogram showing clusters of water sampling points with similar heavy metal in the dry season

Similarly, two main components that explained 66.62% of the overall variance of the parameters analyzed are obtained in the wet season (Table 26). PC1 explained 44.13% of the total variance, with an eigenvalue >2, and indicated a strong positive charge on Cd, Ni, and Cu,

suggesting their sources' similarities and a negative correlation with Pb. The high positive variability of Ni, Cr, and Pb in PC1 suggests that they might mainly originate from anthropogenic activities such as municipal wastewater from the surrounding communities. Pb may also derive from vehicle traffic and deposits from the atmosphere ending up in the water and soil. PC2 showed a positive charge of Cu and Cd (Figure 23) and explained 25.49% of the total variance, with an eigenvalue >1. Given its high loading in PC2, Cd may have originated from the natural background. Like Cr in the dry season, Cu may come from both natural and anthropogenic sources.

Table 26 Factor loadings of the first two PC and their percentage contribution in the wet season

Component matrix	Rotated component matrix	
	PC1	PC2
Cu	0.71	0.59
Ni	0.81	-0.20
Cr	0.79	0.39
Cd	0.31	0.86
Zn	0.18	-0.46
Pb	-0.74	0.19
Eigenvalue	2.47	1.53
Variability (%)	41.13	25.49
Cumulative (%)	41.13	66.62
Values in bold with factor loadings > 0.55		

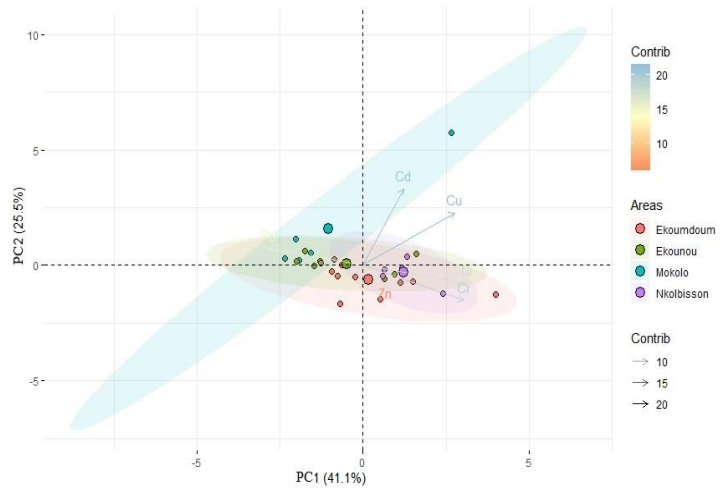


Figure 23 Factor loading plots for PC1 and PC2

The dendrogram of irrigation water samples shows 3 clusters in the wet season. The variables that describe each cluster are summarized in Table 27. Similar to the dry season, cluster I showed a high variation (Figure 24). High similarities of heavy metals were found across sites 29 and 9, sites 11 and 7, sites 25 and 10, and sites 26 and 13. The common criteria that the samples in each cluster have is their similarity of the heavy metal's concentration (Cf. Table 28).

Table 27 Description of each cluster by quantitative variables in the wet season

Variables	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Pb	0.003	0.001	0.002	0.002	0.000
Ni	0.000	0.001	0.001	0.001	0.002
Cu	0.001	0.003	0.001	0.003	0.002
Cr	0.000	0.003	0.001	0.003	0.000
Cluster II					
Cr	0.006	0.003	0.001	0.003	0.000
Ni	0.002	0.001	0.001	0.001	0.003

Pb	0.000	0.001	0.000	0.002	0.000
Cluster III					
Cd	0.000	0.000	0.000	0.000	0.000
Cu	0.012	0.003	0.000	0.003	0.001

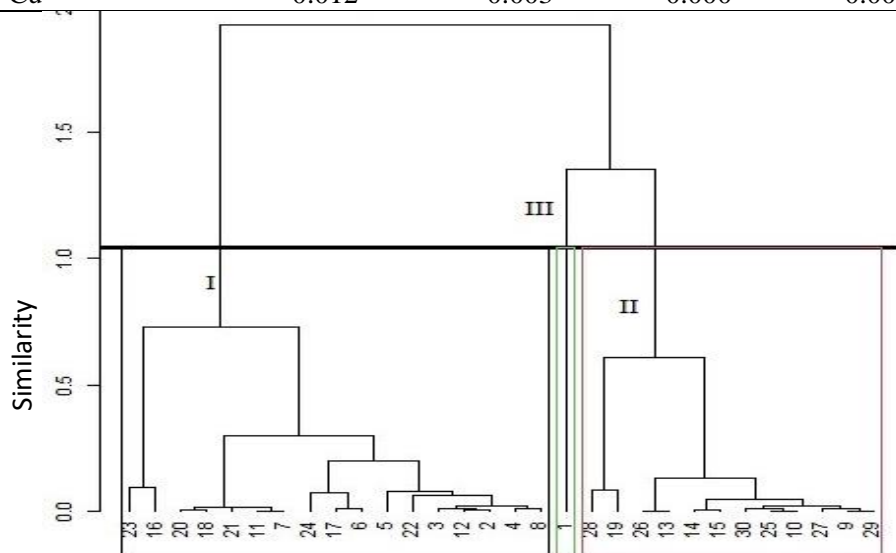


Figure 24 Dendrogram showing clusters of irrigation water sampling points based on analyzed variables in the wet seasons.

Summary

The irrigation water was slightly acid to neutral. All the values of pH and EC recorded were within the WHO/FAO recommended thresholds for irrigation water. Zn had the highest concentration in the irrigation water and Cd the lowest in all the study areas during both dry and wet seasons. The contamination was higher during the dry than the wet season, suggesting that rainfall might dilute the irrigation water, reducing the heavy metal concentrations. The level of contamination varied among areas depending on the heavy metals. The highest concentrations of Cu, Ni, and Zn were recorded in Ekounou, and those of Cr and Pb were in Nkolbisson and Cd in Mokolo irrigation water. However, all the concentrations were below the standard limits. Therefore, these water sources seemed appropriate for irrigation and might not pose risks from heavy metal contamination in the vegetables.

Knowing that heavy metals in vegetables may come from contaminated irrigation water and/or are taken up from the soil through the roots, the following section characterizes the physico-chemical and concentration of heavy metals in the agricultural soil of the study areas.

4.3. Quality of the agricultural soils

4.3.1. Physico-chemical characteristics

The pH is one factor that influences the bioavailability and the transport of heavy metals in the soil. Heavy metals are generally more mobile at pH <7 (Adjia et al., 2008). Figure 25 shows

the mean distribution of soil pH. The average soil pH for each site was 7.3, 5.8, 6.2, and 4.9 in the dry season and 7.2, 6.0, 5.9, and 5.5 in the wet season for Mokolo, Ekounou, Ekoumdoum, and Nkolbisson areas, respectively. Both irrigation water and soil samples from Mokolo had the highest pH among all sites.

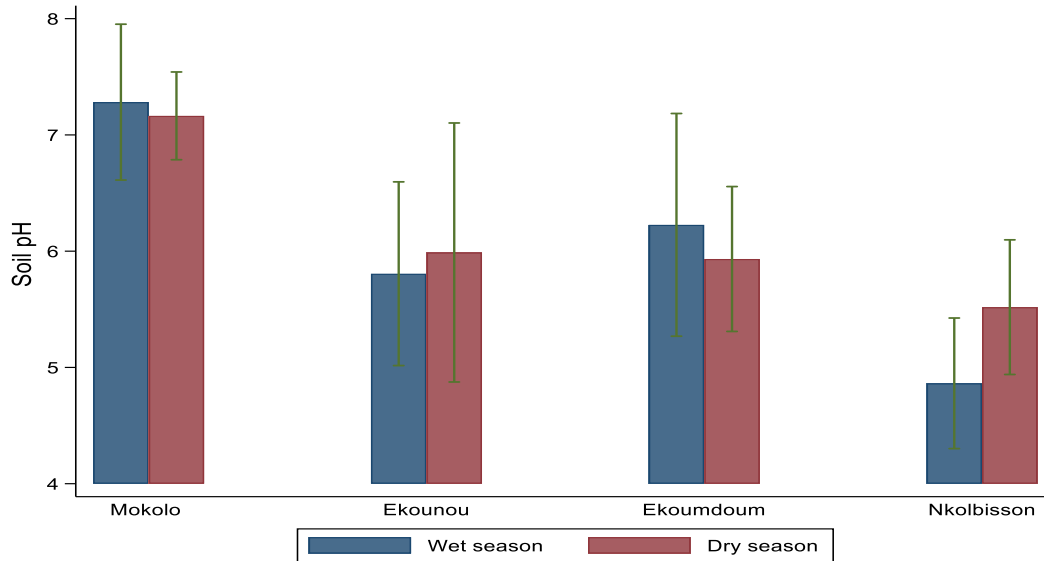


Figure 25 The distribution of pH values (mean \pm SD) in soils during the dry and wet seasons

The pH from the agricultural soil samples in the study areas ranges from acid (>4.9) to slightly neutral (<7.3), which are similar to those obtained in the irrigation water. Only samples from the Nkolbisson area had pH values ranging from 6 to 7.5, which is the recommended range for most plants (FAO, 2021). Soil pH affects the soluble proportion of nutrients and chemicals in the irrigation water and their availability to plants. Some nutrients are more accessible under acid, while others are more available under alkaline conditions. For instance, plants take up essential elements (Ca, K, Mg) less from acidic soil. Meanwhile, some components like Cu, Zn, Mn, and Fe are not easily absorbed in alkaline soils (Kadam, 2016). At very low pH, the solubility of some elements can become toxic to sensitive plants. On the other hand, at high pH values, the solubility of others can become so low that plants cannot obtain adequate supplies from the soil. According to Caporale (2016), soil pH influences the bioavailability and mobility of heavy metals, and their changes depend on the pH of the water used for irrigation. Thus, the precipitation of hydroxides and carbonates increases soil pH and reduces heavy metals' ability to move. The highest pH value obtained in the Mokolo area in both seasons compared to other areas could result from differences in farming practices such as the intensive fertilization of these soils. In addition, farmers in these areas use high quantities of animal manure (cf. Figure 14), which contains lime-like materials such as Ca and Mg that can increase the soil pH level (Bell & Mathesius, 2019; Hailin Zhang, 1998). The pH values obtained in Nkolbisson soil align with those from a previous study in the same area

by Aboubakar et al. (2021) and are slightly higher than those found in the urban watershed of Yaoundé (Defo et al., 2015), where pH ranges between 4.1-5.9 for soil at 0-30 cm depth.

The average soil EC values (as an estimate for soil salinity) were 34.9, 58.5, 50.2, and 50.1 $\mu\text{S}/\text{cm}$ in the dry season and 32.5, 62.1, 64.2, and 66.5 $\mu\text{S}/\text{cm}$ in the wet season for Mokolo, Ekounou, Ekoumdoum, and Nkolbisson, respectively (Figure 26). Nkolbisson soil samples had the highest soil EC during the wet season.

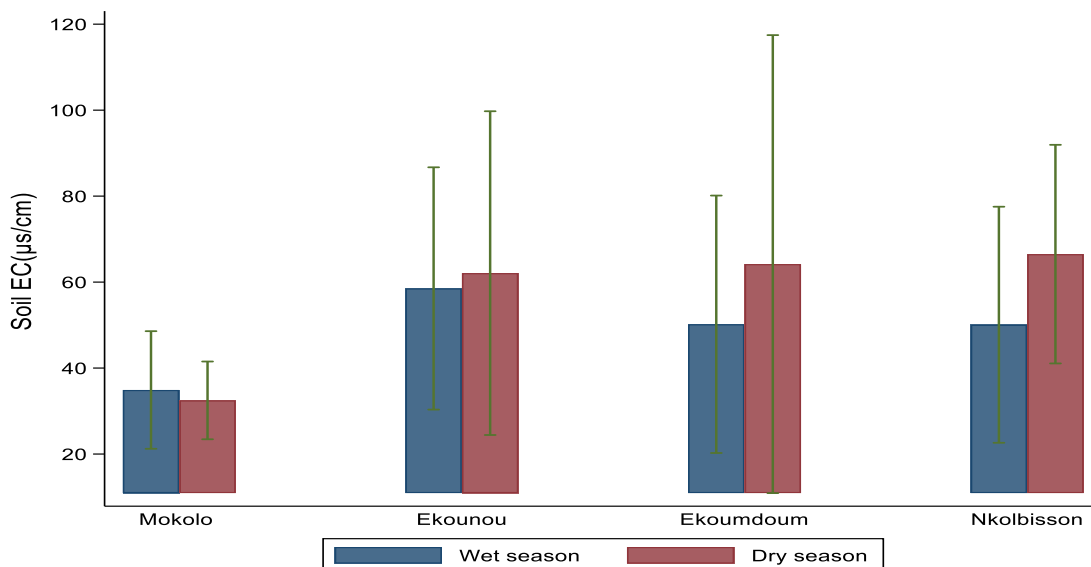


Figure 26 Distribution of EC (mean \pm SD) during the dry and wet season in the studied areas soils

The increase of soil electrical conductivity improves heavy metals solubility and thus enhances the availability of metals from the soil to the plants (Singh & Taneja, 2009). EC values of soil samples varied from 32-66 $\mu\text{S}/\text{cm}$, meaning the soil samples were non-saline (Sparling, 2011) and still below the thresholds of 1,300 $\mu\text{S}/\text{cm}$ for the soil under vegetable crops (USDA, 1994). Mokolo's soil samples had the lowest EC while other areas showed high conductivity values in the rainy season.

The soil CEC were 11.1, 13.5, 32.5, and 11.0 $\text{cmol}(+)/\text{kg}$ in the dry season and 13.6, 11.6, 19.9, and 10.4 $\text{cmol}(+)/\text{kg}$ in the wet season in Mokolo, Ekounou, Ekoumdoum, and Nkolbisson respectively (Figure 27). In the dry season, soil samples from Ekoumdoum had the highest CEC value, indicating that the soil's clay texture, when combined with organic matter, possesses several electrically charged sites that can attract and hold oppositely charged ions (Gebeyehu et al., 2020).

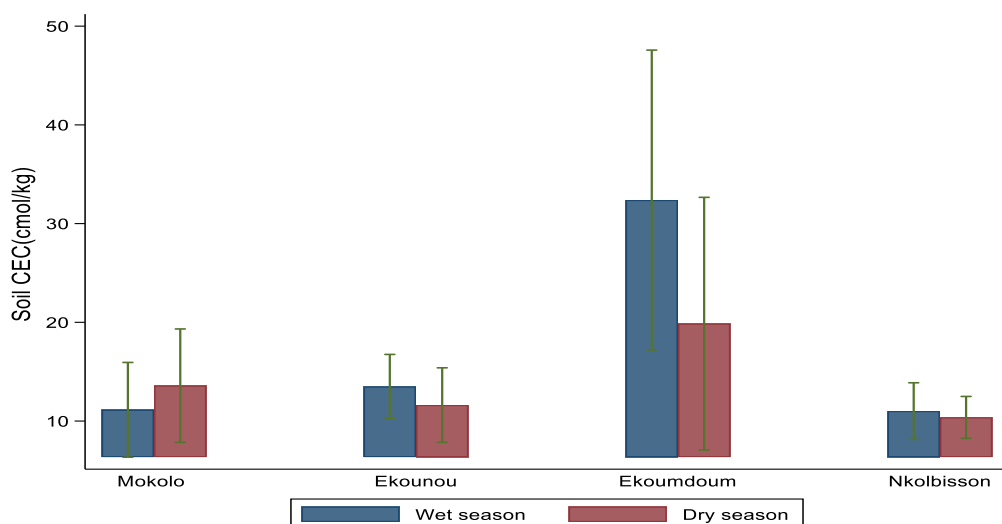


Figure 27 Distribution of CEC values (mean \pm SD) during the dry and wet seasons in the studied area soils

Soils with a high CEC have a lower percentage of cations in the soil water. Thus, they are less susceptible to nutrient loss by leaching (Spargo, 2013). Furthermore, the capacity of the soils to absorb heavy metals is correlated with their CEC. The greater the CEC values, the more exchange sites on soil minerals are available for metal retention (Alamgir, 2016).

In this study, the CEC obtained in Nkolbisson was higher than the value of 8.92 cmol(+)/kg reported in the same area by Aboubakar et al. (2021). The CEC values, ranging from 10.7 to 32.7 cmol/kg, are consistent with the observations of Defo et al. (2015) for some soils in the Ntem watershed in Yaoundé,

The linear mixed-effect models show the differences in the soil pH, EC, and CEC distribution between the areas, seasons, and interactions (Table 28).

Table 28 Contrasts of marginal linear predictions from mixed-effect linear regression.

Variables	df	pH		EC		CEC	
		F	P>F	F	P>F	F	P>F
Areas	3	12.57	0.000	25.16	0.000	21.58	0.000
Season	1	0.26	0.616	1.15	0.283	3.94	0.047
Areas#season	3	1.03	0.386	1.76	0.624	6.93	0.070

Note: Degrees of freedom (df) is the number of independent variables. Number in bold: statistically significant difference at $p < 0.05$.

The results revealed no overall significant difference in the interaction of areas and season for the studied parameters, and that area affected the distribution of pH and EC. However, both area and season affected CEC (Table 29).

Table 29 Seasonal and areas difference in pH, EC, and CEC distribution in the studied soils.

Areas	Seasons	pH		EC		CEC	
		Contrast	P>t	Contrast	P>t	Contrast	P>t
Mokolo	Dry vs Wet	-0.118	0.812	-2.436	0.743	2.447	0.521
Ekounou	Dry vs Wet	0.183	0.642	3.563	0.842	-18.746	0.057
Ekoumdoum	Dry vs Wet	-0.294	0.382	14.007	0.355	-12.523	0.010
Nkolbisson	Dry vs Wet	0.655	0.152	16.437	0.309	-0.641	0.593
Seasons	Areas						
Dry	Ekounou vs Mokolo	-1.476	0.002	23.621	0.032	2.352	0.293
Dry	Ekoumdoum vs Mokolo	-1.056	0.015	15.283	0.141	21.246	0.000
Dry	Nkolbisson vs Mokolo	-2.419	0.000	15.173	0.199	-0.129	0.954
Wet	Ekounou vs Mokolo	-1.175	0.011	29.610	0.025	-1.970	0.459
Wet	Ekoumdoum vs Mokolo	-1.231	0.005	31.717	0.048	6.276	0.155
Wet	Nkolbisson vs Mokolo	-1.646	0.001	34.025	0.001	-3.217	0.193
Dry	Ekoumdoum vs Ekounou	0.420	0.252	-8.338	0.518	18.894	0.000
Dry	Nkolbisson vs Ekounou	-0.943	0.030	-8.448	0.548	-2.481	0.108
Wet	Ekoumdoum vs Ekounou	-0.056	0.878	2.097	0.917	8.246	0.037
Wet	Nkolbisson vs Ekounou	-0.470	0.270	4.416	0.781	-1.247	0.407
Dry	Nkolbisson vs Ekoumdoum	-1.363	0.001	-0.111	0.994	-21.375	0.000
Wet	Nkolbisson vs Ekoumdoum	-0.414	0.301	2.319	0.899	-9.493	0.013

Number in bold: statistically significant difference at $p < 0.05$.

4.3.2. Heavy metals in agricultural soils

4.3.2.1. Spatial distribution of heavy metals in Yaoundé

The spatial distributions of the heavy metals during the dry and wet seasons are shown in Figures 28 and 29, respectively. These maps visualize the potential distribution of the selected heavy metals in Yaoundé soils. The concentrations of heavy metals were extrapolated based on the results of the specifically mentioned sites, i.e., Mokolo, Nkolbisson, Ekoumdoum, and Ekounou. The findings indicate that Zn and Cu distributions were similar during dry and wet seasons. Their concentrations were higher in the central part of Yaoundé (around Mokolo). Cr, Ni, and Pb were distributed relatively differently over the whole city but showed slightly higher levels in the dry season.

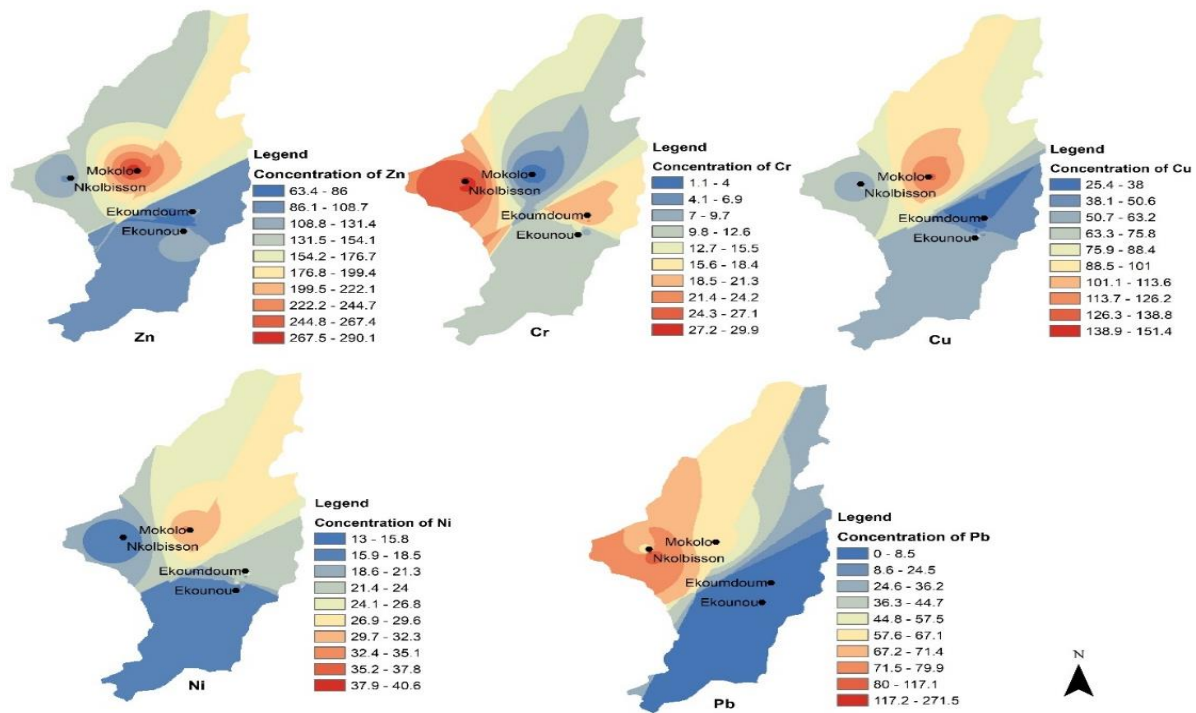


Figure 28 Spatial distribution of Zn, Cr, Cu, Ni, and Pb concentrations (mg/kg) during the dry season in Yaoundé.

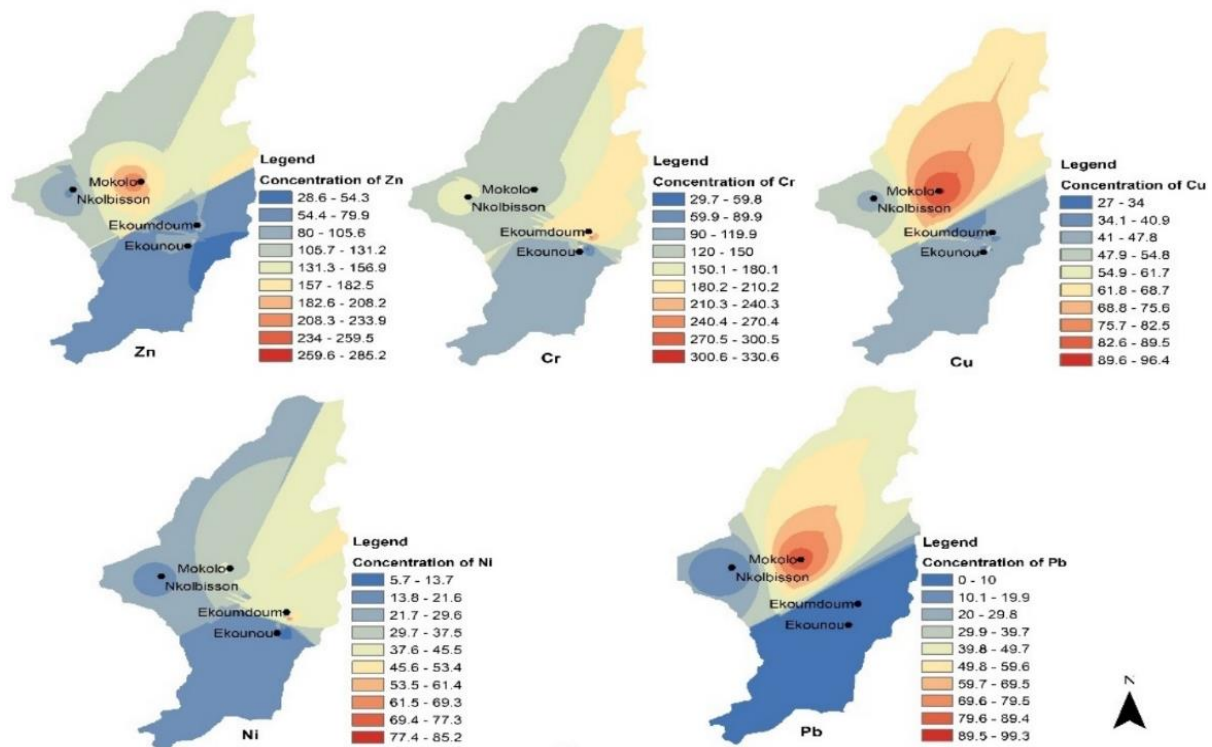


Figure 29 Spatial distribution of Zn, Cr, Cu, Ni, and Pb concentrations (mg/kg) during the wet season in Yaoundé.

4.3.2.2. Heavy metal in the study areas

The concentration of the six selected heavy metals (Cr, Ni, Cu, Pb, Zn, and Cd) in the agricultural soil samples are presented in Table 30 and Figure 30. The highest concentrations of Cu, Pb, and Zn were recorded in the dry season in the Mokolo soil. Similar to the soil pH, the highest concentration of Cu, Ni, Pb, and Zn obtained in the Mokolo area can be explained by the significantly higher amount of fertilizers (cf. Figures 15 & 16) and pesticides farmers applied in their farms compared to other sites.

The highest concentrations of Cr and Ni were recorded in the wet season in soils from Nkolbisson and Mokolo, respectively. A high concentration of Cr was also found during the wet season by Oluyemi et al. (2008) in Nigeria. The run-off might affect the capacity of removing heavy metals from farmlands that led to the high concentration of metals in the dry season. Evaporation is also more intense during the dry season, leading to a more concentrated soil solution. Meanwhile, rainfall might contribute to more leaching of heavy metals from the soil to groundwater and hence dilute the soil solution in the wet season. The total concentration of heavy metals varied among the study areas, following in descending order of Mokolo > Nkolbisson > Ekounou > Ekoumdoum. Similarly, the mean concentrations of heavy metals in these soils were in the following order of Zn > Cr > Cu > Ni > Pb > Cd, in the dry, and Cr > Zn > Cu > Pb > Ni > Cd in the wet season. These orders of metals concentrations are different from those found in the irrigation water, except for Zn and Cd, which showed again the highest and lowest concentrations, respectively. The concentrations of other metals ranked differently, suggesting that heavy metals found in soils do not originate or accumulate solely from the irrigation water but also from fertilizers, pesticides, or other external inputs.

High variability was observed within the samples from the same area and between the samples of different areas. This large variability was confirmed by the coefficient of variation (CV). Based on the CV values, the soil sample can be classified as having low ($CV \leq 25\%$), moderate ($25\% < CV \leq 75\%$), or high ($CV > 75\%$) variability (Mamut et al., 2018). With CVs of 35, 42, 43, and 46 for Zn, Cr, Cu, and Ni, respectively, fell into the moderate and with 85 Pb in the high variability group. Low CV values of heavy metals in the soil indicate that natural sources are dominant, whereas high CV values point to anthropogenic sources that affect heavy metals distribution (Baltas et al., 2020). The moderate to high variability observed in the heavy metal distribution in this study might be due to anthropogenic activities, including the use of untreated water for irrigation, pesticide and fertilizers usage, and the informal industries in the surrounding areas.

Table 30 Average heavy metal concentration in mg/kg (mean±SD) in the agricultural soils versus other studies.

Country/city	Areas	Seasons	Cu	Pb	Ni	Cr	Zn	Cd	Authors
Cameroon/ Yaoundé	Mokolo	Dry	122.2±40.8	65.1±28.9	31.4±9.9	133.5±21.6	276.6±56.4	nd	This study
		Wet	87.7±28.5	83.1±35.2	37.0±24.9	132.1±42.5	258.9±85.5	nd	
	Ekounou	Dry	36.9±12.1	1.4±3.7	22.8±5.9	135.2±46.6	104.1±55.5	nd	
		Wet	41.2±23.2	3.6±3.8	21.9±9.7	147.1±48.0	89.6±29.1	nd	
	Ekoumdoum	Dry	54.4±31.9	2.0±3.0	17.9±6.8	87.6±48.6	108.6±36.2	nd	
		Wet	44.8±21.2	4.0±4.7	15.2±9.7	103.6±76.5	92.4±39.7	nd	
	Nkolbisson	Dry	59.7±17.4	8.8±12.3	16.9±3.4	106.1±34.5	114.4±47.0	nd	
		Wet	46.5±6.4	12.5±9.8	20.5±3.0	151.6±43.2	82.9±9.3	nd	
Cameroon/ Yaoundé	Kolomdom	Dry	5.7±0.2	10.9±2.9	5.9±0.2	2.4±0.6	27.5±1.6	0.07±0.01	(Aboubakar et al., 2021)
	Nkolbisson	Dry	6.7±0.7	16.5±3.4	11.9±1.3	16.5±3.5	27.6±1.1	0.04±0.01	
Cameroon/ Bamenda	-		-	30.6	-	35.7	-	0.4	(Godswill A. Asongwe & Yerima, 2016)
Cameroon/ Ngaoundere	Bali	-	10.3±0.3	17.5±0.1	24.6±0.0	-	30.6±2.06	nd	(Noubissié et al., 2016)
	Sabongari	-	19.7±1.2	53.4±6.0	26.3±1.3	-	164.7±8.7	0.02±0.0	
Nigeria/ Ife	Obafemi	Dry	875.0 ±0.0	317.5 ±0.0	128.1 ±0.0	107.5 ±0.0	366.2 ±0.0	11.3±65.0	(Oluyemi et al., 2008)
	Awolowo	Wet	844.0 ±0.0	304.5±0.07	117.6±0.0	181.3 ±0.0	206.6 ±0.0	10.4 ±0.0	
Kenya, Homabay	Homahills	-	12.0 - 12.9	9.9-10.8	-	9.7-10.1	0.2- 46.9	0.5- 0.7	(Akenga, et al., 2020)
Threshold values (MEF, 2007)			100	60	50	100	200	1	

Notes. nd: not-detected; -: not-applicable

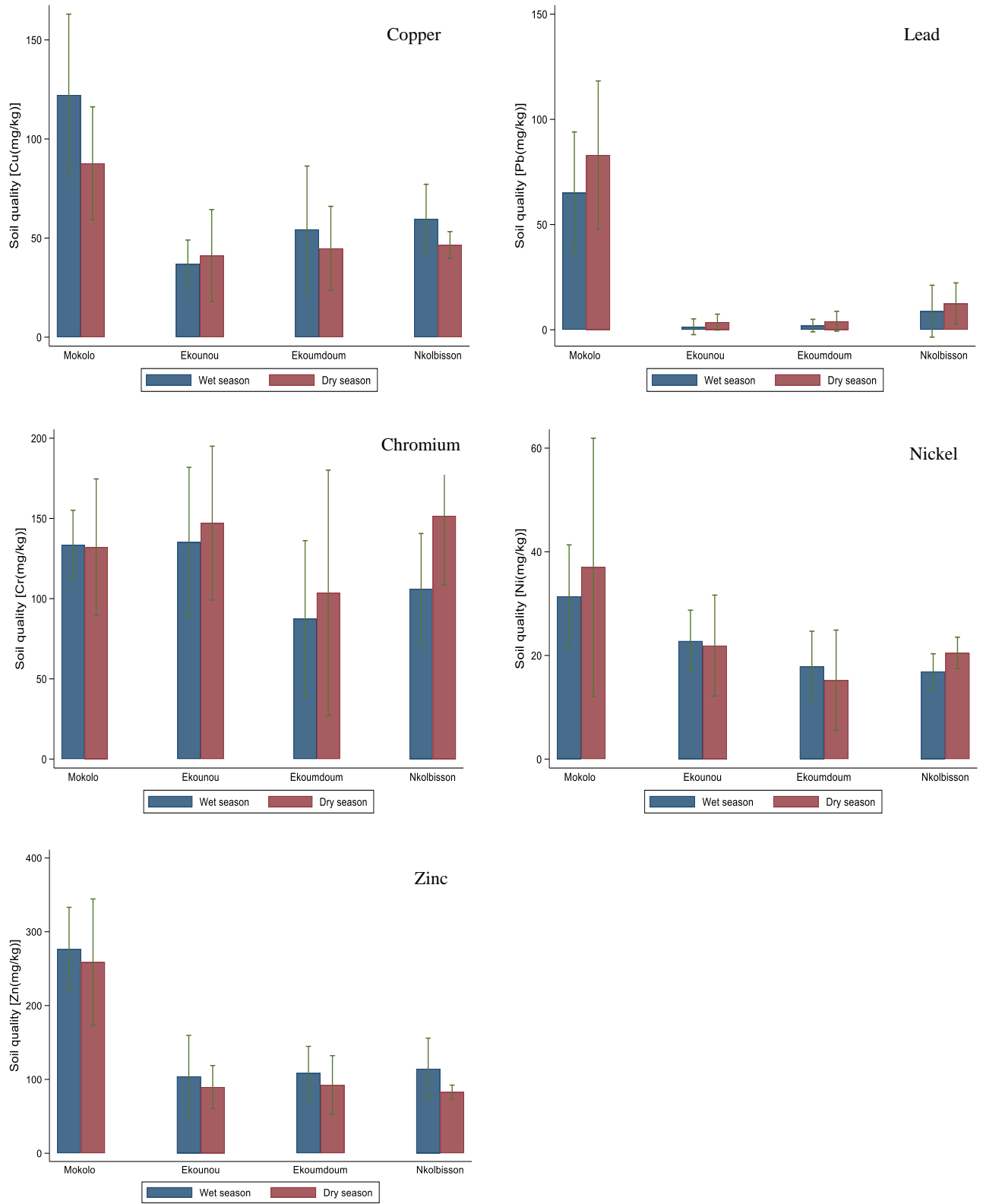


Figure 30 The concentration of heavy metals (mean \pm SD) in Yaoundé in the dry and wet seasons.

The heavy metal concentrations observed in the agricultural soil were compared with the permissible limits in agricultural soils set by the Ministry of the Environment, Finland (MEF, 2007b). These thresholds (Table 31) were chosen within the various options (standards) because they are, according to Tóth et al. (2016), a suitable standard for heavy metals in agricultural soil. These authors reported that the MEF thresholds “represent a good approximation of the mean values of different national systems in Europe (Carlson, 2007) and India (Awashthi, 2000), and they have been applied in an international context for agricultural soils as well (UNEP, 2013)”. Findings of this study suggest that the concentration of Cr was higher, and Ni was lower than the permissible limits in all the areas during both dry and wet seasons.

The concentrations of Cu, Pb, and Zn were higher than the threshold values in Mokolo and were lower in other areas. It can be explained by the high amount of fertilizer applied per square meter of farmland by the farmers in the Mokolo area (cf Figure 15 and 16). Moreover, informal waste disposal can justify the high concentrations of these metals since the Mokolo site was among the most polluted areas, with many informal industries such as tanneries, casting lead and lead products manufacturing, and waste burning activities in the open air during the samples collection.

In this study, Cd, Cu, Pb, Zn, and Cr concentrations were lower than the values (cf. Table 31) reported by Akenga et al. (2020) for the soil under cultivation of *S. nigrum*. These uncontrolled activities might release a considerable amount of Cu, Zn, Ni, and Pb in the environment contaminating the agricultural soils (Luo et al., 2012). Moreover, the high level of Zn, Cu, Pb observed could have emanated from fertilizers and pesticides, domestic inputs, and other building materials. Pb and Cu contributing to the enrichment of heavy metals in the study areas might have come from nearby roads traffic activities and agrochemicals inputs, respectively. Qaswar et al. (2020) reported a significant increase in the concentration of metals such as Cu, Zn, Cr, and Cd in the soil with the combined application of manure and synthetic fertilizers. The same authors demonstrated that high Pb concentrations were linked to farms under inorganic fertilization than farms under mixed fertilizer.

The highest concentration of Cr obtained at the Nkolbisson site (152 mg/kg) could have originated in large parts from the rivers contaminated with domestic wastewater and manure. Although a high percentage of farmers in the Nkolbisson site used fertilizers, they also irrigated the land with water from the rivers that are potentially contaminated by domestic wastewater and sewage sludge from the nearby residential areas. The concentrations of Cr, Ni, Zn, and Cu found in Nkolbisson were higher than those reported by Aboubakar et al. (2021) for the same area (cf. Table 31). Yet, the same authors reported Cd and Pb (except for Mokolo) in higher concentrations than the concentrations obtained in this study. They also noted that Cd in soil varied between 0.02 and 0.25 mg/kg, whereas it was below the detection limits in this study. The results of the linear mixed-effect models are reported in Table 32.

Table 31 Contrasts of marginal linear predictions from mixed-effect linear regression.

Variables	df	Cu		Cr		Ni		Pb		Zn	
		F	P>F	F	P>F	F	P>F	F	P>F	F	P>F
Areas	3	39.77	0.000	8.02	0.046	13.33	0.004	118.62	0.000	89.57	0.000
Season	1	4.12	0.042	2.55	0.110	0.21	0.646	1.24	0.266	2.2	0.138
Areas#season	3	5.15	0.161	1.93	0.587	5.64	0.130	0.58	0.901	0.81	0.846

Note: Degrees of freedom (df) is the number of independent variables. Number in bold: statistically significantly different at $p < 0.05$ and $P < 0.01$.

Although the difference in the interaction between areas and seasons was not significant, the findings suggest an overall significant area difference in the concentrations of Cu, Cr, Ni, Pb, and Zn. The concentrations of other metals were not significantly different, except for Cu, whose concentration significantly differed between seasons. For the specific areas and seasons differences, the results of the analysis are reported in Appendix 5.

4.3.2.3. Multivariate analysis

Principal component and cluster analysis

Table 32 and Figure 31 show the factor loadings for the first two PCs and their percentages of contribution to the total variance for heavy metals in soil in the dry season. The eigenvalues for the first two-component PC 1 and PC2 were >1 and counted for 73.44% of the total variance, suggesting that the two-component model contains all the information. PC1 accounted for 48.23% of the total variance with an eigenvalue >2 and is significantly associated with Zn, Cu, and Ni. PC2 accounted for 25.49% of the total variance, with an eigenvalue >1 , and correlated significantly with Cr.

Table 32 Factor loadings of the first two PC and their contribution percentage in the dry season

Component matrix	Rotated component matrix	
	PC1	PC2
Zn	0.87	0.26
Cr	0.50	0.77
Cu	0.77	-0.54
Ni	0.75	0.49
Pb	0.49	-0.21
Eigenvalue	2.41	1.26
Variability (%)	48.23	25.21
Cumulative (%)	48.23	73.44
Values in bold with factor loadings > 0.55		

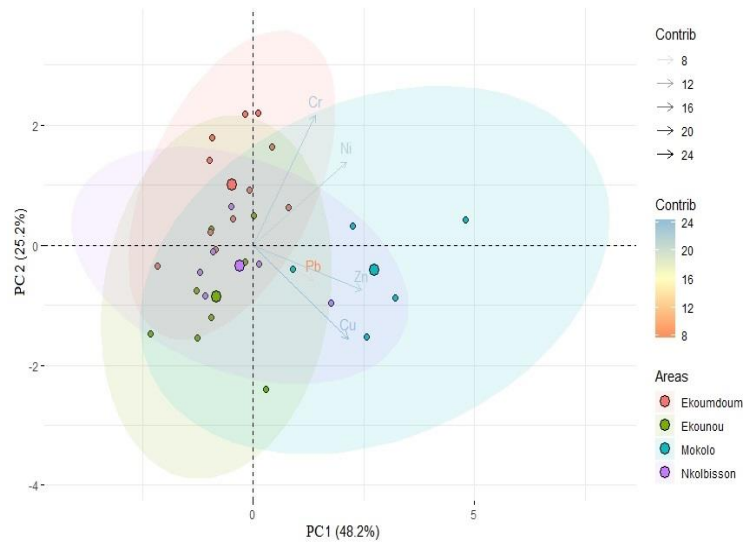


Figure 31 Factor loading plots for PC1 and PC2

The HCA performed using the HCPC method divided soil samples into seven clusters in the dry season. The characteristics of each cluster of the six heavy metals analyzed in the HCA are summarized in Table 33. The lowest rates of Cr and Ni are represented in Cluster I compared to all the other clusters.

Table 33 Description of each cluster by quantitative variables in the soil in the dry season

Variables	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Cr	57.04	111.63	17.67	44.73	0.000
Ni	12.76	21.24	2.28	8.02	0.002
Cluster II					
Cluster III					
Cr	179.76	111.63	12.96	44.73	0.000
Cu	29.76	62.11	7.78	37.97	0.040
Cluster IV					
Zn	226.26	136.57	23.26	76.18	0.013
Cluster V					
Pb	422.21	27.07	0.00	77.85	0.000
Cluster VI					
Cu	155.05	62.10	9.749	37.95	0.000
Zn	371.43	136.57	43.56	76.18	0.000
Cluster VII					
Ni	47.73	21.26	0	8.02	0.000
Zn	371.43	136.57	0	76.18	0.000

Seven main clusters can be distinguished in the dendrogram shown in Figure 32. The high similarities of heavy metal species were found across sites 26, 11, and 27; sites 30 and 22; and sites 23, 9, 25, and 14. The lowest similarity observed in site 29 was interlinked with other study area sites.

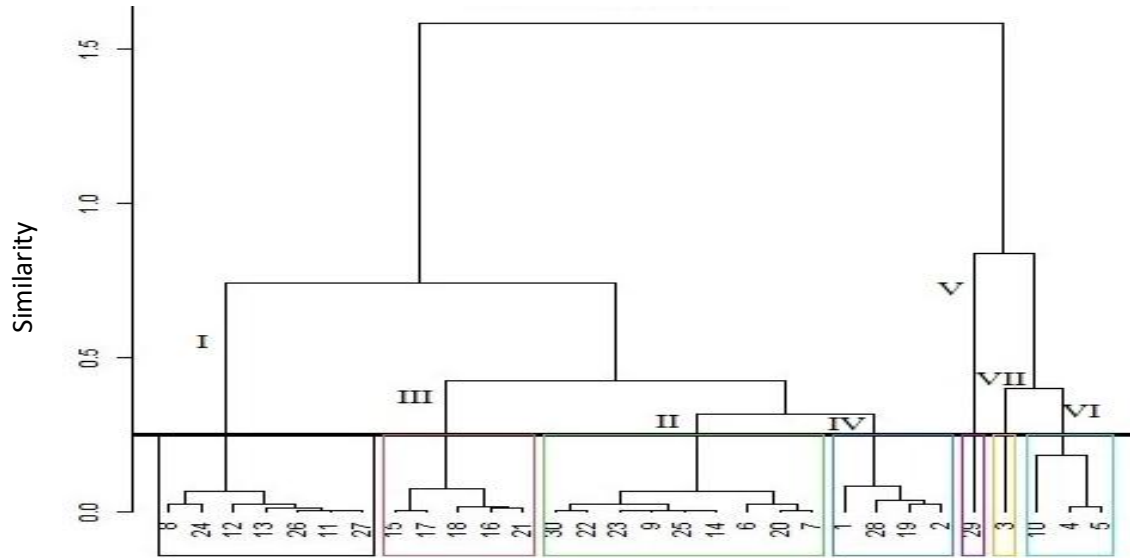


Figure 32 Dendrogram showing cluster of soil sampling points based on analyzed variables.

In contrast, the first two PCs in the wet season accounted for 90.89% of the total variances (Figure 33). The PC1 (52% of the information) with an eigenvalue >2 significantly correlated with Zn, Cu, and Pb. The PC2 (38.17% of the information with an eigenvalue >1) shows strong positive loadings with Cr and Ni (Table 34).

Table 34 Factor loadings of the first two PC and their percentage contribution in the wet season.

Component matrix	Rotated component matrix	
	PC1	PC2
Zn	0.97	-0.05
Cr	0.03	0.98
Cu	0.88	-0.15
Ni	0.21	0.96
Pb	0.93	-0.06
Eigenvalue	2.64	1.91
Variability (%)	52.71	38.17
Cumulative (%)	52.71	90.89
Values in bold with factor loadings > 0.55		

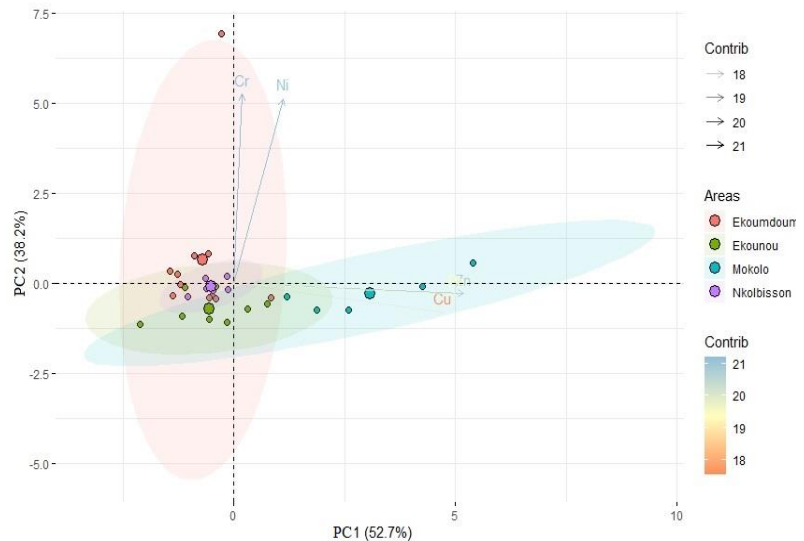


Figure 33: Factor loading plots for PC1 and PC2

The strong positive loadings of Zn and Cu in the PC1 for both dry and wet seasons could come from anthropogenic sources. In addition, PC1 showed high variability (52%). Higher Cu and Zn contents in the sampling sites might have come from various practices and agrochemicals that farmers applied on their farms. For example, the intensive application of mineral (urea, NPK) and organic (animal manures and compost) fertilizers, pesticides, and untreated water used for irrigation could contribute to the enrichment and accumulation of these metals in the soils. Zn and its compounds might originate from manufactured goods such as paints, cosmetics, batteries, automobile tires, and electrical appliances. The primary origin of Pb found in some soils might come from vehicles fume in dense traffic areas and the atmospheric deposition from nearby industrial facilities.

Heavy metals in the PC2, including Cr, could be seen as a natural component due to the low variability of the heavy metals, which seem to be controlled by the parent materials. According to Kabata-Pendias (2011) and Shaheen et al. (2021), Cr has strong lithogenic tendencies and can pass from the parent rocks to agricultural soils. Increased Cr level (above the recommended value) in some cultivated soils can be linked to various polluted sources such as municipal (sewage sludge) and industrial wastes. De Moura et al. (2010) assessed heavy metals in urban agricultural soils using multivariate analysis and found that Cu, Pb, Zn, and Cr were mainly associated with anthropogenic activities while the natural environment controlled the presence of Ni.

The HCA in the wet season shows three main clusters. The characteristics of these main clusters are summarized in Table 35. Similar to the dry season, the lowest rates of all metals, including Cr and Ni, are represented in Cluster I.

Table 35 Description of each cluster by quantitative variables in the soil in the wet season

Wet	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Cr	127.914	148.310	62.337	118.063	0.038
Ni	18.628	28.797	8.673	41.050	0.003
Cu	44.202	51.334	18.478	25.676	0.001
Zn	95.240	117.491	39.488	75.662	0.000
Pb	8.136	18.790	11.811	32.084	0.000
Cluster II					
Ni	236.872	28.797	0.000	41.050	0.000
Cr	699.374	148.310	0.000	118.063	0.000
Cluster III					
Pb	89.867	18.790	31.729	32.084	0.000
Zn	268.064	117.491	83.028	75.662	0.000
Cu	99.993	51.334	7.625	25.676	0.000

These three main clusters can be identified in the dendrogram shown in Figure 34. Unlike the dry season, the lowest similarity was observed in site 22. The seasonal differences observed in the PCA and HCA confirm that the sources of contamination and, consequently, the heavy metal

concentration in the soil samples are variables in the function of the seasons and the sampling sites. These variations were mainly observed for Zn, Cu, and Pb, which are linked to anthropogenic activities. Cr and Ni (mainly from lithogenic backgrounds) proved to be relatively constant.

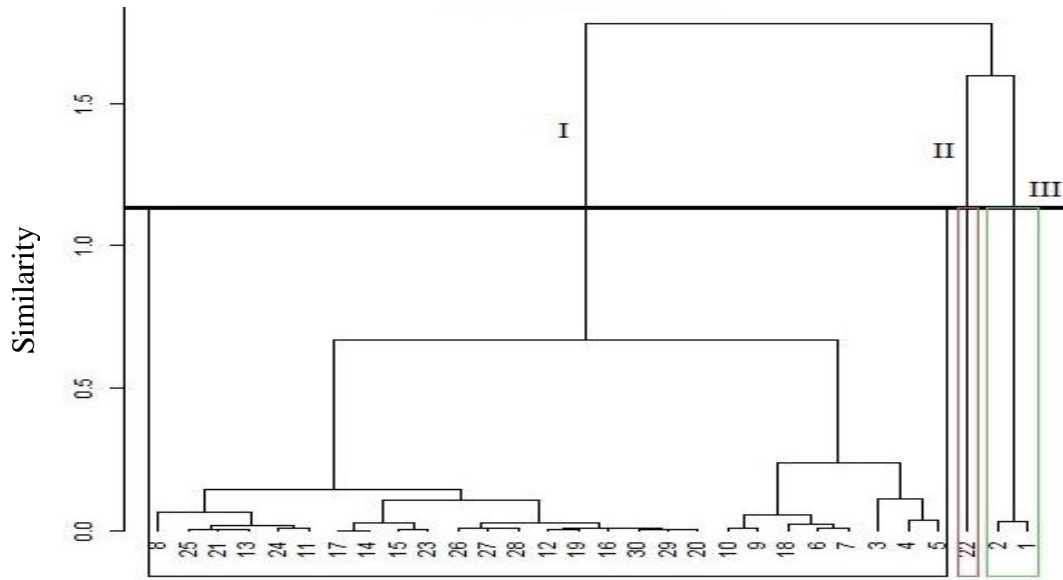


Figure 34 Dendrogram showing cluster of soil sampling points based on analyzed variables

Summary

The main objective of this sub-chapter was to characterize the chemical and heavy metal quality of the agricultural soils in the lowlands of Yaoundé. Except for the soil pH in Nkolbisson that was below the threshold, all the pH values from the other studied sites were within the suitable range for most of them. Like in the irrigation water, the highest heavy metal concentration in the soil was recorded in the dry season, indicating that agricultural runoff might leach heavy metals in soils that ended up in the water sources. The composition of soils revealed that Cr concentrations exceeded the permissible limits while the Ni concentration was below that level in all studied areas in both seasons. Except for Mokolo, Cu, Zn, and Pb were below the thresholds limits for agricultural soils. The variability of these metals was classified as moderate to high, suggesting their anthropogenic sources like fertilization, pesticides, wastewater, and other human activities. All studied metals exhibited the area effects in their distribution, while the seasonal effect was noticeable only for Cu. The levels of heavy metals in the agricultural soil were much higher than those recorded in the irrigation water, suggesting that besides irrigation waters, other sources might add heavy metals to the agricultural soils.

The absence of Cd in the soil samples contrasted with their presence in traces in the irrigation water samples. Presumably, Cd from irrigation water was not accumulated in the soil, and from the soil, it was absorbed or accumulated in the plants. To investigate this in more detail, the following part of this study assessed the concentration of Cd, among other heavy metals, in *S. nigrum* plants grown in these soils.

4.4. Heavy metals in the vegetable crops (*S. nigrum*)

The average concentration of heavy metals in the edible parts of *S. nigrum* during the dry and wet season is shown in Figure 36 and summarized in Table 38. The concentration of heavy metals in plants varied within the same area and between the areas. Similar to the concentration of heavy metals found in soils, the highest concentration of Cu, Cd, Pb, Cr, Ni, Zn was recorded in *S. nigrum* samples from the Mokolo area, with higher concentrations during the dry compared to the wet season.

Table 36 Average concentration of heavy metals (mg/kg) in *Solanum nigrum* (mean \pm SD).

Areas	Season	Cu	Cd	Pb	Ni	Cr	Zn
Mokolo	Dry	22.2 \pm 6.8	1.7 \pm 0.3	1.8 \pm 0.8	3.2 \pm 1.2	2.6 \pm 1.1	68.7 \pm 17.4
	Wet	22.1 \pm 6.3	1.5 \pm 0.1	0.7 \pm 0.2	1.1 \pm 0.6	1.8 \pm 0.2	65.4 \pm 27.2
Ekounou	Dry	13.2 \pm 2.7	1.0 \pm 0.1	0.2 \pm 0.1	1.0 \pm 0.4	1.8 \pm 0.4	55.7 \pm 12.9
	Wet	7.9 \pm 1.8	1.6 \pm 0.3	0.03 \pm 0.1	2.6 \pm 1.8	1.7 \pm 0.3	48.2 \pm 11.8
Ekoumdoum	Dry	16.1 \pm 3.4	1.3 \pm 0.2	0.7 \pm 0.2	2.2 \pm 0.8	1.4 \pm 0.9	62.3 \pm 17.9
	Wet	9.7 \pm 3.9	1.4 \pm 0.1	0.1 \pm 0.1	1.8 \pm 1.1	1.6 \pm 0.7	56.7 \pm 20.7
Nkolbisson	Dry	19.8 \pm 5.2	0.9 \pm 0.1	0.2 \pm 0.2	1.4 \pm 0.6	1.8 \pm 0.3	63.9 \pm 19.5
	Wet	5.2 \pm 2.3	0.8 \pm 0.1	–	0.1 \pm 0.2	1.2 \pm 0.2	25.3 \pm 7.6
Treshold values		10	0.2	0.3	1	1	50

The trend of heavy metal concentrations in the studied areas was classified as Mokolo>Ekoumdoum>Ekounou>Nkolbisson. The high concentration of heavy metals in *S. nigrum* plants from Mokolo is probably associated with the high heavy metal concentrations found in the soil as growing plants bioaccumulate heavy metals in their tissue. The highest concentration of metals obtained in Mokolo reinforces the assumption that fertilization is one of the main sources of heavy metals in soils and plants.

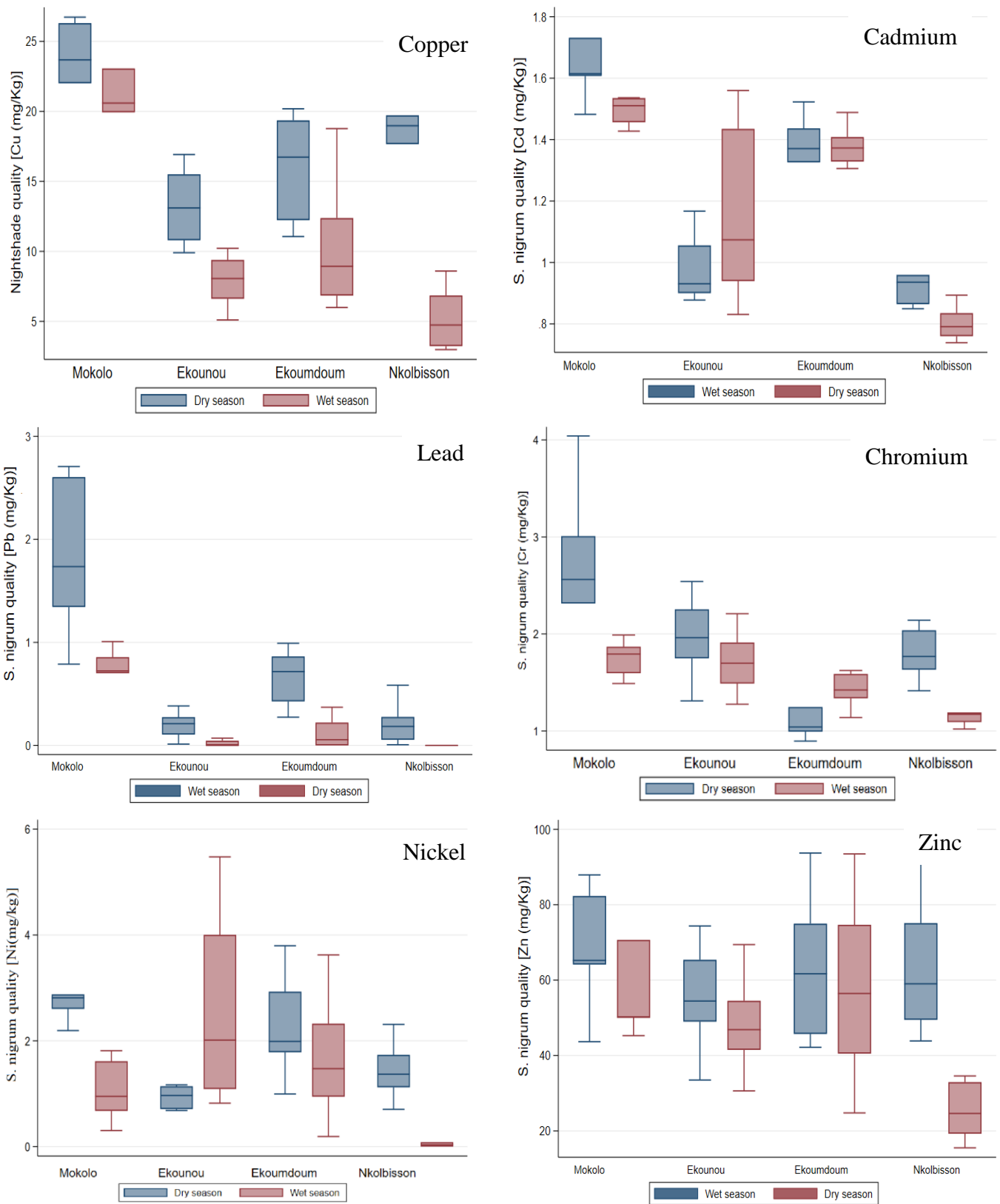


Figure 35 The concentration of heavy metals in *Solanum nigrum* in the dry and wet seasons.

The highest amount of fertilizer applied in the Mokolo farms might explain the highest concentration of elements recorded in the plant collected in that area. Besides, higher concentrations of all six tested heavy metals were found in *S. nigrum* plants during the dry season, similar to the analyses of water and soil samples, suggesting a strong dilution effect of heavy metal concentrations during the wet season. The following consideration can explain this low concentration of heavy metal: rainfall occurs in the wet season, which dilutes the irrigation water, making the heavy metal concentrations lower; the lower amount of heavy metals are absorbed in soil particles due to the diluted irrigation water; and the diluted irrigation water contributes to the lower heavy metal concentrations in vegetables in the wet season (Ahmed et al., 2019).

In addition, during the dry season, open dumping and burning of solid wastes are common near the area. The farmland being too close to the refuse dump, ash and atmospheric particulates loaded with heavy metals might be readily deposited on the vegetables' leaves and later translocated into the plants' system through foliar absorption. This process of foliar absorption is more pronounced in the dry season due to the persistence of these airborne ash and atmospheric particulates on the plants' leaves and the absence of the washing of the leaves by rainfall, contributing to increasing heavy metal concentrations in crops during the dry season. Furthermore, soil water evaporation and evapotranspiration could increase the leaves' heavy metals concentration.

The studied heavy metals ranked in the following order, $Zn > Cu > Ni > Cr > Cd > Pb$ in both dry and wet seasons. Like for the water and soil samples, Zn had the highest concentration in the vegetable samples, suggesting that *S. nigrum* has a higher accumulation capacity for essential metals such as Zn and Cu than for toxic ones like Ni, Cr, Cd, and Pb. Onakpa et al. (2018a) reported that the general transfer factors (i.e., the potentiality of heavy metals to be absorbed by plants) for vegetables are in the order of $Cd > Zn > Hg > Pb$. The authors explained that this classification is due to the higher mobility of Cd occurring naturally in soil and the low retention of Cd in the soil than other toxic cations. However, the rankings were similar for the water, soil, and plant samples, with high Zn and low Cd contamination. Besides, the plant samples showed higher heavy metals contamination levels than soil and irrigation water samples water. These findings suggest that plants take up heavy metals from both the soil and irrigation water, causing higher concentrations (Balkhair & Ashraf, 2016; Gupta et al., 2010). In addition, atmospheric deposition (dust) can also add heavy metals to plants (Luo et al., 2011). Furthermore, some factors such as the soil properties, total metal content, and the chemical form of the elements might be influencing the distribution of heavy metals in the soil and plants. Many authors demonstrated that soil chemical and physical properties affect the bioavailability and movement of heavy metals to be taken up by the plants. Similarly, toxic metal properties, precipitation reactions, soils characteristics, adsorption-desorption, and environmental factors, plus heavy metal concentration, may condition their bioavailability for plants (Li et al., 2003). Meite et al. (2018) showed that the mobility of heavy metals can be affected by severe rainfall events. The high heavy metal concentration found in *S. nigrum* in this study is also due to the plant's high bioconcentration factor. According to Yu et al.

(2015), *S. nigrum* can uptake Cu, Zn, and Pb from contaminated soil besides its capacity to accumulate Cd. Therefore, a high concentration of these elements could be expected in this species.

Except for Cr, the interactions between sites and seasons were significantly different for all the metals (Table 37).

Table 37 Contrasts of marginal linear predictions from robust mixed-effect linear regression.

Variables	Cu		Cd		Pb		Ni		Zn		Cr		
	df	chi2	P>chi2	chi2	P>chi2	chi2	P>chi2	chi5	P>chi2	chi2	P>chi2	chi2	P>chi2
Areas	3	30.0	0.000	293.1	0.000	71.82	0.000	56.4	0.000	9.9	0.019	11.33	0.010
Season	1	30.1	0.000	0.6	0.440	37.39	0.000	7.1	0.008	10.9	0.001	6.09	0.014
Areas#season	3	14.8	0.002	15.4	0.002	30.27	0.000	31.7	0.000	16.5	0.001	7.1	0.069

Note: Degrees of freedom (df) is the number of independent variables. Number in bold: statistically significant difference at $p < 0.05$.

This suggests that only Cr has overall significant areas and seasonal differences. The specific seasonal and areas variations are summarized in Appendix 6.

In general, almost all the heavy metal concentrations found in *S. nigrum* exceeded the FAO/WHO acceptable limits. The accumulation and excessive use of agrochemical inputs can explain the concentration of heavy metals exceeding the permissible limits. According to many authors, agricultural activities, such as pesticide applications, polluted irrigation water, municipal waste used for fertilization, and even mineral fertilizer containing traces of heavy metals, increased the background concentration of heavy metals and thus their presence in the environmental media (Akhtar et al., 2021; Chaoua et al., 2018). Agenin (2002) demonstrated that manure application significantly increases the Zn concentration in agricultural soils, which plants are then efficiently taking up. According to Rehman et al. (2017), heavy metal concentration in the soil medium is the prime factor conditioning their uptake by plants.

Leafy vegetables can accumulate a high concentration of heavy metals from atmospheric deposition, especially in smelting areas (Zhou et al., 2016). Thus, the elevated values of heavy metal recorded in the *S. nigrum* might be related to contaminated soils, untreated irrigation water, fertilization, and pesticides applications. The observed high concentration of heavy metals in edible parts of *S. nigrum* irrigated with untreated or wastewater in this study corroborates results from earlier investigations with leafy vegetables (Chaoua et al., 2019; Edogbo et al., 2020), though concentrations of Cu, Zn, and Cr in Nkolbisson reported here were higher than those by Aboubakar et al. (2021). These authors reported concentrations of 9.3, 44.8, 1.0 mg/kg respectively for Cu, Zn, and Cr in the same area during the dry season. Similarly, they also reported Ni (9.3mg/kg) and Cd (1.4 mg/kg) concentration in *S. nigrum* higher than the permissible limits but lower than the values obtained in this study. Akenga et al. (2020) also found a high concentration of Cd and Pb above the allowable limit in *S. nigrum* cultivated in urban irrigated soils in Kenya. Although Ni, Cu, and Zn are micronutrients classified as essential elements for plants at low concentrations (Ali et al., 2019), they may negatively affect human health at a concentration above the standard. In addition, the presence of Cd, Cr, and Pb, which are classified as toxic and have no known biological roles, is at risk for humans when they enter the food chain and pose many health problems, including cancers (Järup, 2003; WHO, 2007; Solomon, 2008; Steenland et al., 2017,

2019). Previous studies demonstrated that plants grown on soils irrigated with untreated or wastewater are generally contaminated with heavy metals, thus posing a major health concern (Ahmed et al., 2019; Sultana et al., 2019).

4.4.1. Multivariate analysis

Principal component and cluster analysis

The factor loading for the first two PCs and their percentage of contribution to the total variance for heavy metals contents in *S. nigrum* during the dry is reported in Table 38 and Figure 36. In this model, the heavy metals in the plant samples can be aggregated in the first two PCs and explained 72.35% of the total variance. PC1 (52.76 %) with an eigenvalue >3 had high positive loadings for Cu, Cd, Pb, Ni, and Zn during dry seasons, while PC2 (19.58%) with an eigenvalue >1 had a high favorable loading for Cr only. These results indicate that samples of *S. nigrum* located on the right side of PC1 contained high concentrations of Cu, Cd, Ni, Pb, and Zn. Accordingly, those found on the positive side of PC2 had high Cr. The positive correlation of the first component with Cu, Zn, and Ni and the strong correlation of Cr with the second component is similar to the result obtained in the soil samples. This finding suggests that the presence of these metals in the plant's samples may originate from the soil on which they are grown.

Table 38 Factor loadings of the first two PC and their percentage contribution in the dry season.

Component matrix	Rotated component matrix	
	PC1	PC2
Cu	0.71	0.29
Cd	0.88	0.33
Pb	0.83	0.17
Cr	0.26	0.91
Ni	0.88	-0.36
Zn	0.60	0.05
Eigenvalue	3.17	1.18
Variability (%)	52.76	19.58
Cumulative (%)	52.76	72.35
Values in bold with factor loadings > 0.55		

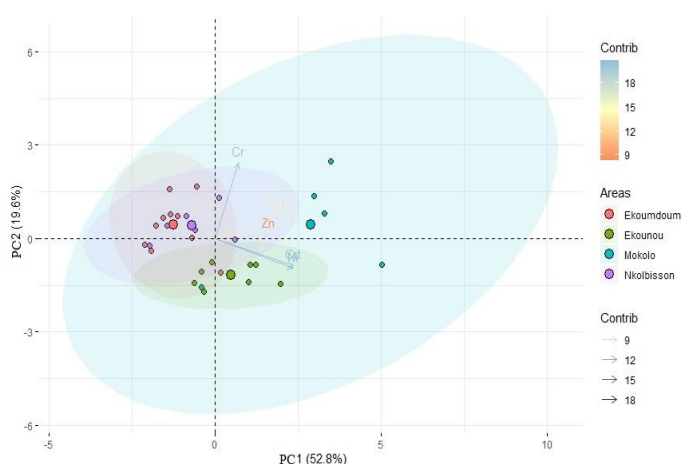


Figure 36 Factor loading plots for PC1 and PC2

The HCA performed using the HCPC method divided plant samples into three clusters. The characteristics of each cluster for the six analyzed heavy metals are described in Table 39. The lowest rate of Cr and Ni is represented in Cluster I compared to all the other clusters.

Table 39 Description of each cluster by quantitative variables in the soil in the dry season

Dry	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Pb	0.219	0.652	0.157	0.668	0.000
Ni	1.128	1.887	0.359	1.013	0.000
Cd	0.953	1.223	0.082	0.325	0.000
Cluster II					
Cd	1.382	1.223	0.101	0.325	0.045
Ni	2.377	1.887	0.599	1.013	0.048
Cr	1.111	1.843	0.232	0.789	0.000
Cluster III					
Pb	2.098	0.652	0.576	0.668	0.000
Cd	1.798	1.223	0.258	0.325	0.000
Cu	24.683	17.013	1.935	5.077	0.001
Ni	3.385	1.887	1.079	1.013	0.002
Cr	2.981	1.843	0.661	0.789	0.002

Three main clusters can be identified in the dendrogram shown in Figure 37. The heavy metals showing higher and constant similarities were in sites 24 and 22, sites 17 and 16, and sites 1 and 9. The lower similarity observed in sites 5 and 26 was interlinked with other sites of the study areas.

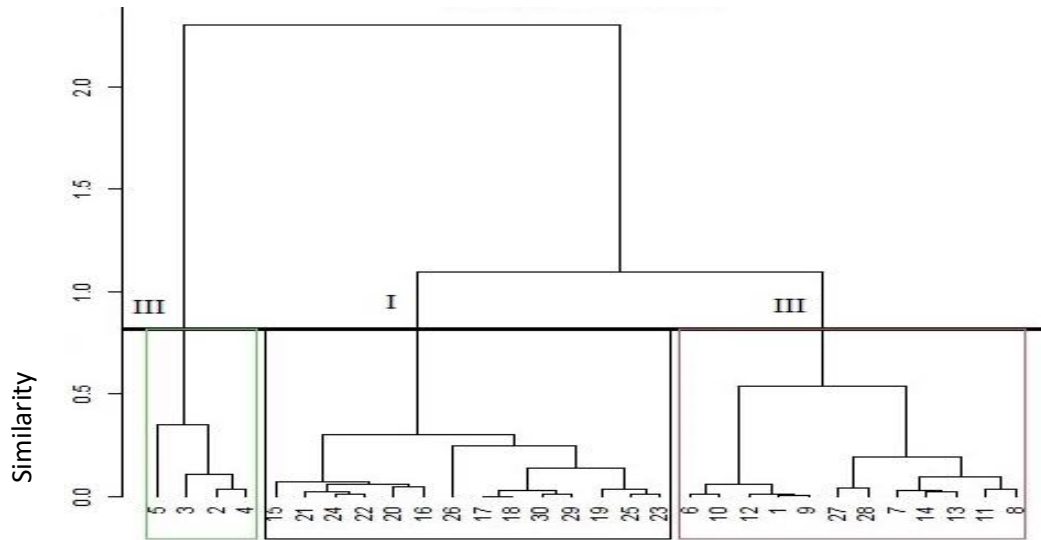


Figure 37 Dendrogram showing cluster of soil sampling points based on analyzed variables

PC1 accounted for 45.42% of the variance in the wet season with an eigenvalue >2. This PC1 has a positive factor loading of Cu, Cd, Pb, and Zn (Table 40 and Figure 38). In contrast, PC2 with 20.6% of the total variance explained is positively associated with Ni and Cr. The positive correlations of Cu, Cd, Pb, and Zn with the first component suggest that these metals originate from the same sources of contamination.

Table 40 Factor loadings of heavy metal in *Solanum nigrum* in the wet seasons

Component matrix	Rotated component matrix	
	PC1	PC2
Cu	0.93	0.03
Cd	0.75	0.14
Pb	0.88	0.24
Cr	0.27	0.71
Ni	-0.08	0.81
Zn	0.68	0.004
Eigenvalue	2.72	1.24
Variability (%)	45.42	20.60
Cumulative (%)	45.42	66.03
Values in bold with factor loadings > 0.55		

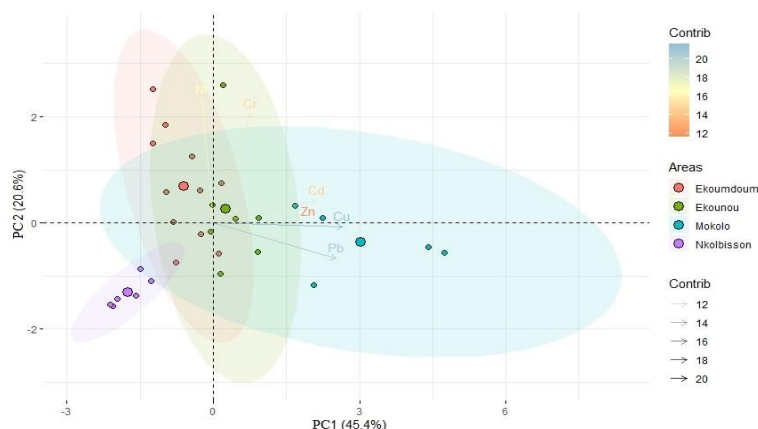


Figure 38 Factor loading plots for PC1 and PC2

The association of these heavy metals in the plant samples was similar to those observed in the soil samples. Similar heavy metal characteristics of the agricultural soils accounted for the similarity in their concentrations in the plant samples. The description of each cluster by quantitative variables in the wet season is shown in Table 41.

Table 41 Description of each cluster by quantitative variables in plants in the wet season

Wet	Mean in category	SD	Overall Mean	SD	p-value
Cluster I					
Cr	1.200	1.582	0.170	0.475	0.031
Cu	5.189	10.400	2.078	6.482	0.030
Ni	0.107	1.526	0.176	1.377	0.006
Cd	0.802	1.225	0.051	0.279	0.000
Cluster II					
Ni	3.879	1.526	1.084	1.377	0.000
Cluster III					
Cd	1.387	1.225	0.098	0.279	0.006
Cluster IV					
Cr	3.628	1.582	0.000	0.475	0.000
Cluster V					
Cu	0.747	0.172	0.225	0.281	0.000
Pb	21.885	10.400	6.310	6.482	0.000
Cluster VI					
Zn	426.670	61.477	0.000	71.037	0.000

The highest level of Ni and Cr are represented in Clusters II and IV, respectively, suggesting that these clusters are more likely associated with the PC2. In contrast, clusters III, V, and VI seem to represent more PC1 due to their high Cd, Pb, and Zn concentrations. Contrary to

the dry season, six main clusters were identified in the wet season (Figure 39). The heavy metals showing higher and constant similarities were in sites 29 and 30, 17 and 10, and 28 and 25. The lowest similarity was observed in sites 1 and 7 and was interlinked with other sites of the study areas.

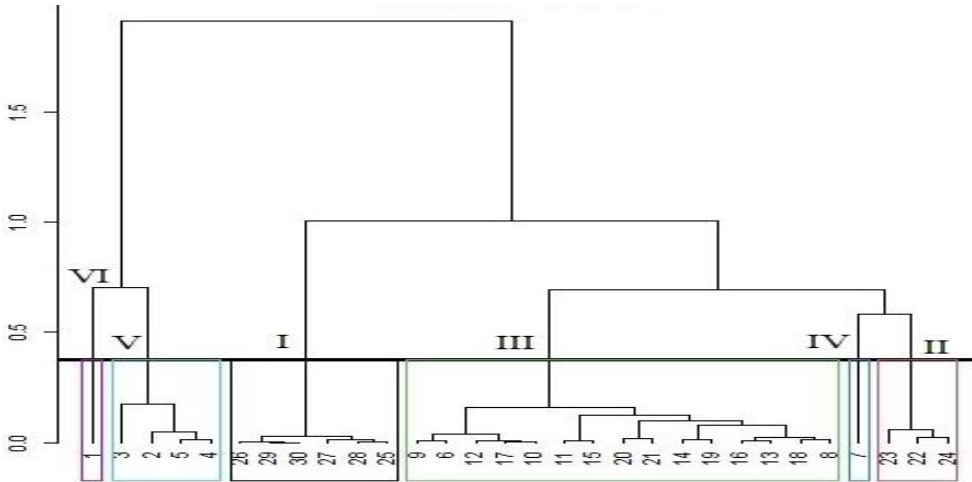


Figure 39 Dendrogram showing clusters of vegetable sampling points based on analyzed variables during the wet season.

4.4.1. Correlation analysis

Pearson's correlation analysis was used to estimate the inter-metal relationship in the systems between water and plant samples, soil and plant samples, and each sample type. These inter-metal associations help to understand whether the heavy metals in the plants' samples originated from the irrigation water or the soil in which they are cultivated (Mishra et al., 2018).

4.2.4.1. Water-plant systems

Figure 40 shows Pearson's correlation coefficient calculated from the observed elemental concentrations in the collected water (e) and plant (n) samples in the dry season. Significant negative relationships were found between Cr in the irrigation water (C_{re}) and Ni, Cd, Cu, and Pb in *S. nigrum* samples (Ni_n , Cd_n , Cu_n , and Pb_n). This implies that the contents of these heavy metals have not been supplied from the same sources of contamination.

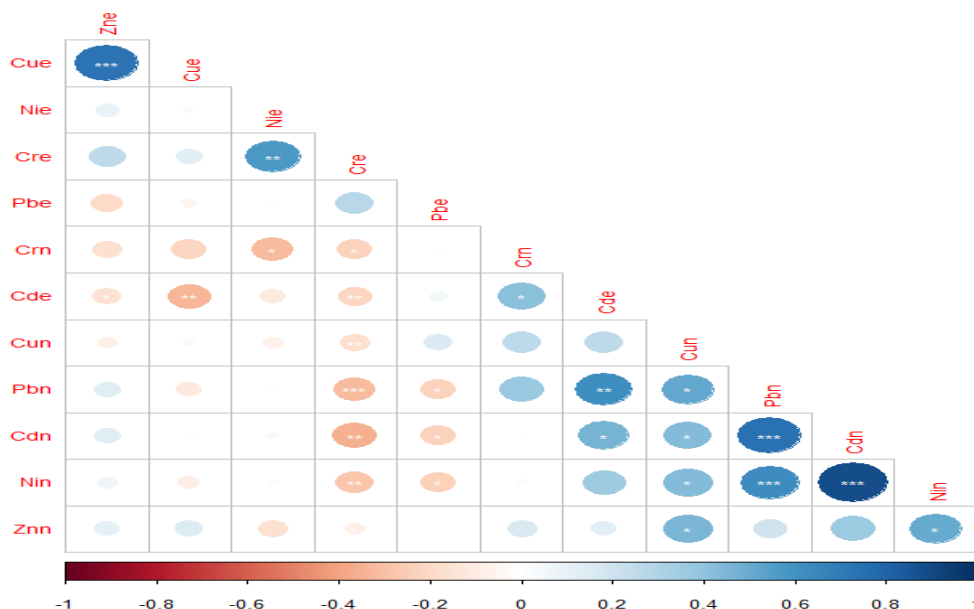


Figure 40 Correlation matrix showing the relationship between the metals in water and plants in the dry. (Blue: positive correlations; red: negative correlations. Colour intensity and the circle size are proportional to the correlation coefficients. Legend color shows the correlation coefficients and the corresponding colors; significance level: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$; water (e) and plant (n)).

Similarly, Cr in water samples (Cr_e) significantly negatively correlated with Cr, Pb, Cd, and Cu in vegetable samples during the wet season (Figure 41). This result suggests that Cr in the plant did not originate from the irrigation water. The same holds true for Ni in water (Ni_e) and Cr in plant (Cr_n) samples. On the contrary, significantly positive correlations were found between Cd in water (Cd_e) and Zn in plants (Zn_n) and Pb in water (Pb_e) and Cu in the plant (Pb_e). The significant positive relationship between these metal groups indicates some common sources of origin (Gupta et al., 2018; Shaheen et al., 2021).

Significant positive correlations were found between Cd_e and Zn_n as well as Pb_e and Cu_n , while the rest of the metals had significant negative associations. There was no significant correlation between an element in the water sample and the same element in the plant sample, indicating that the studied heavy metals in *S. nigrum* were unlikely to have originated from the irrigation water. These results corroborate similar findings of Zhang et al. (2010), who found no correlation between heavy metal concentrations in the water and plants. The authors concluded that the poor correlations for metal concentrations between plants and water implied that other factors such as air pollution, including traffic emissions and the wastes discharged from the nearby industry, affected the accumulation of heavy metals in the plants.

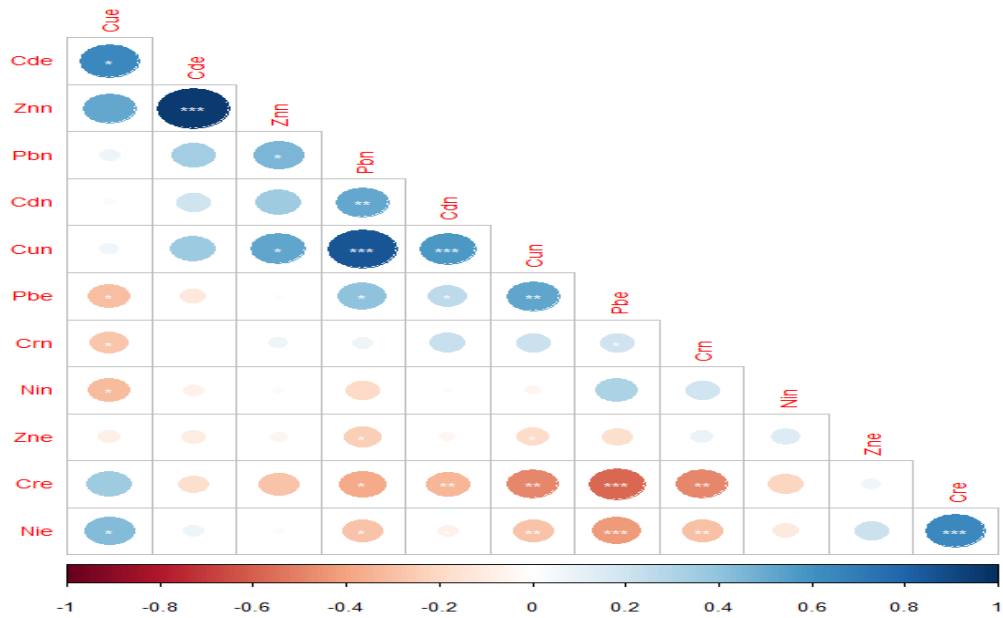


Figure 41 Correlation matrix showing the relationship between the metals in water and plant during the wet season. (Blue: positive correlations; red: negative correlations. Colour intensity and the circle size are proportional to the correlation coefficients. Legend color shows the correlation coefficients and the corresponding colors; significance level: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$; water (e) and plant (n)).

4.2.4.1. Soil-plant systems

Figure 42 shows significant positive correlations between Zn in the soil (Zn_s) and Ni, Cd, and Pb in the plant samples (Ni_n , Cd_n , Pb_n) during the dry season. Cu in the soil sample (Cu_s) positively correlated with Ni, Cd, Pb, and Cu in those from the plants (Ni_n , Cd_n , Pb_n , and Cu_n).

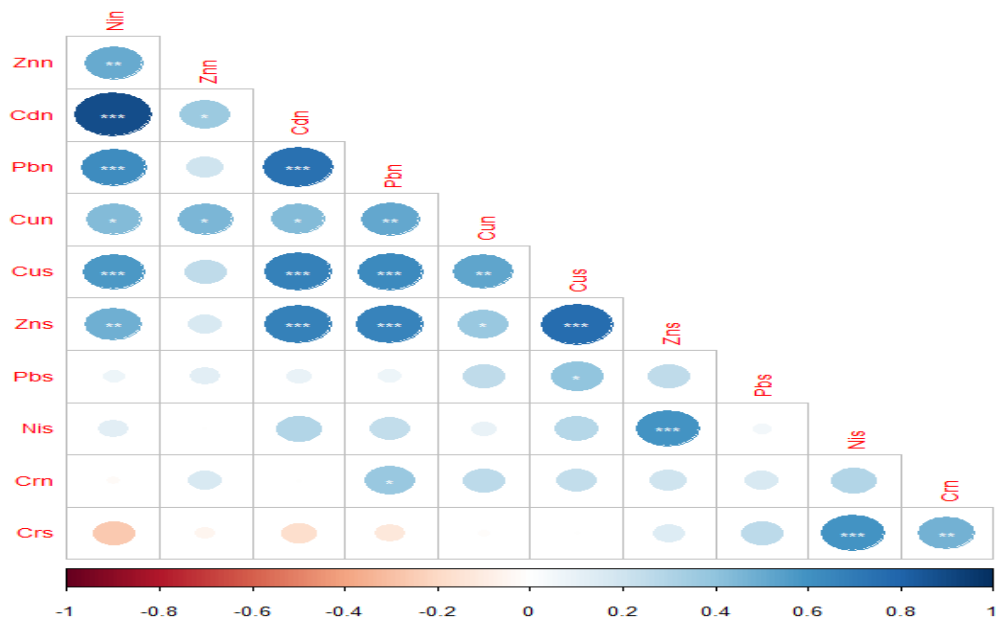


Figure 42 Correlation matrix showing the relationship between the metals in soil and plants during

the dry. (Blue: positive correlations; red: negative correlations. Colour intensity and the circle size are proportional to the correlation coefficients. Legend color shows the correlation coefficients and the corresponding colors; significance level: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$. soil (s) and plant (n)).

Similarly, strong significant and positive associations were found between Pb in plants and Zn, Pb, Cu in soil samples (Figure 43). Cu in plant samples correlated significantly with Zn and Pb in soil samples, while Zn in plant samples (Zn_n) was associated with Pb and Zn in soil samples (Pb_s and Zn_s).

Overall, most of the studied six heavy metals in *S. nigrum* samples were positively correlated with those from the soil in which the plants were cultivated. These results suggest that increasing heavy metal contents of soils might increase the mobility and plant uptake of these metals. The strong correlations between samples from the soil and *S. nigrum* indicate that metals accumulated in plants might have predominantly originated from the farm soils. The strong dependence of two elements may be an occurrence of the strong dependence of both variables on the same causal factor, probably due to their common derivation from the geogenic basement (Osobamiro & Adewuyi, 2015). Strong correlations between Cu, Zn, and Pb in soil and plants were also reported by Hołtra and Zamorska-Wojdyła (2020).

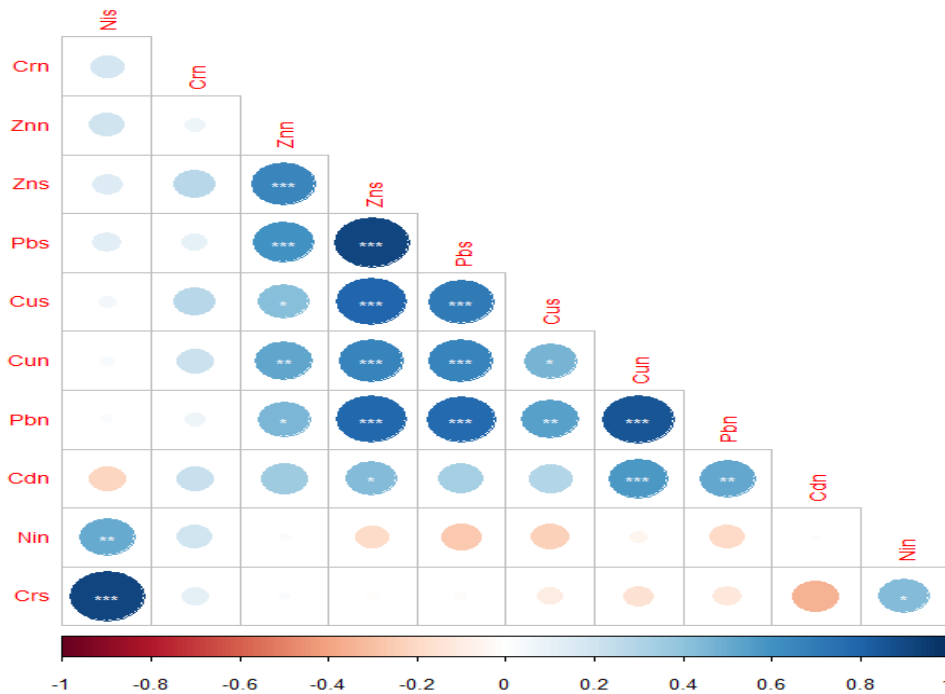


Figure 43 Correlation matrix showing the relationship between the metals in soil and plant during the wet season. (Blue: positive correlations; red: negative correlations. Colour intensity and the circle size are proportional to the correlation coefficients. Legend color shows the correlation coefficients and the corresponding colors; significance level: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$; soil (s) and plant (n)).

Despite their variabilities, a significant correlation was recorded between the two-component systems. However, the strength of the relationships between metals in the two media (soil and plants) was higher in the dry season than in the wet season, which was expected given

that the metals' concentrations also increased during the dry compared to the wet season. The lower association of heavy metals with plant uptake in the wet season is probably caused by the rain when it precipitated the run-off of metal elements from the soil to the water and the dilution of the metals.

Summary

This study was conducted to assess the heavy metal contamination in *S. nigrum* plants grown in lowlands areas in Yaoundé during the dry and wet seasons. Similar to the irrigation water and soil, a higher concentration of heavy metals was recorded in the plants during the dry season, confirming the dilution effect previously mentioned. Like for the agricultural soils, higher heavy metals contamination was found in the Mokolo area, suggesting that the higher heavy metal pollution in the soil then led to a high concentration in the *S. nigrum* plants via bioaccumulation processes. Heavy metals were ranked in the order of Zn > Cu > Ni > Cr > Cd > Pb in both dry and wet seasons. As with the water and soil samples, contamination with Zn was highest in the plants, with concentrations in plant samples higher than those found in the soil. Moreover, heavy metals concentrations in plant and soil samples were higher than in the irrigation water, suggesting that heavy metals in the plant might originate from irrigation water and soil and possibly atmospheric deposition. Most of the studied heavy metals exceeded the FAO/WHO thresholds throughout the study areas. Therefore, the edible parts of *S. nigrum* harvested in these lowlands may be unsafe for human consumption. The positive correlations observed between heavy metals in vegetable plants indicated contamination from the same sources.

The significant association between the heavy metals in the soil and plant analyses calls for more investigations regarding the degree of plant contamination. Thus, as this study's third objective, the following part investigates and classified the degree of crop contamination by heavy metals (crop pollution index) and identifies heavy metals that can easily be taken up by the plants (Bioaccumulation factor).

4.5. Contamination level of *Solanum nigrum*

4.5.1. Crops pollution index (Pi)

The crops pollution index (Pi) was used to explore the degree of contamination of heavy metals in crops and includes some elements of descriptive statistics like the mean, median, standard deviation (SD), Min, Max, and coefficient of variance (CV) (Appendix 7 and 8). It was calculated by dividing its concentrations in the plant samples by its standard value (cf. Equation 1). In the dry season the Pi values in descending order were the following, Cd (6.37), >Pb (2.58), >Ni (2.05), >Cr (1.89), >Cu (1.79), and >Zn (1.26) and in the wet season, Cd (6.12), > Cr (1.46), >Zn (1.35), > Ni (1.31), > Cu (1.14), and > Pb (0.76).

Based on the single crop pollution index classification, heavy metals with $Pi \leq 1$ was classify as safe (class I); $1 < Pi \leq 2$ slightly pollution (class II); $2 < Pi \leq 3$ mild pollution (class III); $3 < Pi < 5$ moderate pollution (class IV) and $Pi > 5$ strong pollution (class V) (cf. Table 12). The results show that Cd was the strongest polluting heavy metal (class V) in *S. nigrum* both in the dry and wet seasons. This suggests that the plant can easily accumulate the heavy metal in its leaves. In their studies in India and Pakistan, respectively, high Cd accumulation in *S. nigrum* leaves has also been reported by Dwivedi et al. (2014) and Khan et al. (2013). Pb in the samples from Ekoumdoum and Ekounou collected in both seasons was classified as safe (class I).

The degrees of Cu, Zn, Cr, Ni, Cd, and Pb pollution in *S. nigrum* leaves are shown in Figure 44. The Pi of Cu, Cr, Cd, Ni, and Pb in *S. nigrum* from Mokolo varied from strong to moderately polluted, suggesting that those vegetables may not be safe for human consumption.

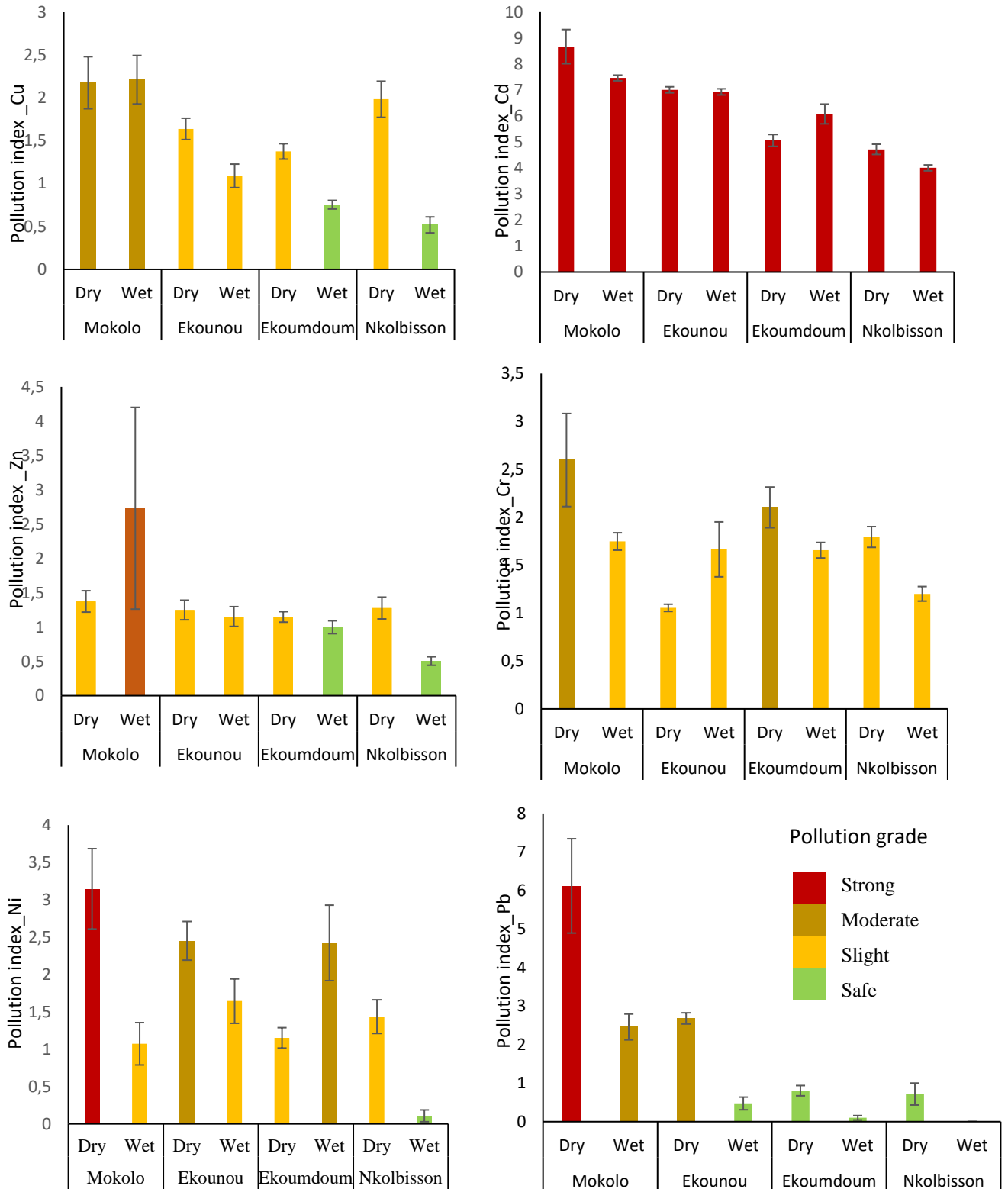


Figure 44 Crop pollution indexes (Pi) of heavy metals in *Solanum nigrum* from Yaoundé lowlands (mean \pm SE). (with $Pi \leq 1$ =safe; $1 < Pi \leq 2$ =slightly pollution; $2 < Pi \leq 5$ moderate pollutions and $Pi > 5$ strong pollutions).

The composite indexes (Ps) is the sum of the Pi of a single heavy metal in *S. nigrum* from the four studied lowlands in the dry and wet seasons are reported in Figure 45. Ps is calculated by summarizing the Pi of heavy metals and classified similarly to the Pi of single metals: $Ps \leq 1$ =safe; $1 < Ps \leq 2$ = slight pollution; $2 < Ps \leq 5$ moderate pollutions and $Ps > 5$ strong pollutions. Like for the Pi, the *S. nigrum* samples from Mokolo had the highest pollution Ps grade, classified as strong to moderate, while those from Nkolbisson had the lowest (classified as slight to safe).

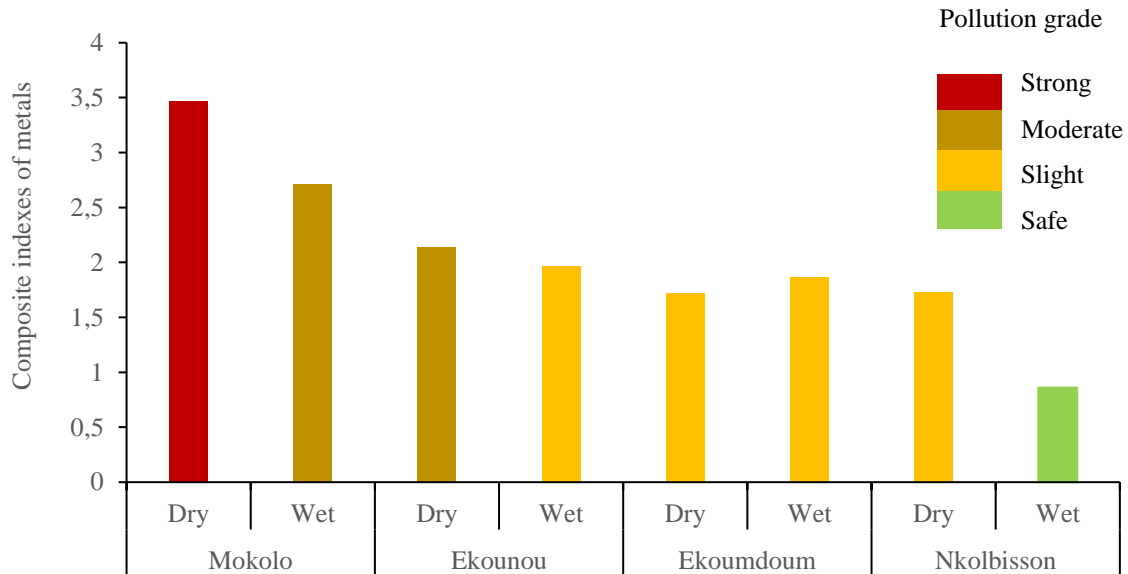


Figure 45 Composite indexes (Ps) of heavy metals pollution in *Solanum nigrum* from four Yaoundé lowlands; $Ps \leq 1$ =safe; $1 < Ps \leq 2$ = slightly pollution; $2 < Ps \leq 5$ moderate pollutions and $Ps > 5$ strong pollutions.

4.5.2. Bioaccumulation factor (BAF)

The bioaccumulation factor (BAF) was used to show the magnitude of heavy metal accumulation in *S. nigrum* plants relative to that in the environment, i.e., the soil they had been grown in. BAF was used to assess the transfer of heavy metals from soil to plant, and this factor can be obtained by dividing the concentrations of heavy metals in the plants by the corresponding soils samples (cf. Equation 2). The BAF varies between areas depending on the heavy metal concentrations and can be graded as $BAF < 1$ plant are excluders; $BAF > 1$ plant are accumulators, and $BAF > 10$ plants are hyperaccumulators for this metal. Figure 46 shows the BAF of heavy metals during the dry season. The average BAF of heavy metals throughout the study areas in descending order are Zn (0.53), > Cu (0.32), > Pb (0.21), > Ni (0.10), and > Cr (0.02). The highest BAF value of 0.72 for Zn was found in *S. nigrum* samples from Ekoumdoum during the dry season.

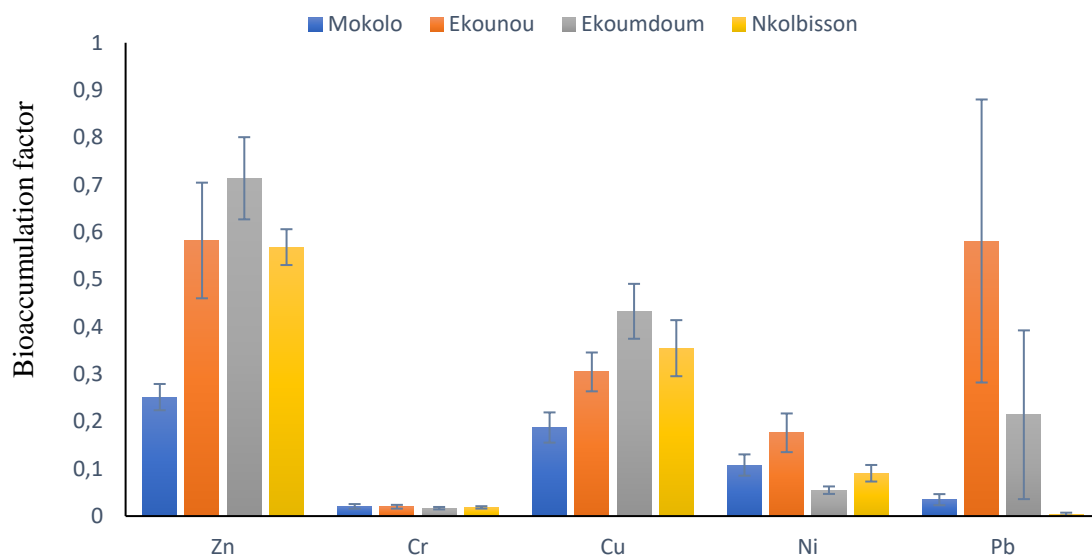


Figure 46 Bioaccumulation values of heavy metals (mean \pm SE) in *Solanum nigrum* during dry.

In the wet season, the average BAF values were Zn (0.67), > Cu (0.26), > Ni (0.08), > Pb (0.01), and > Cr (0.01). Like the dry season, *S. nigrum* samples from Ekounou had the highest BAF value of 1.33 for Zn (Figure 47).

During both seasons, Zn and Cu were easier transferred from the soil to the edible parts of *S. nigrum* than Cr. The high BAFs for Zn and Cu indicates their high absorbability by *S. nigrum*, suggesting a high absorption capacity for these metals in different parts of *S. nigrum*. High BAF values indicate low retention of heavy metals in the soil and a high absorption capability of plants. In contrast, low BAF shows high retention of heavy metals in the soil and a low absorption capability of the plants (Akenga et al., 2020). According to Luo et al. (2012), heavy metals with high BAFs can be easier absorbed from soils by vegetables than those with low BAFs.

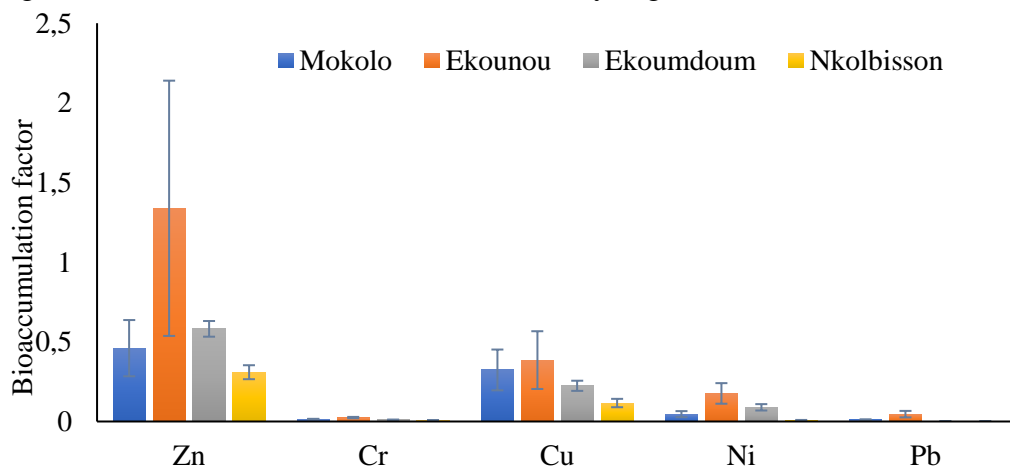


Figure 47 Bioaccumulation factor of heavy metals in *Solanum nigrum* (mean \pm SE) during the wet seasons.

The metabolism in *S. nigrum* can possibly explain the high BAF value for Zn and the high Pi for Cd. Zn is easily absorbed by the plants from the soil but not accumulated in the plants' tissues, while Cd has the highest accumulation capacity. Since essential elements for the plant's physiology and growth, such as Zn and Cu, are more easily absorbed (Rieuwerts et al., 2015), the higher transfer capacities of Zn and Cu can be explained by the nutritional needs of the plants (Ogunkunle et al., 2013). However, different heavy metals have different mobility and bio-availability. According to Mishra & Pandey (2019), heavy metal transfer and accumulation vary between plant and metal species. Depending on the plants' physiology, various plant species have been shown to uptake heavy metals differently (Hu et al., 2017; Onakpa et al., 2018a).

The BAF values of the studied metals were higher during the dry than wet season, suggesting that the heavy metal uptake was more accessible from soils during the former than the latter season. The low absorption of heavy metals during the wet season could be due to the dilution of heavy metals in the water column and the ability of stronger plants to grow in the wet season than in the dry season. Meanwhile, the BAF depends on the heavy metal concentrations in the plants and the source media, i.e., the soil, and both were higher in the dry season (cf. Figures 29 and 34). Because of the enhanced evapotranspiration during the dry season, heavy metals are transported faster from the soil to the edible parts of plants. Higher temperatures may also accelerate physical, chemical, and biological processes, thereby increasing the availability of heavy metals in the substrate and the internal mobility of heavy metals in the plants. According to Rehman et al. (2016), heavy metal bioavailability in the soil is greatly influenced by pH, which controls their mobility, though their concentration in the soil remains the main factor for heavy metals uptake by plants (Wei et al., 2016; Rehman et al., 2017).

The BAF values for most of the heavy metals were <1 (Figures 45 and 46) in all four areas and during both seasons, suggesting that heavy metals can only be absorbed but cannot be accumulated in the plant tissues. BAF values in *S. nigrum* <1 have also been reported by Akenga et al. (2020) in Kenya with values of Zn (2.42×10^{-5}), Cu (1.97×10^{-5}), Pb (1.63×10^{-5}), and Cr (1.01×10^{-5}) that were lower than those obtained in this study. Meanwhile, Zn and Cu were the most accumulated metals similar to this study. The ability of *S. nigrum* to accumulate certain heavy metals compared to other plants has been previously studied. For example, Malik et al. (2010) evaluated the heavy metal concentrations in 16 native plant species collected from industrial areas in Islamabad (Pakistan). They found that *S. nigrum* could be used for phyto-stabilization Pb- and Cu-contaminated soils. Kumar et al. (2013) studied metals accumulation in twelve weeds grown on heavy metal-contaminated soils in India and reported that *S. nigrum* had a higher heavy metal BAF than most other studied plants. Similarly, Singh et al. (2016) investigated the heavy metals concentrations in different weed species collected from metal-contaminated soils in India and found that *S. nigrum* contained higher Cd and Ni concentrations than the other tested plant species.

Summary

The pollution grade of heavy metals in *S. nigrum* varied from slight to strong in all studied areas of the Yaoundé lowlands during the dry and wet seasons except for Nkolbisson, where it was slight to safe. The highest pollution grade was recorded in Mokolo and confirmed the comparatively high contamination of *S. nigrum* grown in this area. Cd showed the highest accumulation capacity in *S. nigrum* in both seasons, unlike other heavy metals. In general, *S. nigrum* grown in these lowlands is contaminated by the six studied heavy metals posing risks for farmers and consumers once eaten.

The BAF results indicated that the absorption capacity of *S. nigrum* differs among the studied heavy metals. Cr had the lowest BAF, while the BAF for Zn was the highest in both seasons, suggesting that plants absorb Zn stronger than the other heavy metals. Although Zn is essential for various processes and plant growth, its excessive accumulation in edible plant parts may lead to human health risks. In this study, *S. nigrum* accumulated heavy metals in the edible part of vegetables above the recommended limits, potentially affecting human health.

Since the bioaccumulation and Pi of heavy metals are among the main components controlling human exposure to heavy metals through the food chain, and diet is the primary pathway of human exposure to heavy metals, it is necessary to assess the degree of human exposure via the consumption of *S. nigrum* cultivated in these areas. The following part (fourth objective) estimates the human health risk due to exposure to heavy metals through the diet.

4.6. Health risk assessment

4.6.1. Non-carcinogenic risk

4.6.1.1. Daily intake of heavy metals

Among various existing exposure pathways of heavy metals to humans, ingestion via the food chain is the most significant one (Kamunda et al., 2016; Hu et al., 2017; Kacholi, 2018). Since the degree of heavy metals' harmfulness to humans is cumulative and depends on their daily intakes, estimating the degree of heavy metal exposure through the diet is essential when assessing human health risks. In this study, the estimated dietary intakes (EDIs) of Cr, Cd, Zn, Pb, Ni, and Cu were calculated based on the consumption of 400g (WHO/FAO minimum recommended vegetable quantity per day) of *S. nigrum* per day for a 70 kg adult body weight (Table 42).

For non-carcinogenic effects, the EDIs of Cu, Cd, Pb, Cr, Ni and Zn were 0.068, 0.006, 0.002, 0.008, 0.008 and 0.306 mg/kg.bw/day, respectively. The highest EDIs of Cd, Pb, Ni, and Cr were recorded in Mokolo in the dry season and the highest EDI of Cu and Zn in the wet season and in the same area. These results possibly reflect the high concentrations of these heavy metals in *S. nigrum* during the two seasons. The EDIs of Cr and Cd from *S. nigrum* consumption was found to be higher than the provisional tolerable daily intake (PTDI) in all areas. In addition, Pb in Mokolo exceeded the PTDI thresholds of FAO/WHO (2011). Based on these findings, these elements may threaten the health of farmers and consumers of *S. nigrum* cultivated in the studied lowlands.

Table 42 Estimated daily intake (EDI in mg/kg.bw/day) for heavy metal in *Solanum nigrum* compared to previous studies in Yaoundé, Cameroon.

Areas	Season	Cu	Cd	Pb	Ni	Cr	Zn	Authors	
Mokolo	Dry	0.103	0.008	0.009	0.015	0.012	0.324	This study	
	Wet	0.105	0.007	0.003	0.005	0.005	0.645		
Ekoumdoum	Dry	0.076	0.006	0.003	0.010	0.007	0.294		
	Wet	0.046	0.007	0.001	0.008	0.008	0.268		
Ekounou	Dry	0.062	0.005	0.001	0.005	0.009	0.263		
	Wet	0.037	0.005	0.000	0.012	0.012	0.228		
Nkolbisson	Dry	0.094	0.004	0.001	0.007	0.008	0.302		
	Wet	0.025	0.004	0.000	0.001	0.001	0.119		
Previous studies in Yaoundé, Cameroon									
Nkolbisson	-	0.007	0.001	0.001	0.007	0.001	0.034		(Aboubakar et al., 2021)
Nkolondom	-	0.005	0.000	0.000	0.016	0.004	0.038		
PTDI (mg per kg of body weight)								(FAO/WHO, 2011)	
		0.5	0.001	0.004	0.02	0.003	0.3-1		

PTDI – Provisional Tolerably Daily Intake

The overall average daily intakes of heavy metals in *S. nigrum* were in the order of Zn > Cu > Cr > Ni > Cd > Pb. Ingestion of Zn was highest, followed by Cu, while those of Pb were lowest. The high EDI values of Zn and Cu indicate that vegetable consumption was the main source of these essential elements for humans, as illustrated by the high concentration of these elements in the plant samples. Except for the EDI of Pb, all other EDIs recorded in this study were above those obtained from two communities in Yaoundé in a previous study (Aboubakar et al., 2021).

Cd, Cr, and Pb (the latter in Mokolo only) were consumed daily via *S. nigrum* and were above the PTDI in all areas. Cd and Pb are non-essential metals and may affect human health. Mainly, Cd is a cumulative toxin with unknown benefits for the human body. Once it has entered human organs, it may remain in the metabolism for 16 to 33 years and can be linked to several health problems (Guerra et al., 2012). According to WHO, a high intake of Cd-contaminated food causes acute gastrointestinal effects such as diarrhea and vomiting.

Moreover, chronic exposure to Cd may lead to kidney damage with a perturbation of phosphorus and calcium metabolism and a possible higher risk of kidney stones. It also affects the reproduction and endocrine systems of women. The level of Cd intake found in this study was higher than that reported in the literature, ranging between 0.0004 and 0.0019 mg per day (Okereke, 2016; Ngweme et al., 2020). The EDI values of Cd from Mokolo, Ekoumdoum, Ekounou, and Nkolbisson sites indicated that high consumption of *S. nigrum* can possibly be associated with human health risks.

Pb is poisonous for the human body and affects many organs such as the liver, kidney, lungs, and spleen, causing severe biochemical defects. Adults exposed occupationally or

accidentally to excessive levels of Pb can exhibit neuropathology. The literature stresses an association between Pb in the human body and adult high blood pressure (Ametepey et al., 2018). Except for the EDI of Pb in Mokolo (0.006 mg/kg.bw/day), it was below the recommended limit in the other areas and also below the 0.009 to 0.194 mg/kg.bw/day reported in previous studies (Guerra et al., 2012; Sultana et al., 2019; Ngweme et al., 2020; Gebeyehu et al., 2020).

4.6.1.2. Hazard quotient

The hazard quotient-based risk indicates the level of risk due to exposure to contaminants that are unlikely for even sensitive populations to experience an adverse health effect (Chary et al., 2008; USEPA, 2010). It represents the level at which no harmful effects are expected (Kacholi & Sahu, 2018). The Hazard quotients (HQ) of individual heavy metals from the studied areas in the lowlands of Yaoundé are presented in Figure 48. HQ is obtained by the ratio between the exposure to toxicity (EDI) and the reference dose (RfD), as in equation 4. According to the United States Environmental Protection Agency (USEPA), the HQ of metal above one (HQ>1) implies the potential for adverse health effects from the exposure to the element (USEPA, 2014, 2018b).

Except for Pb and Ni, almost all the HQ values were higher than one (>1) in *S. nigrum* during both seasons in all studied areas. The highest HQs of Cu (2.57), Cd (8.20), Cr (4.09), and Zn (2.15) were recorded in vegetable samples from Mokolo. In general, HQ values in all areas were in the following descending order of Cd > Cr > Cu > Zn > Ni > Pb.

The high HQ values found in Mokolo suggest that consuming *S. nigrum* from this area may substantially increase the risk of illnesses associated with heavy metals like Cd, Cr, and Cu and, to some extent, Zn. These results suggest that the exposure concentration of Cd, Cr, Cu, and Zn exceeded the USEPA limits for a potential human health risk. Therefore, the consumption of leafy vegetables grown in this area should be avoided. These findings corroborated an earlier report by Ngweme et al. (2020), who found HQ values >1 in leafy vegetables from peri-urban areas of Kinshasa, DRC.

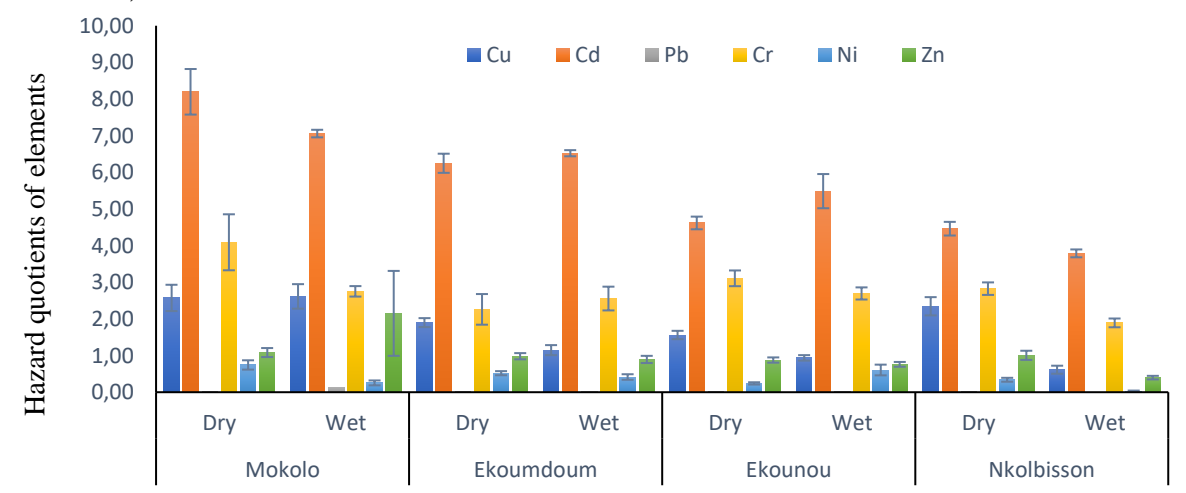


Figure 48 Hazard quotients (HQs) of heavy metals (mean ± SE) in *Solanum nigrum*

4.6.1.3. Hazard index

HI is the key variable to assess the overall potential hazards for multiple health effects posed by more than one heavy metal. The hazard index (HI) is treated as the arithmetic sum of the individual risk (HQ) described by USEPA (Equation 5) and assumes that the consumption of targeted food crops would result in concurrent exposure to numerous potentially toxic elements (Sanaei et al., 2021). If the HI is <1, it is unlikely that there will be any obvious adverse effects, whereas an HI >1 indicates the probability of adverse effects. When the HI is >10, the risk is considered high and can be chronic or even acute (Lei et al., 2015). Figure 49 summarizes the HIs of the different heavy metals studied in *S. nigrum*. The HI for the six heavy metals ranked in descending order in the dry season were Mokolo (16.7), >Ekoumdoum (11.9), >Ekounou (10.4), and >Nkolobisson (11.0), and in the wet season Mokolo (15.0), >Ekoumdoum (11.6), >Ekounou (10.5), and >Nkolobisson (6.7). With 16.7, the highest HI was found in *S. nigrum* from Mokolo during the dry season.

In most areas, HI values >10 suggest that farmers and other consumers of *S. nigrum* are highly exposed to potentially non-carcinogenic adverse effects that may include but are not limited to serious damages to their organs. These health effects may be chronic or acute. A HI >1 for *S. nigrum* has also been reported by Nabulo et al. (2010) and for *Amaranthus hybridus*, a similar leafy vegetable, by Kacholi (2018). Moreover, Aboubakar et al. (2021) found a HI >4.23 for *S. nigrum* in the Nkolobisson area of Yaoundé in both dry and wet seasons.

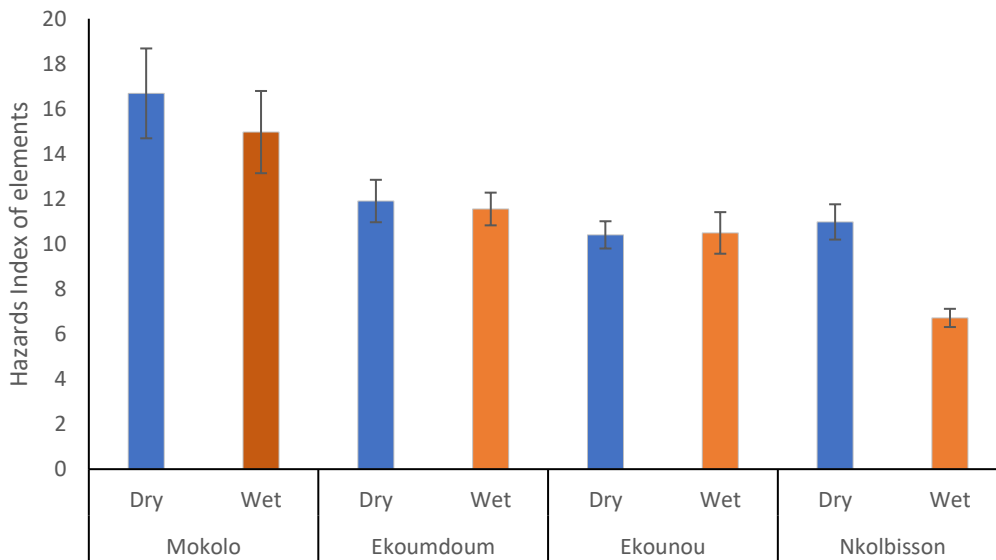


Figure 49 Hazard index (HIs) of heavy metals (mean ± SE) in *Solanum nigrum*

4.6.2. Carcinogenic risk

The target carcinogenic risk (TCR) of heavy metals refers to the lifetime cancer risk, and in the context of this study, for farmers due to their exposure via their diets. The TCR is calculated by using the carcinogenic risk values of the EDI presented in Appendix 9. Due to the lack of the

carcinogenic slope factors of Cu and Zn (cf. Table 14), only the TCRs of Pb, Cr, Cd, and Ni could be estimated. The results are summarized in Table 43. TCR values for Cd, Cr, and Ni exceeded the range of 1×10^{-6} to 1×10^{-4} , which is the acceptable limit of no carcinogenic risk concern set by USEPA (USEPA, 2018a). The highest cancer risks of Cd (5.3×10^{-2}), Cr (2.6×10^{-3}), and Ni (1.1×10^{-2}) from *S. nigrum* consumption were recorded in the dry season in Mokolo. Except for Mokolo, the TCRs of Pb were within the acceptable range during the dry and wet seasons. These results suggest potential carcinogenic concerns of Cd, Cr, Pb, and Ni for farmers and consumers of *S. nigrum* grown in these lowland areas, and thus, the consumption of *S. nigrum* cultivated in the areas should be avoided. Yet only the TCR values for CR and Pb from Mokolo were above the acceptable USEPA range (see section 2.5.2.), and consumption of *S. nigrum* grown by the Ekounou, Ekoumdoum, and Nkolbisson farmers had little to no Pb-related cancer risks.

Table 43 Carcinogenic risk (TCR) values of heavy metal in *Solanum nigrum* and the cumulative cancer risk (CCR).

Areas	Seasons	Target Carcinogenic risk (TCR)				CCR
		Cd	Pb	Cr	Ni	
Mokolo	Dry	5.27E-02	3.16E-04	2.63E-03	1.08E-02	6.65E-02
	Wet	4.54E-02	1.27E-04	1.27E-04	3.69E-03	4.93E-02
Ekoumdoum	Dry	4.02E-02	1.18E-04	1.45E-03	7.56E-03	4.93E-02
	Wet	4.19E-02	1.86E-05	1.86E-05	6.04E-03	4.80E-02
Ekounou	Dry	2.97E-02	3.39E-05	2.00E-03	3.50E-03	3.52E-02
	Wet	3.53E-02	5.92E-06	5.92E-06	8.83E-03	4.41E-02
Nkolbisson	Dry	2.87E-02	3.70E-05	1.82E-03	4.94E-03	3.55E-02
	Wet	2.44E-02	0.00E+00	0.00E+00	3.69E-04	2.47E-02

Exposure to multiple pollutants may result in interactive or additive adverse effects. The cumulative cancer risk (CCR) or total cancer risk is calculated by summing the individual lifetime cancer risk (TCR) as described by equation 7 (USEPA, 2018c). Table 47 shows the cumulative cancer risk (CCR) of heavy metals through the consumption of *S. nigrum* during the dry and wet seasons. The highest value of CCR was estimated for Mokolo (1.2×10^{-1}), followed by Ekoumdoum (9.7×10^{-2}), Ekounou (7.9×10^{-2}), and Nkolbisson (6.0×10^{-2}). Cd had the highest CCR, and Pb had the lowest (Cd > Ni > Cr > Pb). All CCRs obtained were above the USEPA and WHO acceptable range of 10^{-6} , 10^{-5} , or 10^{-4} (see section 2.5.2), suggesting that consuming *S. nigrum* grown in these lowlands might cause a risk of cancer for farmers and consumers.

This research presents the cancer risks of contaminants to farmers through the cumulative ingestion of carcinogenic metals in *S. nigrum* cultivated in the four lowland areas of Yaoundé, Cameroon. Overall, three out of the four analyzed heavy metals, corresponding to 80% of the carcinogenic heavy metals ingested via the consumption of *S. nigrum*, may increase the cancer risks to farmers and other consumers of the vegetables grown in these areas.

Summary

The objective of these investigations was to assess heavy metals' non-carcinogenic and carcinogenic risks via the consumption of *S. nigrum* contaminated with heavy metals grown in the dry and wet seasons in four lowland areas of Yaoundé. The results show that heavy metals' non-carcinogenic and carcinogenic risks depend on site-specific factors such as heavy metal concentration in the vegetables. The EDIs of Cd and Cr were beyond the provisional tolerable daily intake. Similarly, the HQs of Cd, Cr, Cu, and to some extent Zn were >1, suggesting the probability of adverse effects. The health risk is likely to occur for consumers of *S. nigrum*. The non-carcinogenic combined impact of multiple metals (HI) was classified as follows descending order: Mokolo > Ekoumdoum > Ekounou > Nkolobisson. The HI >10 for most areas suggests a high probability of health risk that can be chronic or acute. The carcinogenic risk analysis indicated that the TCR levels for Cd, Cr, Ni, and Pb (the latter heavy metal only for Mokolo) due to the consumption of *S. nigrum* were also higher than the recommended thresholds and could be ranked according to the area as Mokolo, >Ekoumdoum, >Ekounou, and >Nkolobisson. The findings suggest a probability of cancer risks for consumers via consuming *S. nigrum* cultivated in the four lowlands areas of Yaoundé. The presence of non-essential heavy metals in vegetables, even in traces, is unacceptable. Consequently, further effective measures are necessary to manage heavy metal contamination in soils and plants to reduce metal translocation from soils to the edible parts of crops.

Limitations

This study appraised the EDI, HQ, HI, TCR, and CCR values based on the estimated daily consumption of vegetables of 400 g per day of *S. nigrum*. The study considered the total metal and no edible metal effectively absorbed by the human body. Thus, it is likely that the values of EDI and HQ obtained could be over or underestimated, and that could have possibly affected the HI, TCR, and CCR values. The study had only considered *S. nigrum* (the edible parts) to estimate the possible non-carcinogenic and carcinogenic health hazards among Mokolo, Ekoumdoum, Ekoumdoum, and Nkolobisson lowlands farmers, but not the total risk to the population. For this reason, the potential health risks to the local population via the exposure to heavy metals through the consumption of leafy vegetables might be underestimated as the cooking might include other vegetable fruits and/or herbs whose levels of heavy metal contamination are unknown.

5. Conclusion

This study aims to assess the heavy metal contamination in irrigation water, agricultural soils, and plants and the cancer risk associated with exposure to vegetables grown in the lowlands of Yaoundé. The study found that farming practices in the lowlands were mostly similar across the study areas in that farmers practiced polyculture with a predominance of leafy vegetables using mineral and organic fertilizers and various pesticides. Despite the small farm size in the Mokolo area, farmers there applied the highest amount of organic and mineral fertilizers. This practice can lead to soil degradation, and the fertilizer and pesticides affect the physio-chemical properties of the surrounding water bodies. Moreover, the often-inadequate applications may lead to soil degradation. The amounts of fertilizers applied and the sources of irrigation water were the most critical factors that differentiated the study areas. Farmers in the Mokolo areas used only GW for irrigation while those in Ekounou used GW and SW equally, and the ones in Nkolbisson and Minkoameyos irrigated mainly with SW. Topical irrigation with untreated water is a path in which pathogens and chemicals, including heavy metals, are transferred to the food crops. They might lead to heavy metals contamination in the environment and end up in the food chain. For this reason, this study monitored potential risks for farmers and consumers via the water, soil, and production quality.

The level of contamination in irrigation water in the study area was in the following descending order: Ekounou > Ekoumdoum > Mokolo > Nkolbisson. The Zn concentration in the irrigation water was highest during both seasons and in all areas, while that of Cd was the lowest. The concentrations of heavy metals found in the irrigation water were Zn > Cu > Ni > Cr > Pb > Cd in the dry and Zn > Cu > Cr > Pb > Ni > Cd in the wet season. All heavy metals concentrations were below the FAO/WHO recommended thresholds for irrigation during dry and wet seasons, with the highest concentrations of Cu, Ni, and Zn recorded in Ekounou, while Cr and Pb were found in higher concentrations in Nkolbisson and Cd in Mokolo. Despite these findings, water in all four areas of Yaoundé was safe for vegetable irrigation. Still, continuous monitoring of the heavy metals in the water is recommended as they are cumulative and non-degradable.

The concentrations of heavy metals in soil samples in the studied areas in Yaoundé can be ranked in the following descending order, i.e., Mokolo > Nkolbisson > Ekounou > Ekoumdoum, with Zn > Cr > Cu > Ni > Pb for the dry and Cr > Zn > Cu > Pb > Ni for the rainy season. Unlike in the irrigation water samples, the highest Cu, Zn, Ni, and Pb values were recorded in soil samples from Mokolo during both seasons. Cr concentration exceeded the recommended limit in all samples during the dry and wet seasons. Except for Mokolo, Cu, Zn, and Pb, recorded in the three other study areas were below the thresholds, and the concentration of Cd in the soil samples was below the detection limit during both seasons. Meanwhile, Cd was present at trace levels in the irrigation water. This difference suggested that although the heavy metals in agricultural soils might also originate from the irrigation water, their accumulation and availability in the agricultural soil as affected by soil physico-chemical properties such as pH, OM, CEC etc. (Kabata-pendias, 2011; Kim et al., 2015). Due to the moderate to high variability of these metals

in the agricultural soils, these heavy metals might derive mainly from anthropogenic sources, including fertilization, pesticide, wastewater, tanneries, and other human activities.

Similar to the irrigation water and the cultivated soils, high heavy metals concentrations were recorded in the *S. nigrum* samples during the dry season, confirming the dilution effect in the wet season. The level of heavy metals in the plants was in the descending order as $Zn > Cu > Ni > Cr > Cd > Pb$ in both seasons. Most of these heavy metals were found in the plant samples at concentrations that exceeded the FAO/WHO recommended limits. Zn was dominant in plant samples, as it was in the irrigation water and soil samples. Furthermore, like for the soil analyses, plant samples from Mokolo had the highest heavy metals concentrations, suggesting that their high concentration in the soil then leads to a high concentration in the growing plants. The significant positive correlation between heavy metals in the soils and plants indicates that they were likely to come from the same contamination sources. It also showed that heavy metals in the plants originated from the soils. While Cd was absent in the soil samples, traces of it were detected in the irrigation water, and higher concentrations were found in *S. nigrum* samples. Vegetables uptake nutrients and other elements, including heavy metals, from the soil cultivated in via their roots, thereby accumulating soil pollutants in the process. The analyses also suggested that heavy metals in the plants did not only originate from the water and soil but also from the atmosphere through atmospheric deposition (air).

Zn had a high metal bioaccumulation factor (>1), suggesting that plants can easily absorb it from the soil. Plants in small amounts need Zn as an essential element in various development processes, including plant internode elongation and growth hormone production. High accumulation of Zn in edibles part of the vegetables may harm both plant health and its consumers. While Zn is easily absorbed from the soil and used in various plant development processes, Cd is non-essential and considered a crop contaminant. The presence of non-essential metals such as Cd in the edible parts of vegetables can directly affect human health. Of all heavy metals in this study, Cd showed the highest degree of contamination in *S. nigrum* in both seasons, indicating that the atmospheric deposition (air) rather than soil might be the main route for its transfer to the vegetables. Overall, the six (6) studied heavy metals in plants varied from a slight pollution grade (in most plants from Nkolbisson) to strong pollution grade (in plants cultivated in Mokolo). These pollution grades helped classify crops in Nkolbisson as slightly clean and those in Mokolo as severely polluted.

The consumption of food contaminated with heavy metals is the primary exposure pathway of humans. Therefore, assessing the human health risk through the consumption of heavy metal contaminated crops is essential to establish the level of risks. In this study, the daily intakes of Cd and Cr from food exceeded the tolerable daily intakes, indicating that the consumption of *S. nigrum* grown in these areas might pose a significant risk to human health. Similarly, the HQ >1 of Cd, Cr, Cu, and Zn (in Mokolo) indicated consumers' probability of adverse health effects. Except for the Nkolbisson area in the wet season, the hazard index of the combined heavy metals >10 in

Mokolo, Ekoumdoum, and Ekounou suggested a high probability of chronic or acute human health risks. The target cancer risks of Cd, Cr, Ni, and Pb (the latter only for Mokolo) were higher than the recommended thresholds, indicating a probable cancer risk for farmers and consumers of *S. nigrum* cultivated in the Mokolo, Ekoumdoum, Ekounou, and Nkolbisson lowlands areas of Yaoundé. The order of cancer risk level from *S. nigrum* consumption was Mokolo > Ekoumdoum > Ekounou > Nkolbisson. The probability of risks was higher for farmers and consumers of *S. nigrum* produces in Mokolo than in other areas.

Recommendations

We found that the heavy metal contamination was higher in environments where there were a lot of anthropogenic activities such as tanneries, open car repairs, untreated waste from metal workshops, waste incinerations, vehicles fumes, and other human activities. Untreated waste fumes from different industries may add to the natural emission of heavy metals, specifically in metropolitan areas of developing countries like Yaoundé. Future research needs to estimate the extent that these activities affect the environmental media as follows:

- Experimental studies should be undertaken to evaluate the amount of heavy metals uptake by plants from fertilizers.
- Experimental studies should be conducted to identify vegetable species or cultivars that accumulate the least heavy metals for preferential cultivation in urban areas.
- The bioaccumulation values rather than the total contents of heavy metals should be taken into consideration.

Assessing these critical aspects will be helpful to understand and manage the relatively high level of heavy metals in urban areas in Cameroon.

The following recommendations can be addressed to:

1. Policymakers

- Increase awareness of the use of occupational health and safety measures and the need to refrain from burning waste by farmers;
- Provide more training on safe urban farming techniques and the management of agrochemicals; Educating people, improving the quality of cultivation methods, and controlling pesticides emissions can reduce the risk effects of heavy metals;
- Regularly monitor to alert farmer's awareness of the levels of heavy metals in agricultural soils and vegetables produced in these lowlands;
- Help create public awareness to avoid consuming vegetables grown in contaminated areas, hence reducing health risks;
- Regulating and adjusting agronomic measures and planting patterns, and land-use types changes in areas where soil heavy metal contents are high could contribute to minimizing the human health risk by controlling the pollution source;
- Soil physico-chemical properties strongly affect the dynamics of heavy metals in agricultural soil and their uptake and bioavailability to plants. Thus, future research should place more emphasis on soil properties.

- Take adequate measures concerning proper assessment by scientists and researchers regarding monitoring and assessing the impact of human activities on heavy metal pollution.

2. Farmers

- Prioritize growing vegetables during the wet season to reduce the use of irrigation water and thus diminish the risk of contamination. Adjust the planting structure and spatial distribution of crops based on the distributions of the different elements in the soil and the growing season.
- Adopt more sustainable agricultural practices like organic farming to achieve sustainable development.

3. Consumers

- Wash vegetables thoroughly before cooking to decrease the intake of heavy metals as it mechanically removes heavy metals deposited on the surfaces of the vegetables.
- Avoid consuming vegetables grown in areas identified as contaminated.

This study, for the first time, attempted to intensively monitor heavy metals in three environmental media (water, soil, and plants) and their associated health risks via the food pathways. It will be important for future research to expand such monitoring on heavy metal concentrations in animals, including humans (blood and urine) in urban environments. Future work should involve bigger sample sizes and a more long-term follow-up that this study could not achieve due to the limited timeframe and funding. It will also be useful to compare results between different metropolitan areas in Africa and other developing countries and evaluate the effects of gender and age.

This study points out that it is crucial to treat industrial and municipal wastes to control heavy metal contaminations. Thus, developing countries like Cameroon must adopt eco-friendly and economically feasible technologies for better waste management.

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7. Appendices

Appendix 1. Farmer questionnaires



GENERAL INFORMATION

Section / _____ /	Code / ___ /	Coordinates N: /-----/ E: /-----/
Interview date	/ ___ / ___ / ___ /	
Name of investigator	/ _____ /	Code / _____ /

100- FARMER IDENTIFICATION

101- Sex	1- Male	2- Female		
102- Marital status	1- Single	2- Married		
	3- Widow(er)	99- Other (Specify)		
103- Education level	1- Primary	3- Secondary	4-	
	University	99- Other (Specify)		
104- Age / _____ / years				
105- Main Occupation	1-Landowner	2-Farmer	3- Housewife	4- Unemployed
	5-Student	6- Laborer	7-Seller	99-
	other(specify)			
106- Region of origin	1- Adamawa North	2- Center	3- East	4-Far
	5- Littoral West	6-North	7-North- West	8- South-
	9-South	10- West	99-Other (Specify)	
108- Number of people involved in lowland agriculture / _____ / years				
109- Membership of a NGO or association / ___ /	1- Yes	2- No		
110- If yes, name	/ _____ /			
111- Mains motivations to do farming?	1-Family habits	2- Income enhancement		
	3- Unemployment	4- Other (Specify)		
112- Farming frequency	1- Permanent	2- Temporary		
113- Sponsorship of this farming activity?	1- Public	2- Private		
	4- Loans	3- Auto-financing		
	99- Other (specified)			

200/IDENTIFICATION OF THE AREA

201- Farm characteristics	1- Mainland	2- Temporarily flooded	3- Permanently flooded
202- Do you own the land?	1- Yes	2- No	99- Other (Specify)
203- If yes, how?	1- Heir	2- Donation	3- Purchase with title
	4- Purchase without title	99-Other (specify)	
204- If No, Is it?	1- Rent	2- Free installation	
205- Farm size	/ _____ / m ²		
206- Year of cultivation	/ ___ / years		

207- Distance house- farm / _____ / m

300-CROPS CHARACTERISTICS

- 301-Cropping phase / ____/ 1- Dry season (specify) 2- Wet season 3- Year-round 4-Other
- 302-Type of crops / ____/ 1- Only vegetables (specify). 2- Only Cereals 3- Both 99- Other
- 401- Do you use selected seeds 1- Yes 2- No
- 402- Origin of the seeds 1- Old crops 2- NGO/donations 3-Purchase 99- Other (specify)
- 403- Post-harvest activity 1- None 2- Wash with irrigation water 4- Packaged 3- Wash with tap water 99- other (specify)

Crop Type	Planting period	Fertilization period	Irrigation period	Irrigation frequency	Harvest period	Output (kg)	Main purpose of growing

- 304-Primary growing purpose 1- Consumption only 2- Sale only 3- Consumption/Sale other (specify) 99-
- 305- If consumption, what proportion of Nightshade do you eat? 1- All the production 2- Half of the products 99- other (specify) 3- 2/3 of the products 4- 3/4 of the products

307- What foods have you eaten in the past 24 hours?

Foods	Days	Mode ate*	Amount prepared (kg)	Average intake	Origin**
Maize					
Rice					
Wheat					
Amaranth					
Nightshade					
Other vegetable leaves					
Root Vegetables					
Other Vegetables					
Fruits					
Fish/Meat					
Eggs					
Other Products					
*Mode ate: 1-Cooked; 2- Raw; 99-other (Specify)					
**Origin: 1-own produce; 2- Gifts; 3- Market; 4- Supermarket; 5-other (Specify)					

400- AGRICULTURAL INPUTS

- 401- Do you use any soil amendments? 1- Yes 2- No
- 402- If yes, what kind of amendment is it 1- Only organic 2- Only chemical 99- 3- Organic and Chemical Other (specify)
- 403- Characteristics of the amendments used

N°	Chemical amendments	Amount	N°	Organic amendments	Amount
1	NPK		1	Manures	

2	Urea		2	Fecal sludge	
3	Ammonium sulfate		3	Compost	
4			4		
5			5		
6			6		

406- Do you use any pesticides? 1- Yes 2- No

407- If yes, what type is it?

Type of pesticides	Yes	No	Utility	Cost
Callomil				
Hydrox				
Banco				
Cypercal				

408- Do you use any machinery? 1- Yes 2- No

409- If yes, which one? 1- Hoe/Machete 2- Binate
3- Diesel pump 99- other (specify)

410- When working, do you ingest some soil? 1- Never 3- Rarely
2- Often/Always 4- Other (Specify)

411- Do you use any protective equipment? 1- Yes 2- No

412- If yes, what? 1- Gloves 3- Raincoats
2- Gumboots 4- Nose mask
5- Glasses 99- Other (Specify)

500- WATER, SANITATION and HYGIENE

501- Origin of water used for watering 1- Surface water 2- Groundwater
99- Other (specify)

502- What irrigation materials do you use? 1- Watering cans 2- Buckets
3- None 99- other (specify)

503- Do you clean your hand after farming activities? 1- Yes 2- No

504- If yes, which water do you use? 1-Irrigation water 2- Drinking water
3-Other (specify)

505- During irrigation, do your clothes become wet with water? 1- Never 2- Rarely
4- Always 99- Other (specify)

506- Do you walk in the irrigation water? 1- Never 2- Rarely
3-Always/Often 99- Other (specify)

507- Do you use irrigation water for any other purposes? 1- Yes 2- No

508- If yes, which ones? 1- Laundry 2- Swimming 99-
3- Fish farming Other (specified)

509- Do you have any inconvenience when using these waters 1- Yes 2- No

510- If yes, which ones? 1- Poor odor 2- Itching
3- Skin burns 99- Other (specify)

511- Do you know any health risks of using poor-quality water? 1- Yes 2- No

512- If yes, which ones? 1- Disease risk 2- Crop contamination 99-
3- Soil pollution Other (specify)

513- What is your primary source of drinking water? 1- Tap water 3- Tube/ bore well
2- Spring 99- Other (specify)

514- What type of toilet facility do you use? 1-Pit latrine 2- Flush toilet
3- Open defecation 99- Other (specify)

515- Do you have any problems with your toilet facility? 1- Yes (specify) 2- No

600 / HEALTH

601- Did you experience any health problems in the last seven days? 1-Yes 2- No

602- if yes, could you describe the symptoms/disease of that?

Disease\ symptoms	Loose stool	Muscle aches	Loose stool with blood	Stomach pain	cough	Fatigue	Fever

603- Do you have any long-term health problems?

1- Yes

2- No

604- If Yes, what?

1- Skin rashes

3- Heart problem

2- Lung problems 99-

4- Cancer

Other (specify)

605-Do you know anybody who has cancer in your family?

1- Yes

2- No

606-If yes was he works as a farmer

1- Yes

2- No

607- When you are sick, what treatment do you have?

1- Self-medication 3-

2- Traditional herbs

Health center

99- other (specify)

608- If it is the health center, how far is it?

700-OTHERS

701- Do you think lowland farming is essential?

1- Yes

2- No

702- If yes, why is this important?

1- Food security

2-Against unemployment

3- Increase income 4-

99- Other (specify)

Lowland protection

703- Have you been trained in urban farming?

1-Yes

2- No

704- If Yes, what is the duration of this training?

1- One week

2- Two weeks

3- One month

99- Other (Specify)

705- Which Institution did the training?

1- Public

2- Private

3- NGOs

99- Other (specify)

706- Which constraints do you face during the farming activities?

1- Access to land 3-

2- Poor water quality

Agricultural inputs

4- Flooding

5- Soil exhaustion

6- Marketing policy

7- Water scarcity

99- Other (specify)

9- Lack of supervision

707- What do you think can be done to improve urban farming?

Thank you for your participation

Appendix 2. Consent Form

Purpose of Research

Assess the heavy metal contamination in plant soil and water and the human health risks in Yaoundé. The health risks associated with agricultural inputs will be highlighted, and the bioaccumulation of heavy metals estimated.

Methods

You will be interviewed three times (during seeding, weeding, and harvesting), and the questionnaire may take at least 30 minutes. During the interview, you will also give some information about your diet, and your body weight will be taken with the scale. Heavy metal will be analyzed in irrigation water, soil, and crops using an Atomic Absorption Spectroscopy.

Duration

This study will take nine months: you will be visited in the field during the first three months for the farm survey. Then during the six last months, your farm will be visited during the planting and the harvest to pick up the samples to analyze.

Benefits

This study will identify the level of heavy metal contamination of irrigation water, crops, and soil.

Risks

There are risks free through participating in this study.

Confidentiality

Information collected in this questionnaire is strictly confidential and will be used only for statistical purposes for the student thesis and related publications. This information will not be shared with any other institution or administration; it will only be used for academic purposes. Additionally, the final document will represent the overall results (No farmer will be cited individually).

Voluntary nature of participation

I am free to accept or refuse to participate in this project. If I start, I am free to withdraw my participation at any time without any penalty.

Human Subject statement

Suppose you have any problems or concerns that occur due to your participation. In that case, you can report them to Annie Stephanie Nana, responding to +237 6 75 34 04 22, PO Box: 8250, University of Yaoundé I, Wastewater Research Unit.

I freely consent to participate in this research study, and I have read the preceding information, or it has been read to me. I understand that I can withdraw at any time without penalty.

Participant's name and signature

Date

Researcher's signature

Date

Appendix 3. The difference in the proportion of farmers in the studied areas using various farming practices.

Variables	Use of fertilizers		Use groundwater		Use of pesticide		Orga_ferti kg /m ²		Mine_ferti kg/m ²	
	Contrast	P.value	Contrast	P.value	Contrast	P.value	Contrast	P.value	Contrast	P.value
Mokolo vs Emana	0.12	0.373	0.46	0.008	-0.21	0.222	1.76	0.11	0.10	0.035
Minkoameyos vs Emana	0.15	0.303	-0.36	0.045	-0.72	0.000	-0.56	0.349	0.03	0.382
Nkolbisson vs Emana	0.19	0.156	-0.17	0.301	-0.24	0.145	-0.19	0.76	-0.02	0.169
Ekoumdoum vs Emana	0.06	0.634	0.06	0.700	-0.18	0.252	1.37	0.059	-0.01	0.85
Ekounou vs Emana	0.16	0.233	-0.05	0.780	-0.29	0.075	0.38	0.602	-0.03	0.132
Minkoameyos vs Mokolo	0.03	0.838	-0.81	0.000	-0.51	0.001	-2.23	0.015	-0.07	0.147
Nkolbisson vs Mokolo	0.07	0.547	-0.63	0.000	-0.03	0.809	-1.95	0.043	-0.12	0.007
Ekoumdoum vs Mokolo	-0.06	0.549	-0.39	0.003	0.03	0.833	-0.39	0.703	-0.10	0.025
Ekounou vs Mokolo	0.03	0.75	-0.50	0.000	-0.09	0.532	-1.37	0.184	-0.13	0.006
Nkolbisson vs Minkoameyos	0.04	0.724	0.19	0.200	0.48	0.001	0.37	0.157	-0.05	0.062
Ekoumdoum vs Minkoameyos	-0.09	0.434	0.42	0.003	0.54	0.000	1.93	0.000	-0.03	0.283
Ekounou vs Minkoameyos	0.01	0.934	0.31	0.001	0.43	0.004	0.94	0.049	-0.05	0.05
Ekoumdoum vs Nkolbisson	-0.13	0.19	0.23	0.058	0.06	0.62	1.56	0.001	0.02	0.119
Ekounou vs Nkolbisson	-0.03	0.757	0.13	0.329	-0.05	0.691	0.57	0.001	-0.01	0.753
Ekounou vs Ekoumdoum	0.10	0.314	-0.11	0.371	-0.11	0.39	-0.99	0.113	-0.02	0.092

Notes: Orga_ferti: Organic fertilizers; Mine_ferti: mineral fertilizers. Numbers in bold represent the statistical significance level at 5%.

Appendix 4. Seasonal and areas difference in the concentration of heavy metal in the irrigation water

Variables		Cu		Cd		Pb		Cr		Ni		Zn	
Areas	Season	Cont	P>t	Cont	P>t	Cont	P>t	Cont	P>t	Cont	P>t	Cont	P>t
Mokolo	Dry Vs Wet	-0.003	0.187	-0.001	0.000	0.003	0.000	-3E-04	0.854	-0.006	0.000	-0.109	0.000
Ekounou	Dry Vs Wet	-0.009	0.004	-0.002	0.000	0.001	0.074	-5E-04	0.703	-0.006	0.000	-0.131	0.000
Ekoumdoum	Dry Vs Wet	-0.008	0.000	-0.002	0.000	9E-04	0.067	-2E-04	0.829	-0.005	0.000	-0.096	0.000
Nkolbisson	Dry Vs Wet	-0.002	0.104	-0.003	0.000	-0.003	0.029	0.003	0.068	-0.005	0.000	-0.08	0.000
Season	Areas												
Dry	Ekounou vs Mokolo	0.0051	0.068	-0.001	0.000	7E-05	0.668	0.002	0.125	0.0009	0.268	0.028	0.114
Dry	Ekoumdoum vs Mokolo	0.003	0.059	-0.001	0.000	-1E-04	0.204	0.003	0.049	0.0004	0.615	0.006	0.665
Dry	Nkolbisson vs Mokolo	-4E-04	0.731	-0.001	0.000	0.002	0.062	0.002	0.106	0.0003	0.731	-0.022	0.043
Wet	Ekounou vs Mokolo	-0.001	0.571	-0.001	0.000	-0.002	0.078	0.002	0.159	0.0003	0.72	0.005	0.111
Wet	Ekoumdoum vs Mokolo	-0.002	0.357	-0.001	0.000	-0.002	0.021	0.003	0.044	0.0013	0.083	0.02	0.009
Wet	Nkolbisson vs Mokolo	0.0005	0.811	-0.002	0.000	-0.003	0.000	0.005	0.001	0.0013	0.138	0.007	0.175
Dry	Ekoumdoum vs Ekounou	-0.002	0.594	2E-06	0.984	-2E-04	0.137	5E-04	0.67	-5E-04	0.435	-0.021	0.291
Dry	Nkolbisson vs Ekounou	-0.005	0.054	-6E-05	0.763	0.002	0.072	3E-04	0.844	-6E-04	0.43	-0.05	0.004
Wet	Ekoumdoum vs Ekounou	-7E-04	0.479	0.0002	0.621	-5E-04	0.536	7E-04	0.524	0.001	0.113	0.014	0.076
Wet	Nkolbisson vs Ekounou	0.0017	0.085	-0.001	0.011	-0.002	0.006	0.003	0.014	0.001	0.197	0.002	0.752
Dry	Nkolbisson vs Ekoumdoum	-0.004	0.046	-6E-05	0.727	0.003	0.048	-2E-04	0.856	-9E-05	0.901	-0.028	0.053
Wet	Nkolbisson vs Ekoumdoum	0.002	0.003	-0.001	0.002	-0.001	0.016	0.003	0.039	-6E-05	0.933	-0.013	0.167

Number in bold: statistically significant difference at p<0.05.

Appendix 5. Seasonal and areas difference in the concentration of heavy metal in the agricultural soil of the study areas.

Areas	Variables Season	Cu		Pb		Cr		Ni		Zn	
		Contrast	P>t	Contrast	P>t	Contrast	P>t	Contrast	P>t	Contrast	P>t
Mokolo	Dry Vs Wet	-34.47	0.118	17.96	0.427	-1.35	0.967	5.62	0.612	-17.81	0.712
Ekounou	Dry Vs Wet	4.26	0.534	2.21	0.070	11.88	0.646	-0.90	0.839	-14.50	0.284
Ekoumdoum	Dry Vs Wet	-9.592	0.345	2.07	0.024	16.07	0.467	-2.68	0.235	-16.20	0.208
Nkolbisson	Dry Vs Wet	-13.22	0.051	3.64	0.489	4.548	0.132	3.59	0.022	-31.52	0.041
Season	Areas										
Dry	Ekounou vs Mokolo	-85.24	0.000	-63.68	0.000	1.728	0.953	-8.62	0.056	-172.56	0.000
Dry	Ekoumdoum vs Mokolo	-67.78	0.000	-63.12	0.000	-45.9	0.104	-13.50	0.003	-168.10	0.000
Dry	Nkolbisson vs Mokolo	-62.44	0.000	-56.28	0.000	-27.4	0.384	-14.50	0.001	-162.22	0.000
Wet	Ekounou vs Mokolo	-46.51	0.001	-79.44	0.000	14.95	0.613	-15.13	0.155	-169.26	0.000
Wet	Ekoumdoum vs Mokolo	-4.29	0.001	-79.02	0.000	-28.5	0.309	-21.81	0.038	-166.49	0.000
Wet	Nkolbisson vs Mokolo	-41.19	0.001	-70.61	0.000	19.47	0.535	-16.53	0.105	-175.93	0.000
Dry	Ekoumdoum vs Ekounou	17.46	0.086	0.56	0.716	-47.6	0.052	-4.89	0.082	4.47	0.835
Dry	Nkolbisson vs Ekounou	22.8	0.003	7.40	0.127	-29.1	0.300	-5.88	0.013	10.34	0.672
Wet	Ekoumdoum vs Ekounou	3.61	0.717	0.41	0.826	-43.4	0.075	-6.67	0.123	2.76	0.856
Wet	Nkolbisson vs Ekounou	5.32	0.516	8.83	0.025	4.516	0.872	-1.40	0.687	-6.68	0.522
Dry	Nkolbisson vs Ekoumdoum	5.343	0.64	6.85	0.150	18.56	0.481	-1.00	0.673	5.88	0.756
Wet	Nkolbisson vs Ekoumdoum	17.1	0.799	8.42	0.034	47.96	0.072	5.28	0.084	-9.44	0.438

Number in bold: statistically significant difference at p<0.05

Appendix 6. Seasonal and area differences in the concentration of heavy metal found in *Solanum nigrum*

Areas	Season	Cu		Cd		Pb		Ni		Zn		Cr	
		Contrast	P>t	Contrast	P>t	Contrast	P>t	Contrast	P>t	Contrast	P>t	Contrast	P>t
Mokolo	dry Vs Wet	0.35	0.920	-0.24	0.073	-1.10	0.000	-2.07	0.000	-3.29	0.785	-0.85	0.018
Ekounou	dry Vs Wet	-5.28	0.000	0.18	0.077	-0.16	0.000	0.55	0.005	-7.46	0.174	-0.26	0.358
Ekoumdoum	dry Vs Wet	-6.36	0.000	0.06	0.288	-0.57	0.000	0.47	0.349	-5.53	0.461	0.19	0.440
Nkolbisson	dry Vs Wet	-14.66	0.000	-0.14	0.001	.	.	0.23	0.000	-38.60	0.000	-0.59	0.071
Season Areas													
Dry	Ekounou vs Mokolo	-8.57	0.003	-0.76	0.000	-1.64	0.000	-2.13	0.000	-12.97	0.118	-0.62	0.055
Dry	Ekoumdoum vs Mokolo	-5.70	0.052	-0.41	0.002	-1.15	0.001	-0.95	0.079	-6.39	0.469	-1.16	0.000
Dry	Nkolbisson vs Mokolo	-1.93	0.569	-0.79	0.000	-1.62	0.000	-1.71	0.001	-4.80	0.639	-0.80	0.020
Wet	Ekounou vs Mokolo	-14.19	0.000	-0.33	0.001	-0.70	0.000	1.49	0.020	-17.14	0.144	-0.03	0.911
Wet	Ekoumdoum vs Mokolo	-12.41	0.000	-0.11	0.000	-0.63	0.000	0.68	0.092	-8.63	0.494	-0.12	0.685
Wet	Nkolbisson vs Mokolo	-16.93	0.000	-0.69	0.000	-	-	-0.96	0.000	-40.11	0.000	-0.55	0.113
Dry	Ekoumdoum vs Ekounou	2.87	0.035	0.34	0.000	0.49	0.000	1.18	0.000	6.58	0.333	-0.54	0.042
Dry	Nkolbisson vs Ekounou	6.64	0.002	-0.03	0.514	0.02	0.842	0.42	0.086	8.17	0.339	-0.18	0.561
Wet	Ekoumdoum vs Ekounou	1.78	0.163	0.22	0.022	0.07	0.096	-0.81	0.224	8.52	0.240	-0.09	0.739
Wet	Nkolbisson vs Ekounou	-2.74	0.009	-0.36	0.000	-	-	-2.46	0.000	-22.97	0.000	-0.51	0.097
Dry	Ekoumdoum vs Nkolbisson	3.77	0.086	-0.38	0.000	-0.47	0.000	-0.76	0.015	1.58	0.861	0.36	0.215
Wet	Ekoumdoum	-4.52	0.001	-0.58	0.000	-	-	-1.65	0.000	-31.49	0.000	-0.42	0.145

Number in bold: statistically significant difference at p<0.05

Appendix 7: Descriptive statistics of crops pollution index during the dry season

Variables	Dry	PiCu	PiCd	PiPb	PiCr	PiNi	PiZn
Mokolo	Mean	2.18	8.67	6.12	2.60	3.15	1.37
	SD	0.68	1.47	2.74	1.08	1.20	0.35
	Median	2.37	8.07	5.78	2.56	2.81	1.30
	Min	1.01	7.41	2.63	1.06	2.19	0.87
	Max	2.67	11.18	9.02	4.04	5.25	1.76
	CV(%)	31.19	16.97	44.73	41.77	38.25	25.37
Ekounou	Mean	1.64	7.01	2.68	1.05	2.45	1.25
	SD	0.35	0.33	0.41	0.11	0.73	0.40
	Median	1.70	6.94	2.62	1.03	2.18	1.15
	Min	1.11	6.63	2.14	0.90	1.78	0.84
	Max	2.02	7.61	3.30	1.25	3.80	1.87
	CV(%)	21.45	4.7	15.33	10.09	29.87	32.3
Ekoumdoum	Mean	1.36	5.06	0.80	2.10	1.15	1.15
	SD	0.30	0.76	0.44	0.70	0.45	0.25
	Median	1.39	4.76	0.89	1.96	1.079	1.14
	Min	0.99	4.39	0.042	0.94	0.68	0.67
	Max	1.94	6.92	1.43	3.24	2.04	1.50
	CV(%)	21.64	14.92	54.77	33.5	39.4	22.09
Nkolbisson	Mean	1.98	4.72	0.72	1.79	1.44	1.28
	SD	0.52	0.48	0.70	0.27	0.55	0.39
	Median	1.90	4.69	0.61	1.77	1.37	1.18
	Min	1.42	4.25	0.02	1.41	0.70	0.88
	Max	2.95	5.60	1.95	2.14	2.31	1.93
	CV(%)	25.97	10.23	97.18	14.81	38.47	30.47

Appendix 8: Descriptive statistics of crops pollution index during the wet season

	Wet	PiCu	PiCd	PiPb	PiCr	PiNi	PiZn
Mokolo	mean	2.21	7.47	2.46	1.75	1.07	2.73
	sd	0.63	0.24	0.75	0.20	0.63	3.29
	p51	2.06	7.55	2.41	1.79	0.95	1.00
	min	1.49	7.14	1.33	1.49	0.30	0.91
	max	3.21	7.68	3.36	1.99	1.81	8.53
	CV(%)	28.62	3.28	30.58	11.62	59.1	120.38
Ekounou	mean	1.09	6.93	0.47	1.66	1.64	1.15
	sd	0.39	0.34	0.47	0.81	0.84	0.41
	p53	0.99	6.87	0.41	1.41	1.39	1.03
	min	0.68	6.53	0	1.14	0.82	0.70
	max	1.88	7.44	1.23	3.63	3.37	1.87
	CV(%)	35.53	4.84	98.44	48.65	51.19	52.95
Ekoumdoum	mean	0.75	6.08	0.10	1.66	2.42	1.00
	sd	0.17	1.26	0.19	0.27	1.68	0.31
	p55	0.73	6.64	0	1.59	2.29	1.00
	min	0.51	4.15	0	1.28	0.19	0.50
	max	1.02	7.80	0.62	2.21	5.48	1.49
	CV(%)	22.03	20.75	190.66	16.24	69.17	31.21
Nkolbisson	mean	0.52	4.01	0	1.20	0.11	0.51
	sd	0.23	0.28	0	0.19	0.19	0.15
	p57	0.47	3.96	0	1.17	0.03	0.49
	min	0.30	3.69	0	1.02	0	0.31
	max	0.86	4.47	0	1.56	0.49	0.69
	CV(%)	43.88	6.93	0	1.55	180.02	30.05

Appendix 9. Estimated daily intake (EDI) for carcinogenic risks.

Variables	Season	Cu	Cd	Pb	Ni	Cr	Zn
Mokolo	Dry	0.044	0.004	0.004	0.006	0.005	0.139
	Wet	0.045	0.003	0.001	0.002	0.004	0.277
Ekoumdoum	Dry	0.033	0.003	0.001	0.004	0.003	0.126
	Wet	0.02	0.003	0	0.004	0.003	0.115
Ekounou	Dry	0.027	0.002	0	0.002	0.004	0.113
	Wet	0.016	0.002	0	0.005	0.003	0.098
Nkolbisson	Dry	0.04	0.002	0	0.003	0.004	0.129
	Wet	0.011	0.002	0	0	0.002	0.051
Total intake	Dry & Wet	0.029	0.002	0.001	0.003	0.004	0.131
PTDI (mg per kg of body weight)		0.5	0.001	0.004	0.02	0.003	0.3-1