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Trace Metal Elements (Pb, Cd and As), 15N Nitrogen and Phosphorus Isotopes Accumulation in Three Varieties of Tomato from Conventional and Agro-Ecological Farming Systems in Burkina Faso

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Abstract

Tomato is a market gardening product that plays an important nutritional and economic role in Burkina Faso. However, the bad use of pesticides for its production could have negative impacts consumers' health. The objective of the present study was to assess the concentration of trace metal elements (Pb, Cd and As), 15N isotopes (NO_2^-, NO_3^-, NH_4^+) and phosphorus (PO_4^{2-}) in tomatoes according to cultivation practices. Thus, three tomato varieties (Mongal F1, F1 Cobra 26 and Roma F1) were grown using organic and conventional production methods on 2 sites at Nongr Massom, (commune of Kadiogo province). The trace metal elements were analyzed using atomic absorption spectrophotometry, the isotopes 15N (NO_2^-, NO_3^-, NH_4^+) and phosphorus (PO_4^{3-}) using standardized methods. Tomatoes from conventional agriculture had higher levels of trace metal elements (0.163 - 0.298 mg/Kg, 0.082 - 0.146 mg/Kg and 0.018 - 0.032 mg/Kg respectively for Pb, Cd and As) than those from organic agriculture samples. Concentration of trace metal is lower for organic production with a reduction of 19.02%, 19.69% and 20.77% for Pb, Cd and As respectively compared to conventional production.

High levels were recorded for the Roma F1 variety. The concentrations of trace metal elements in organic production are lower than the codex standards (2010) while those in conventional production are higher. These results could be due to the use of chemical inputs that could have a negative impact on the nutritional quality of these tomatoes. The concentration of trace metal elements in tomato fruits is strongly correlated by production method and less by the variety (genetic).

Keywords

Tomato, Metal Trace, Farming Practices

1. Introduction

Nowadays, agriculture including market gardening, is facing many challenges such as the increase in food supply and the need for agricultural production that guarantees food safety, nutritional quality and respect for the environment [1]. Indeed, market gardening production, particularly that of tomatoes in Burkina Faso plays an important nutritional and economic role, with annual production estimated at 300,000 tons, including an export of 100,000 tons [2]. Thus, its consumption contributes to the intakes of fibre, antioxidant compounds and minerals [3]. The profitability of the sector requires an intensive use of phytosanitary products to ensure good physical quality of the tomato without trace of pests. Studies on some market garden sites revealed bad phytosanitary practices such as the non-respect of prescribed doses and protection and hygiene rule during treatments, and mismanagement of empty pesticide packaging [4]. The bad use of pesticides in this intensive agricultural practice is now being questioned by research and civil society, given the negative impacts of market gardening on the environment, producers and consumers' health. Among these risks, the bioaccumulation of trace metal elements (lead, cadmium and arsenic) by market gardening products, including tomatoes, can lead to serious health risks (cancer, cardiovascular and hematological diseases). To face this situation, groups of stakeholders have emerged and are advocating sustainable agriculture through agroecological practices and effective consideration of the harmful environmental impacts of current forms of agriculture [5]. In Burkina Faso, the national council for organic agriculture (CNABio) is working to promote biological agriculture through the certification of several tomato production sites, including the agroecological school farm Béo-Neeré. Indeed, the practice of ecological market gardening produces fruits and vegetables with better micronutrient contents compared to conventional agriculture [6]. Moreover, the physico-chemical and nutritional characterization, particularly trace metal elements, 15N nitrogen and phosphorus isotopes contents, can be considered as an index of quality and authenticity of tomato varieties [7]. There is no comparative study on the above-mentioned parameters of tomato between an organic (BioSPG) and conventional site in Burkina Faso. The present study on three varieties of tomato aims to assess the influence of biological and conventional agriculture practices on the bioaccumulation of trace metal, 15N nitrogen and phosphorus isotope in tomato.

2. Materials and Methods

2.1. Sites

This study was carried out from July to October 2018 on 2 sites in Nongr Massom, commune of Kadiogo province. Organic production was installed on the Béo-Neeré site (Latitude 12˚28'38.45"N, Longitude 1˚29'5.60"W) and conventional production on the Doumtenga site (Latitude 12˚28'55.00"N, Longitude 1˚28'22.54"W) as presented in Figure 1. The two sites were 1.6 km apart in order to avoid any contamination.

2.2. Experimental Device and Agronomic Tests

The experimental device on the 2 sites is a Block Fisher with 3 repetitions (Figure 2). The elementary plots, separated by 1.5 m, are made up of 4 lines, each 3 m long. The distance between 2 contiguous lines is 0.8 m and the distance between plants is 0.5 m at the level of each elementary plot. The cultivation operations (soil preparation, nursery, watering, mulching, transplanting, weeding/weeding, hoeing, fertilization, phytosanitary treatment and harvesting) have been carried out according to the indications in the variety's technical data sheet. Organic production consisted in the use of organic manure: animal excrements,

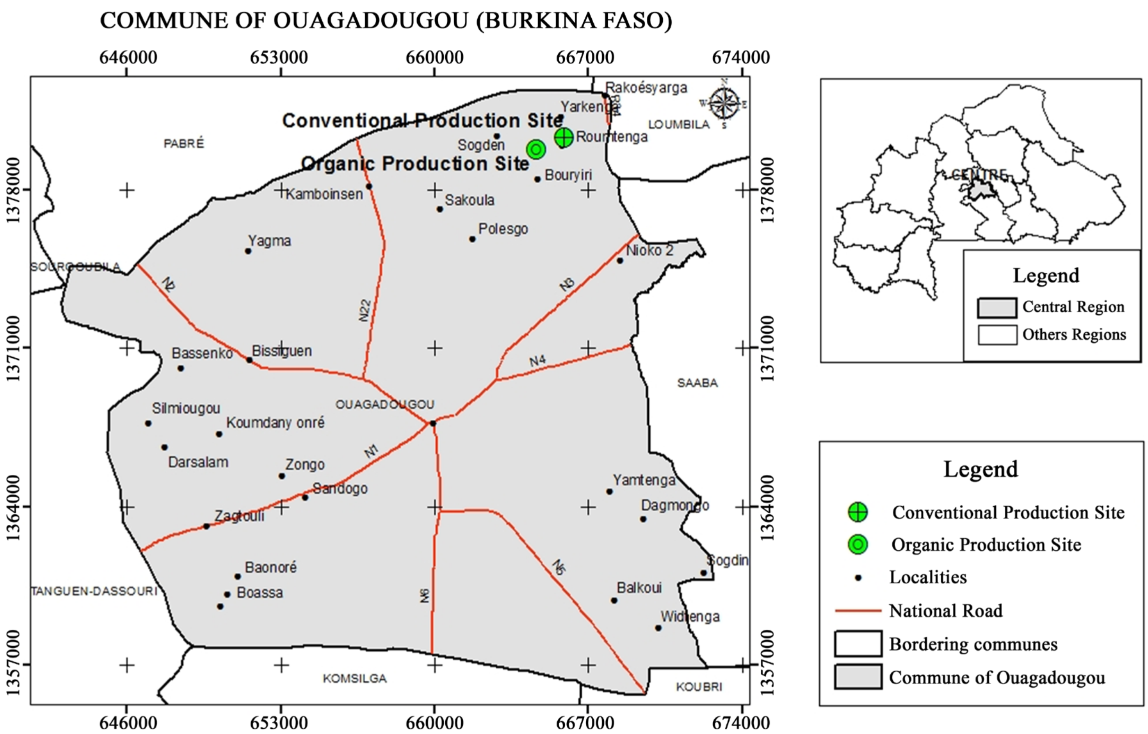
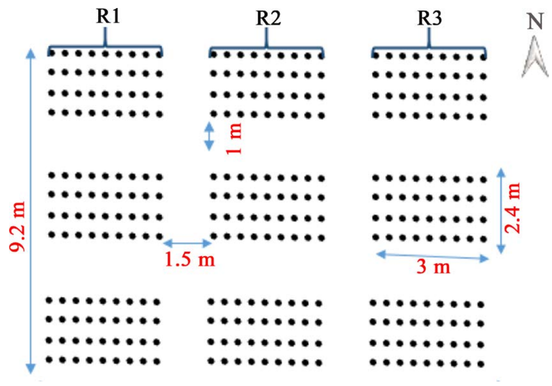


Figure 1. Studies sites localization.



R1: First repetition, R2: Second repetition and R3: Third repetition.

Figure 2. Experimental device.

ashes, neem leaves and biopesticide decoction based on *Azadirachta indica*, *Carica papaya* and *Capsicum frustescens*, respectively replacing the synthetic fertilizers and pesticides used in conventional agriculture.

2.3. Plant Material

Three varieties of tomato (Mongal F1, F1 cobra 26 and Roma VF) from organic and conventional production were used in the study. The tomatoes were harvested at maturity (90 days), bagged, labelled and led to the laboratory for future analysis. The labels MongBio, CobBio and RomBio referred respectively to the variety Mongal F1, F1 cobra 26 and Roma F1 for organic production while the labels MongConv, CobConv and RomConv referred respectively to the variety Mongal F1, F1 cobra 26 and Roma F1 for conventional production. For analysis, the samples are first washed, disinfected with 2% bleach and rinsed with distilled water and then crushed with an electric mill. samples thus treated have been stored in jars at −4˚C for future analyses.

2.4. Quantification of Mineral Trace Elements

Atomic absorption spectrophotometry (AAS) flame was used for the determination of cadmium, lead and arsenic in extraction solvents, according to the method described by Walsh, (1955). It allows the single-element determination of cations in trace amounts of the order of µg/L. Indeed, 0.2 g of each sample was mineralized in 10 ml of concentrated nitric acid HNO_3 (70%) for 24 h beforehand. The mineralized sample had, following a 1/100 dilution with MilliQ water used to quantify the Cadmium, Lead and Arsenic contents with the atomic absorption spectrometer. The commercial solutions of cadmium (Cd), lead (Pb) and arsenic were used for the calibration curves.

2.5. Quantification of 15N Nitrogen and Phosphorus Isotopes

Visible molecular absorption spectrometry was used for the determination of nitrates, nitrites and orthophosphates in tomato extracts.

Nitrates (NO_3^-)

The nitrate content was determined according to the method described by Rodier and al., (2009). The presence of nitrates was revealed by adding 0.5 ml of sodium acid solution (0.5 mg/ml) and 0.2 ml of acetic acid solution to 10 ml of extract. After dry evaporation in a water bath (75˚C - 80˚C) for 5 minutes, 1 ml of sodium salicylate solution (10 g/L) was added, then shaken vigorously with a vortex, evaporated and air cooled. The residue is recovered, moistened with 1 ml of concentrated sulphuric acid. To the reaction medium, 15 ml of deionized water was added and then 10 ml of sodium hydroxide solution which develops the yellow color. The nitrate content was measured with a spectrophotometer at a wavelength of 415 nm. A deionized water was used in place of the extract to made a control. The nitrate content (mg/L) was determined using a calibration curve with different concentrations of a nitric acid stock solution (0 - 200 mg/L).

Nitrites (NO_2^-)

The nitrites were determined according to the method described by Rodier and al. (1978). In presence of ammonium, ion and phenol, sulphanilic acid in a hydrochloric medium forms a yellow complex with the nitrite ions, which intensity is proportional to the nitrite concentration. The reaction medium made with 50 ml of the extract, 2 ml of Zambelli's reagent (5 g of sulphanilic acid, 7.5 g of phenol, 135 g of ammonium chloride in 260 ml of hydrochloric acid ml and 625 ml of distilled water) and 2 ml of pure ammonia. The absorbances were read at 630 nm with the spectrophotometer with reference to a blank. The values obtained were directly extrapolated to a standard nitrous nitrogen curve (0 - 200 mg/L).

Ammonium (NH_4^+)

A stock solution of 1 g tomato in 10 ml distilled water has been prepared. The filtrate obtained through GFC membrane filtration (0.2 micrometer diameter) was introduced after adequate dilution into 25 ml test tube. Nessler reagents for NH_4^+ were added to the test tube. The stirring and waiting times were 30 seconds and 1 minute. The reading was taken with the DR 3900 spectrophotometer after first passing a control cell containing the sample without reagents.

Orthophosphates (PO_4^{3-})

The orthophosphate content was analyzed according to the method described by Rodier and al. (2009). In an acid medium containing ammonium molybdate, orthophosphates give a phosphomolybdic complex which could be reduced by ascorbic acid and develops a blue coloration that can be read by a spectrometric (Rodier and al., 2009). The reaction medium is prepared with 20 ml of the extract (pH adjusted between 2 - 7), 1 ml ascorbic acid and 4 ml of the combined reagent (50 ml sulphuric acid at 15%, 5 ml double tartrate of antimony and potassium at 2.8 g/L, 5 ml ammonium molybdate at 40 g/L and 100 ml distilled water). After a 30 minutes' incubation, the absorbances were read with a spectrophotometer at wavelength 800 nm. The absorbance of the control was taken into account and the content of orthophosphates was determined using a calibration curve carried out with different concentrations of a phosphorus stock solution (0 - 200 mg/L).

2.6. Statistical Processing

Microsoft Excel 2019, GraphPad 2016 and XLSTAT-Basic version 2020.3 have been used for processing, data analysis and graph design. The PCA analysis was performed using R Studio software version 3.6.3 with the FactoMinR package in order to see the grouping of the different samples in relation to the studied factors.

3. Results and Discussion

3.1. Results

Influence of agricultural practices

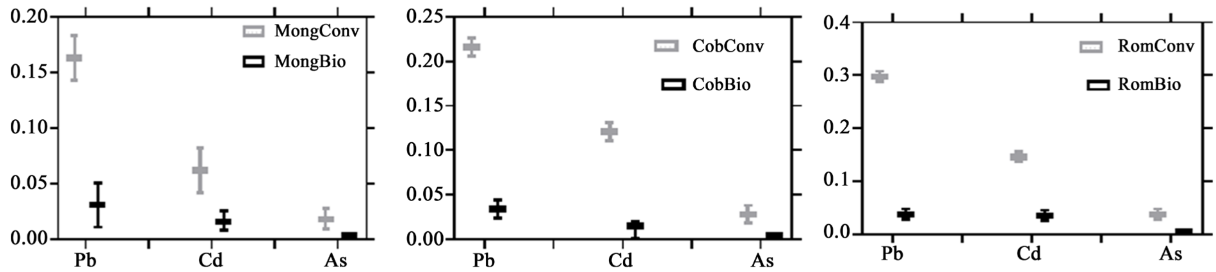
According to agricultural practices, Table 1, summarize the contents of Pb, Cd and As respectively for the tomato varieties Mongal-F1, F1-Cobra-26, and Roma-F1. These results indicate that the concentration of Pb, Cd and As in samples from conventional agriculture were respectively from 0.163 to 0.298 mg/Kg, from 0.082 to 0.146 mg/Kg and from 0.018 to 0.032 mg/Kg. In those from organic agriculture contents varied from 0.031 to 0.038 mg/Kg, from 0.015 to 0.025 mg/Kg respectively for Pb and Cd and were from 0.004 mg/Kg for As. A significant difference was noted between the contents of metallic trace elements in the samples from conventional agriculture and those from organic agriculture (P-value < 0.0001). For each variety, sample from conventional growing had the highest values as presented in Figure 3.

The Principal Component Analysis (PCA) in Figure 4 shows a total percentage of inertia of 99.8% (97.9% for F1 and 1.8% for F2) of the viable results. A good representation of Cd, Pb and As was observed with respect to the main F1 axis for the samples from conventional agriculture. Two distinct groups are observed revealing the correlation between the agricultural practices and their

Table 1. Trace metals contents ($mg \cdot Kg^{-1}$ FW) of conventional and organic tomato.

Varieties	Cultural practices	Pb (mg/Kg)	Cd (mg/Kg)	As (mg/Kg)
Mongal-F1	Conventional	0.163 ± 0.020^a	0.082 ± 0.003^a	0.018 ± 0.001^a
	Organic	0.031 ± 0.001^b	0.016 ± 0.005^b	0.004 ± 0.001^b
	Reduction rate (%)	19.02	19.69	20.77
F1-Cobra-26	Conventional	0.216 ± 0.029^a	0.121 ± 0.006^a	0.028 ± 0.002^a
	Organic	0.034 ± 0.001^b	0.015 ± 0.002^b	0.004 ± 0.001^b
	Reduction rate (%)	15.75	12.80	15.38
Roma-F1	Conventional	0.298 ± 0.024^a	0.146 ± 0.001^a	0.032 ± 0.001^a
	Organic	0.038 ± 0.003^b	0.025 ± 0.002^b	0.004 ± 0.001^b
	Reduction rate (%)	12.90	16.94	13.03
P-value		<0.0001	<0.0001	<0.0001

*In the same column and for the same variety, the values with different superscript letters are significantly different (P < 0.05).



MongConv: Conventional Mongal F1, MongBio: Organic Mongal F1 CobConv: Conventional F1 Cobra 26, CobBio: Organic F1 Cobra 26, RomConv: Conventional Roma F1, RomBio: Organic Roma F1.

Figure 3. Trace metals contents (mg·Kg^{-1} FW) according to tomato varieties: a-Mongal F1, b-F1 Cobra 26, c-Roma F1.

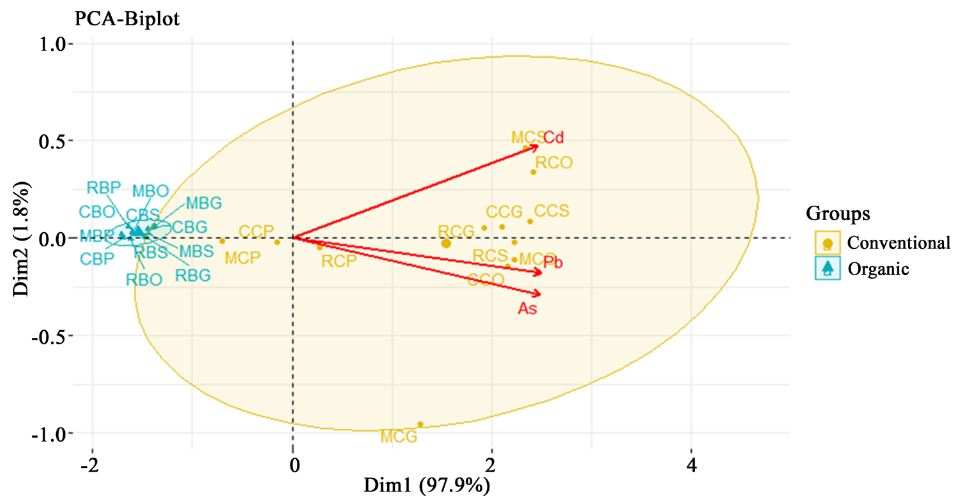


Figure 4. Principal component analysis of trace metals characteristic.

contents of metals traces elements and nitrogen isotope 15N. These results show a positive correlation between the concentration of Pb, As and Cd with conventional agriculture. Samples from conventional growing showed the highest contents as presented in Figure 3.

Table 2 shows the nitrite, nitrate, ortho-phosphate and ammonium content of the three tomato varieties. The concentrations of nitrite, nitrate, phosphate and ammonium varied respectively in samples from conventional growing from 10.400 to 38.200 mg/Kg, from 60.000 to 2420.000 mg/Kg, from 190.333 to 3700.333 mg/Kg and from 100.333 to 305.000 mg/Kg. for the organic growing, contents varied from 16.800 to 55.533 mg/Kg, from 159.667 to 280.000 mg/Kg, from 6320.000 to 61600.000 mg/Kg and from 230.000 to 8800.333 mg/Kg respectively for nitrite, nitrate, phosphate and ammonium. These results show that there is a high accumulation of nitrites, phosphates and ammonium in tomatoes from conventional farming compared to organic farming except nitrates.

Influence of varieties

Figure 5 and Figure 6 show the comparative contents of the varieties Mongal-F1, F1-Cobra-26, and Roma-F1 for conventional and organic farming respectively. For conventional and organic farming, the Roma-F1 variety has the highest content of Pb (0.298 (μg/Kg), Cd (0.146 (μg/Kg) and As (0.032 (μg/Kg).

Table 2. Nitrite, nitrate, ortho-phosphate and ammonium content of the three tomato varieties from organic and conventional practices.

Growing Methods	Samples	Nitrite (mg/kg)	Nitrate (mg/kg)	Phosphate (mg/kg)	Ammonium (mg/kg)
Organic	MongBio	19,000^{d}	380,000^{b}	3,700,333^{d}	200,333^{e}
	CobBio	10,400^{f}	60,000^{f}	190,333^{f}	100,333^{f}
	RomBio	38,200^{c}	2,420,000^{a}	664,667^{e}	305,000^{c}
Conventional	MongConv	55,533^{a}	159,667^{e}	6,320,000^{c}	1,880,000^{b}
	CobConv	16,800^{e}	240,000^{d}	16,900,667^{b}	230,000^{d}
	RomConv	46,000^{b}	280,000^{c}	61,600,000^{a}	8,800,333^{a}

*In the same column the values with different superscript letters are significantly different ($P < 0.05$).

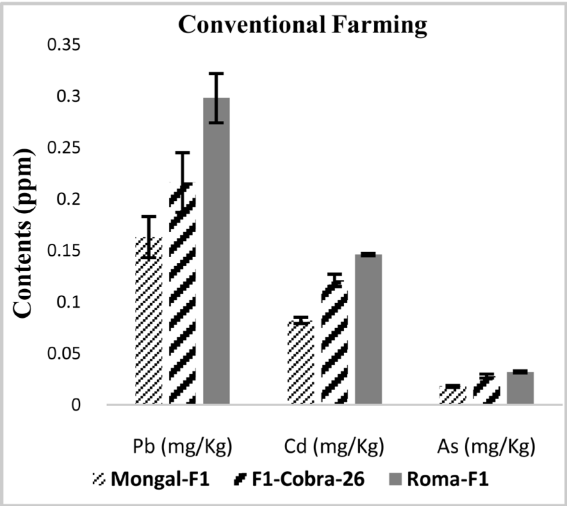


Figure 5. Trace metals (Pb, Cd and As) contents according tomato varieties from conventional farming.

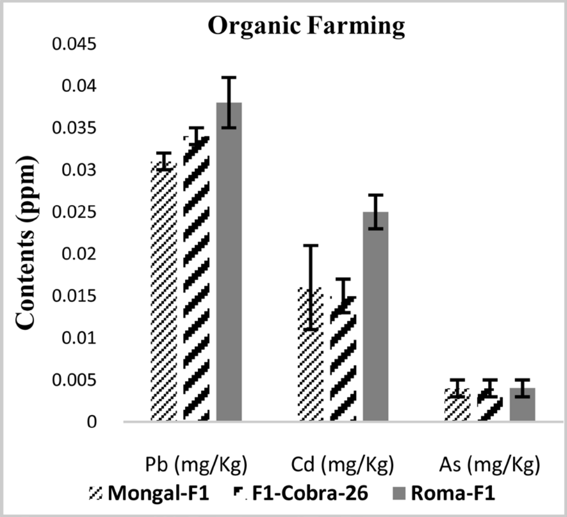


Figure 6. Trace metals (Pb, Cd and As) contents according tomato varieties from organic farming.

For nitrites, nitrates, orthophosphates and ammonium significant difference was noted between varieties. The high concentrations of nitrites, nitrates, orthophosphates and ammonium were respectively 55.33 mg/L (MongConv), 2420 mg/L (RomBio), 61,600 mg/L (RomConv) and 8800 mg/L (RomConv).

3.2. Discussion

The present study shows that conventional agriculture would be responsible for a strong bioaccumulation of trace metal elements (Pb, Cd and As), nitrogen anions and phosphorus compared to organic agriculture. A comparative analysis with the Codex Stan 193 - 1995 standards reveals that the levels of Pb, Cd and As in the three conventional tomato varieties are well above the maximum limits of 0.1 mg/kg, 0.05 mg/kg and 0.05 mg/kg respectively. In contrast, the three organically grown tomato varieties are however below the Codex Stan 193-1995 standards. This high level of conventional agriculture compared to organic agriculture in our samples could be justified by the diversity of inputs used in each production method. The conventional farmers used dam water, commercial fertilizers and pesticides for tomato cultivation. These inputs could be potential sources of contamination among many others. Pb can be found in small amounts in the earth's crust, existing in different chemical forms: metallic (pure metal); inorganic compounds, such as lead oxide, lead sulfate, lead chromates, lead silicates, lead arsenates, and lead chloride; and organic compounds, such as tetraethyl lead [8]. World production amounts to millions of tons and is used in the manufacture of accumulators, batteries, solders, pigments, cables, ceramics, soldering and building materials (because its excellent resistance to corrosion), and anti-rust agents (red lead/lead oxide), and leaded petrol [9]. Phosphate fertilizers, nonferrous metals production, and the iron and steel industry are the sources of Cd in water [10]. Main inputs to agricultural soils, which are of primary relevance to human exposure to Cd, arise from atmospheric deposition, sewage sludge application, insecticides, fungicides and phosphate fertilizer application [8].

The Béo-Neeré site which hosted the organic production was a site certified by the CNABio. In fact, the procedure of certification of BioSPG site follows the standard set up and defining the conditions of organic production [11]. In this production any chemical inputs were excluded. The values reported of conventional growing in this study are low than the results found by Oboulbiga and al. [12] on tomatoes from three conventional production sites (Loumbila, Ouahigouya and Ouagadougou) for the total Pb (1.15 to 1.27 ppm) and As (0.19 and 0.20 ppm) but similar for the total Cd content (0.11 to 0.22 ppm). Indeed, the increased use of pesticides could be the sources of accumulation of Pb, Cd and As in soil and would be bio-assimilated by the plant. In addition, liming materials derived from industrial waste and contained in organic fertilizers may contain a number of heavy metals [13]. These trace metal elements would constitute health risks through the phenomenon of bioaccumulation. Cadmium is particularly dangerous because it can be absorbed from the digestive tract, penetrate through the placenta during pregnancy, and damage membranes and DNA [14]. All three varieties would similarly accumulate As, while the Roma F1 variety would accumulate Pb and Cd to a greater extent than the Mongal F1 and F1-Cobra 26. Tomato quality depends on several factors including the choice of cultivar, cultural practices, harvest time and method, storage, and handling procedures [15].

Urea, ammonium sulfate and ammonium nitrate are the main nitrogen fertilizers for soil application [16]. The present study showed that the conventional agriculture revealed a high concentration of nitrogen and phosphorus isotopes compared to organic agriculture. Indeed, the use of nitrogen and urea fertilizers in conventional agriculture contributes to an increase in the 15N nitrogen and phosphorus isotope content. Whereas natural fertilizers used in organic farming have a low potential for accumulation of nitrite, nitrate, ammonium and phosphorus. The nitrate content in this study was lower than the results found by Zoran and al. [15] on three tomato varieties from conventional and organic growing, range 20 to 27 $mg \cdot kg^{-1}$ and 34 to 45 $mg \cdot kg^{-1}$ for organic and conventional growing respectively. The tomato belongs to the vegetable plants which accumulate less nitrates than other vegetables (100 to 150 $mg \cdot kg^{-1}$). Among the accumulation capacity of the three varieties, the Roma variety showed the highest concentrations of nitrates (2420 mg/kg), phosphates (61600 mg /kg) and ammonium (8800 mg/kg) with the exception of nitrites where the Mongal F1 variety has the highest content (55.533 mg/kg). Nitrate content of vegetables depends on a number of external and internal factors. From external factors should be mentioned, supply of substrate with nitrate, light, day length, temperature, season, supply with water, relative humidity, carbon dioxide concentration in the air, supply with biogenic elements, influence of the accompanying cations, heavy metals, herbicides, chemical properties of the soil, location, time of sowing, time and method of harvest, storage conditions [17]. No studies on nitrite, phosphorus and ammonium content on tomato were founded to compare the results in this study.

The principal component analysis shows a positive correlation between tomatoes from conventional agriculture and a negative correlation with tomatoes from organic agriculture. These results show the influence of agricultural inputs (NPK, urea, phyto-pesticides) on the content of heavy metals, phosphorus and nitrogen isotope compared to those used in organic agriculture (compost, natural fertilizer, biopesticides). On the other hand, many studies have demonstrated the positive impact of organic farming on soil fertility, through an increase in soil organic matter content and available nutrients. Organic farming is believed to have a higher mineral potential compared to conventional agriculture [6]. These results are similar to those obtained by Osma and al. [18] who justified the high content of trace metal elements by the use of fertilizers and the proximity of industries and irrigation water quality.

4. Conclusion

The levels of trace metal elements in tomato fruits are highly dependent on the concentration of these elements in the environment and also on agricultural practices. The results of the present study show that organic farming has a low bioaccumulation of trace metal elements in the three tomato varieties compared to conventional farming. In addition, trace metal element levels in this agriculture practices are in line with the standard requirements of the Codex. The study shows that the production sites in Béo-Neeré where the organic experiments were carried out and the cultivation practices applied offer tomatoes of better sanitary quality with regard to the low levels of trace metal elements in the fruit. Indeed, the massive and repeated use of trace metal elements and their persistence in the environment can lead to the contamination of ecosystems and thus present risks for living organisms.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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