

University of São Paulo  
Luiz de Queiroz College of Agriculture

Bioconcentration and desorption kinetic of potentially toxic elements in soils  
cultivated with horticultural crops in organic and conventional systems

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Thesis presented to obtain the degree of Doctor in  
Science. Area: Soil and Plant Nutrition

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To my family that always gave me roots to grow and wings to fly,  
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*"We must cultivate our garden."*

*Candide: or Optimism – Voltaire*

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## RESUMO

**Bioconcentração e cinética de dessorção de elementos potencialmente tóxicos em solos hortícolas sob sistemas orgânico e convencional**

Elementos potencialmente tóxicos (EPTs) podem estar biodisponíveis para serem absorvidos e acumulados nos tecidos e órgãos vegetais, sendo esta uma importante rota de entrada de EPTs na cadeia alimentar. O manejo do solo pode interferir no comportamento dos EPTs no sistema. Desta forma, o estudo da dinâmica desses elementos no solo é fundamental para melhor entendimento dos fatores que influenciam a transferência solo-planta. No estado de Pernambuco, a produção de hortaliças se concentra em intensos cultivos orgânicos e convencionais localizados em pequenas ou médias propriedades, principalmente na região Agreste e São Francisco. Nossos objetivos neste trabalho foram: i) determinar os teores de EPTs no solo e nas hortaliças produzidas em sistemas convencionais e orgânicos em hortas de Pernambuco; ii) investigar as formas de EPTs e sua absorção pelas hortaliças com base em diferentes fatores de transferência solo-planta; iii) usar os fatores de transferência calculados para investigar o risco de exposição aos humanos; iv) estudar o efeito do tempo e da matéria orgânica (MO) na dessorção de cobre (Cu) em solos de horta; v) determinar se a aplicação de esterco aumenta a concentração de Cu solúvel em água; e vi) verificar a eficiência de técnicas machine learning na classificação de hortaliças produzidas em sistemas orgânicos e convencionais. Soluções ácidas e complexantes foram utilizadas para extrair os teores totais de EPTs nas plantas e os teores pseudototal, reativo e biodisponível dos EPTs no solo. Fatores de transferência solo-planta e avaliação de risco humano foram calculados com base nos teores de EPTs. Estudos de cinética foram desenvolvidos após adição de composto orgânico em solos contaminados com Cu para modelar a dessorção de Cu. Cu solúvel em água e carbono orgânico dissolvido (COD) foram extraídos de solos adubados com esterco, a interação Cu-COD foi estudada usando técnicas espectroscópicas. Folhas de alface (*Lactuca sativa*) apresentaram a maior capacidade de acumular EPTs; o sistema convencional promoveu maiores teores de EPTs nos solos e plantas do que o sistema orgânico; independentemente do cenário, crianças e adultos em contato com vegetais produzidos em sistema orgânico tem um menor risco do que os indivíduos que consomem folhas de alface convencionalmente produzidas. A adição de MO promoveu maior dessorção de Cu, enquanto o aumento no tempo de contato reduziu a liberação de Cu. Os gráficos da espectroscopia de infravermelho com transformada de Fourier mostraram um aumento na presença de grupos funcionais alifáticos e carboxílicos nos extratos de solos adubados com esterco. Esses grupos funcionais no COD formam ligações fortes com Cu, o que promove o transporte do elemento na forma de complexos orgânicos dissolvidos no perfil do solo. O modelo Support Vector Machine apresentou acurácia superior a 90% na classificação das hortaliças em dois grupos (orgânicas e convencionais); e a alta taxa de assertividade mostra o potencial do uso de quantificação elementar e algoritmos como técnicas auxiliares no processo de autenticidade de inspeção de produtos orgânicos.

Palavras-chave: Metais pesados; Solos agrícolas; Fitodisponibilidade; Compostos orgânicos; Sistemas em não-equilíbrio

## ABSTRACT

**Bioconcentration and desorption kinetic of potentially toxic elements in soils cultivated with horticultural crops in organic and conventional systems**

Potentially toxic elements (PTEs) can be absorbed and accumulated in vegetables organ and tissues, and this is an important channel through which PTEs can enter into the food chain. Soil management may interfere in the behavior of PTEs in the system. So, the study of the dynamics of elements in the soil is fundamental to a better understanding of factors that influence soil-plant transfer. In the state of Pernambuco, Brazil, the production of vegetables is mainly concentrated in small or medium organic and conventional farms located in the Agreste and São Francisco regions. Our aims in this research were: i) to determine the contents of PTEs in soil and in vegetables produced under conventional and organic systems in garden soils of Pernambuco; ii) to investigate the forms of the PTEs and their uptake by the vegetables based on different soil-plant transfer factors; iii) to use the calculated transfer factors to assess the human risk exposure; iv) to assess the effect of aging and organic matter (OM) on copper (Cu) desorption in garden soils; v) to determine if manure application to soils increases water soluble Cu concentrations; and vi) to verify the efficiency of machine learning techniques in the classification of vegetables produced under organic and conventional systems. Acid and complexing solutions were employed to extract total contents of PTEs in plants and to extract pseudototal, reactive and bioavailable fractions of the PTEs in soils. Soil-plant transfer factors and human risk assessment were calculated based on the determined levels of PTEs. Kinetic assays were developed using rates of organic composts added to Cu-spiked soils to model the Cu desorption. Water extractable Cu and dissolved organic carbon (DOC) were extracted from soils amended with dairy manure, and the Cu-DOC interactions were studied using spectroscopic techniques. Lettuce (*Lactuca sativa*) leaves had the highest capacity to accumulate PTEs; conventional system promoted higher PTEs values in soils and plants than the organic production; regardless of the scenario, children and adults in contact to vegetables cropped in organic system have a lower health risk than individuals exposed to lettuce leaves conventionally produced. The addition of OM promoted greater Cu desorption, while aging lead to a reduction in the Cu release. The Fourier-transform infrared spectroscopy spectra showed increase in the presence of aliphatic and carboxylic groups in the extracts from the manure-amended soils. Carboxylic acid functional groups on the DOC formed strong bonds with Cu, which can promote Cu transport as dissolved Cu-organic complexes in the soil profile. The Support Vector Machine had an accuracy over 90% to classify the vegetables in the two groups (organic and conventional); and this high assertiveness rate highlights the potential of using elemental quantification and algorithms as support techniques in the process of authenticity and inspection of organic products.

Keywords: Heavy metals; Agricultural soils; Phytoavailability; Organic amendment; Non-equilibrium system



## 1. INTRODUCTION

The state of Pernambuco, Brazil, is responsible for 20% of the horticultural production in Northeast of Brazil (IBGE, 2006). In a detailed study carried out in vegetable-producing properties in the region of Camocim de São Félix (Pernambuco), Embrapa (2000) verified that the farms are small, but intensely cultivated (average of three crops annually). In this region, an abusive use of pesticides and mineral and organic fertilizer has been observed for at least 30 years (Embrapa, 2000). In this way, the contamination and the eutrophication of watercourses are common.

During the last decades, alternative crop systems have been developed in response to an increasing social pressure for better quality and environmental friendly products (de Souza Araújo et al., 2014). The agricultural system called “organic” promotes the production of food free of pesticides or any other synthetically produced compost, and aim to obtain safer products to the consumer. However, de Souza Araújo et al. (2014) found out organic vegetables been sold in the central market of Pernambuco with Cr, Cu, Fe and Zn contents higher than in the conventionally produced vegetables. Gundersen et al. (2000) analyzed the chemical composition of onions (*Allium cepa*) and peas (*Pisum sativum*) produced organically and conventionally in Denmark, and testified the efficiency of elemental composition differentiation for control and authenticity of organic products. Kelly and Bateman (2010) carried this same kind of evaluation but in samples of organic and conventional tomatoes (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*) from United Kingdom and United States of America, and found higher concentrations of Ca, Cu, Zn and Rb in organic tomatoes, while the levels of Cu and Rb were higher in organic lettuce.

The consumption of vegetables is the main source of most of the potentially toxic elements (PTEs) uptake by humans (Chen et al., 2018). To assess human health risk related to vegetable consumption, it is necessary to complement the usual PTEs availability assessment procedures based on total and extractable levels in plants and soils (Antoniadis et al., 2017). Leaf and fruit vegetables usually accumulate more PTEs than tube vegetables, suggesting that there is a larger potential health risk by ingestion of PTEs through fruit and leaf horticultures (Fan et al., 2017). Besides the crop, the production system also can influence the PTEs absorption by plants through modulating their availability (Chen et al., 2014; Fan et al., 2017; Khan et al., 2017).

The bioavailability and mobility of PTEs in soil strongly depends upon the extent of their interaction with soil constituents (Chaturvedi et al., 2006). So, the understanding of the sorption and desorption kinetics of PTEs in soils can help us to model their dynamics in the environment (Shi et al., 2013), and could be very important for the study of risks to ecological receptor and human health (Ming et al., 2016).

The application of organic fertilizers is a routine practice in producing-vegetables systems. The effect of the application of organic compounds on the mobility, migration, reactivity and availability of PTE in the soil depends on the rate and frequency of application, as well as its organo-mineral properties (Cambier et al., 2014). The dissolved organic carbon (DOC) is the most reactive fraction of the organic matter and has different complexation capacities (Hernandez-Soriano et al., 2013). DOC complexed with di- and trivalent metal cations at mineral surfaces might promote detaching of metal complexes and, hence, increase the total dissolved concentration of these cations (Strobel et al., 2001). For example, the complexation of Cu by DOC is relatively strong, and the high concentrations of Cu-DOC complexes in the soil solution may increase Cu leaching through the soil profile (Strobel et al., 2001) or the Cu phytoavailability and uptake by plants (Sun et al., 2017).

Soil chemical process can be studied from both equilibrium and kinetic viewpoints. The study of equilibrium conditions using thermodynamic approach can predict only the final state of the soil system from an initial non-equilibrium state. However, as a result of many phenomena (like slow chemical reactions, plant uptake and fertilizer addition), soils are rarely in a state of equilibrium from the sorption/desorption point of view (Baranimotlagh and Gholami, 2013). Based on this, methods that promote non-equilibrium system could better represent the soil system (Guedes et al., 2016). Continuous influx of solution into a flow cell (as promoted in the stirred-flow chamber) produces steady-state conditions for reactions with the soil sample and allows frequent sampling (Strobel et al., 2001). The use of stirred-flow chamber is vast in literature (Fernández-Calviño et al., 2017; Strawn and Sparks, 2000; Wang et al., 2009) and allow the study of changes in pH, organic matter, aging and many other situations under a non-equilibrium condition.

Understanding quantitatively the kinetic of sorption and desorption of PTEs in soils could help to model their dynamic behavior in the environment (Shi et al., 2013). This kind of study is even more important in garden soils, where the intense use and tillage of the soil, summed to the constant compost application significantly change the dynamic of the elements in the soil and their availability to the plants (Xu et al., 2013).

Once the system applied changes the dynamic of the elements in the soils and their uptake by plants, we hypothesized that it is possible to use elemental composition to differentiate and classify vegetables organically and conventionally produced. However, a simple mean comparison could not be the best approach to compare and differentiate organic and conventional foodstuff. To a better analysis of the chemical composition of the products, statistical and mathematical methods are used to facilitate the interpretation of the results, such principal component analysis (Gundersen et al., 2000; Laursen et al., 2011) or machine learning models (Hernández-Sánchez et al., 2012). For the machine learning models, algorithms are used to classification and regression test from analysis of results and pattern recognition. In this way, this instrument could identify a group in which a new sample belong, based on a preview classification (Barbosa et al., 2015). In other words, machines learning are, basically, a prediction tool (Kotsiantis et al., 2006). In Brazil, this kind of approach is recent. Barbosa et al. (2014) carried out studies with organic coffee (*Coffea*), Barbosa et al. (2015) worked with sugarcane (*Sccharum officinarum*), Borges et al. (2015) classified eggs and Barbosa et al. (2016) evaluated rice (*Oryza sativa*).

Our general aims in this research were: i) to determine the contents of PTEs in soil and in vegetables produced under conventional and organic systems in garden soils of Pernambuco; ii) to investigate the forms of the PTEs and their uptake by the vegetables based on soil-plant transfer factors (TF); iii) to use the calculated TFs to assess the human risk exposure; iv) to assess the effect of aging and organic matter (OM) on copper (Cu) desorption in garden soils; v) to determine if manure application to soils increases water soluble Cu concentrations; and vi) to verify the efficiency of machine learning techniques in the classification of vegetables produced under organic and conventional systems.

## References

- Antoniadis, V., Shaheen, S.M., Boersch, J., Frohne, T., Du Laing, G., Rinklebe, J., 2017. Bioavailability and risk assessment of potentially toxic elements in garden edible vegetables and soils around a highly contaminated former mining area in Germany. *J. Environ. Manage.* 186, 192–200. doi:10.1016/j.jenvman.2016.04.036
- Baranimotlagh, M., Gholami, M., 2013. Time-Dependent Zinc Desorption in Some Calcareous Soils of Iran. *Pedosphere* 23, 185–193. doi:10.1016/S1002-0160(13)60006-5

- Barbosa, R.M., Batista, B.L., Barião, C. V., Varrique, R.M., Coelho, V. a., Campiglia, A.D., Barbosa, F., 2015. A simple and practical control of the authenticity of organic sugarcane samples based on the use of machine-learning algorithms and trace elements determination by inductively coupled plasma mass spectrometry. *Food Chem.* 184, 154–159. doi:10.1016/j.foodchem.2015.02.146
- Barbosa, R.M., Batista, B.L., Varrique, R.M., Coelho, V. a., Campiglia, A.D., Barbosa, F., 2014. The use of advanced chemometric techniques and trace element levels for controlling the authenticity of organic coffee. *Food Res. Int.* 61, 246–251. doi:10.1016/j.foodres.2013.07.060
- Barbosa, R.M., Silva, E., Paula, D., Carolina, A., Moore, A.F., Maria, J., Souza, O., Lemos, B., Campiglia, A.D., Barbosa, F., 2016. Journal of Food Composition and Analysis Recognition of organic rice samples based on trace elements and support vector machines. *J. Food Compos. Anal.* 45, 95–100. doi:10.1016/j.jfca.2015.09.010
- Borges, E.M., Volmer, D. a, Gallimberti, M., de Souza, D.F., de Souza, E.L., Barbosa, F., 2015. Evaluation of macro- and microelement levels for verifying the authenticity of organic eggs by using chemometric techniques. *Anal. Methods* 7, 2577–2584. doi:DOI 10.1039/c4ay02986k
- Cambier, P., Pot, V., Mercier, V., Michaud, A., Benoit, P., Revallier, A., Houot, S., 2014. Impact of long-term organic residue recycling in agriculture on soil solution composition and trace metal leaching in soils. *Sci. Total Environ.* 499, 560–573. doi:10.1016/j.scitotenv.2014.06.105
- Chaturvedi, P.K., Seth, C.S., Misra, V., 2006. Sorption kinetics and leachability of heavy metal from the contaminated soil amended with immobilizing agent (humus soil and hydroxyapatite). *Chemosphere* 64, 1109–1114. doi:10.1016/j.chemosphere.2005.11.077
- Chen, L., Zhou, S., Shi, Y., Wang, C., Li, B., Li, Y., Wu, S., 2018. Heavy metals in food crops, soil, and water in the Lihe River Watershed of the Taihu Region and their potential health risks when ingested. *Sci. Total Environ.* 615, 141–149. doi:10.1016/j.scitotenv.2017.09.230
- Chen, Y., Huang, B., Hu, W., Weindorf, D.C., Liu, X., Yang, L., 2014. Accumulation and ecological effects of soil heavy metals in conventional and organic greenhouse vegetable production systems in Nanjing, China. *Environ. Earth Sci.* 71, 3605–3616. doi:10.1007/s12665-013-2752-x
- de Souza Araújo, D.F., da Silva, A.M.R.B., de Andrade Lima, L.L., da Silva Vasconcelos, M.A., Andrade, S.A.C., Asfora Sarubbo, L., 2014. The concentration of minerals and physicochemical contaminants in conventional and organic vegetables. *Food Control* 44, 242–248. doi:10.1016/j.foodcont.2014.04.005
- Embrapa, 2000. Boletim de Pesquisa. Bol. Pesqui. Número 9 - Embrapa 1–59. doi:1980-6841
- Fan, Y., Li, H., Xue, Z., Zhang, Q., Cheng, F., 2017. Accumulation characteristics and potential risk of heavy metals in soil-vegetable system under greenhouse cultivation condition in Northern China. *Ecol. Eng.* 102, 367–373. doi:10.1016/j.ecoleng.2017.02.032
- Fernández-Calviño, D., Cutillas-Barreiro, L., Paradelo-Núñez, R., Nóvoa-Muñoz, J.C., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., Arias-Estévez, M., 2017. Heavy metals fractionation and desorption in pine bark amended mine soils. *J. Environ. Manage.* 192, 79–88. doi:10.1016/j.jenvman.2017.01.042
- Guedes, R.S., Melo, L.C.A., Vergütz, L., Rodríguez-Vila, A., Covelo, E.F., Fernandes, A.R., 2016. Adsorption and desorption kinetics and phosphorus hysteresis in highly weathered soil by stirred flow chamber experiments. *Soil Tillage Res.* 162, 46–54. doi:10.1016/j.still.2016.04.018
- Gundersen, V., Bechmann, I.E., Behrens, A., Stürup, S., 2000. Comparative investigation of concentrations of major and trace elements in organic and conventional Danish agricultural crops. 1. Onions (*Allium cepa* Hysam) and peas (*Pisum sativum* Ping Pong). *J. Agric. Food Chem.* 48, 6094–6102. doi:10.1021/jf0009652

- Hernández-Sánchez, C., Luis, G., Moreno, I., Cameán, A., González, A.G., González-Weller, D., Castilla, A., Gutiérrez, A., Rubio, C., Hardisson, A., 2012. Differentiation of mangoes (*Mangifera indica* L.) conventional and organically cultivated according to their mineral content by using support vector machines. *Talanta* 97, 325–330. doi:10.1016/j.talanta.2012.04.038
- Hernandez-Soriano, M.C., Peña, A., Mingorance, M.D., 2013. Soluble metal pool as affected by soil addition with organic inputs. *Environ. Toxicol. Chem.* 32, 1027–1032. doi:10.1002/etc.2159
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE. Censo agropecuário: Brasil, grandes regiões e unidades da federação. Rio de Janeiro, p.1-777, 2006.
- Kelly, S.D., Bateman, A.S., 2010. Comparison of mineral concentrations in commercially grown organic and conventional crops - Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*). *Food Chem.* 119, 738–745. doi:10.1016/j.foodchem.2009.07.022
- Khan, Z.I., Ahmad, K., Yasmeen, S., Akram, N.A., Ashraf, M., Mehmood, N., 2017. Potential health risk assessment of potato (*Solanum tuberosum* L.) grown on metal contaminated soils in the central zone of Punjab, Pakistan. *Chemosphere* 166, 157–162. doi:10.1016/j.chemosphere.2016.09.064
- Kotsiantis, S.B., Zaharakis, I.D., Pintelas, P.E., 2006. Machine learning: a review of classification and combining techniques. *Artif. Intell. Rev.* 26, 159–190. doi:10.1007/s10462-007-9052-3
- Laursen, K.H., Schjoerring, J.K., Olesen, J.E., Askegaard, M., Halekoh, U., Husted, S., 2011. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *J. Agric. Food Chem.* 59, 4385–4396. doi:10.1021/jf104928r
- Ming, H., Naidu, R., Sarkar, B., Lamb, D.T., Liu, Y., Megharaj, M., Sparks, D., 2016. Competitive sorption of cadmium and zinc in contrasting soils. *Geoderma* 268, 60–68. doi:10.1016/j.geoderma.2016.01.021
- Shi, Z., Di Toro, D.M., Allen, H.E., Sparks, D.L., 2013. A general model for kinetics of heavy metal adsorption and desorption on soils. *Environ. Sci. Technol.* 47, 3761–7. doi:10.1021/es304524p
- Strawn, D., Sparks, D., 2000. Effects of soil organic matter on the kinetics and mechanisms of Pb (II) sorption and desorption in soil. *Soil Sci. Soc. Am. J.* 64, 144–156. doi:10.2136/sssaj2000.641144x
- Strobel, B.W., Hansen, H.C.B., Borggaard, O.K., Andersen, M.K., Raulund-Rasmussen, K., 2001. Cadmium and copper release kinetics in relation to afforestation of cultivated soil. *Geochim. Cosmochim. Acta* 65, 1233–1242. doi:10.1016/S0016-7037(00)00602-5
- Sun, F., Li, Y., Wang, X., Chi, Z., Yu, G., 2017. Using new hetero-spectral two-dimensional correlation analyses and synchrotron-radiation-based spectromicroscopy to characterize binding of Cu to soil dissolved organic matter. *Environ. Pollut.* 223, 457–465. doi:10.1016/j.envpol.2017.01.046
- Wang, D.Z., Jiang, X., Rao, W., He, J.Z., 2009. Kinetics of soil cadmium desorption under simulated acid rain. *Ecol. Complex.* 6, 432–437. doi:10.1016/j.ecocom.2009.03.010
- Xu, D., Zhou, P., Zhan, J., Gao, Y., Dou, C., Sun, Q., 2013. Assessment of trace metal bioavailability in garden soils and health risks via consumption of vegetables in the vicinity of Tongling mining area, China. *Ecotoxicol. Environ. Saf.* 90, 103–111. doi:10.1016/j.ecoenv.2012.12.018

## 2. TRANSFER FACTORS AND RISK ANALYSIS OF POTENTIALLY TOXIC ELEMENTS GROWN IN ORGANIC AND CONVENTIONAL FARMING SYSTEMS

### Highlights

- The farming system influences the concentrations of potentially toxic elements in soil and plants
- Leafy vegetables are more able to accumulate potentially toxic elements than bulbs and fruit vegetables
- Lettuce grown under the organic system poses a lower risk for human health than conventional farming

### Abstract

Vegetables have different capacities to uptake and accumulate potentially toxic elements (PTEs). Soil attributes and the farming system (conventional or organic) play important roles in PTE phytoavailability and influence the human health risk. The aims of the authors in this study were to: (i) determine the levels of Ba, Cu, Mn, Ni and Zn in soils and in vegetables produced under organic and conventional systems; (ii) investigate the transfer of PTEs to plants through soil-plant transfer factors; and (iii) estimate the human health risks associated with the consumption of these vegetables as affected by the farming systems. Samples of soil (0 – 20 cm) and edible parts of organically and conventionally produced lettuce (*Lactuca sativa*), onion (*Allium sepa*), bell pepper (*Capsicum annuum*) and tomato (*Solanun lycopersicum*) were collected from farms in the state of Pernambuco, northeastern Brazil. Total contents of PTEs in the samples were extracted by HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> digestion. The pseudo-total fraction of the PTEs in the soil was extracted using a HCl:HNO<sub>3</sub> (1:3 v/v) solution; the reactive fraction was extracted with 0.43 mol L<sup>-1</sup> HNO<sub>3</sub>, and the available contents of the PTEs were extracted with DTPA. Soil-plant transfer factors and risk concentration were calculated to evaluate the interaction between PTE concentrations, in plant and soil, and human health risk. In general, vegetables accumulated Zn and Cu, and excluded Ba, Mn and Ni. Lettuce leaves presented the highest capacity to accumulate PTEs. PTE content was higher in vegetables grown under the conventional system when compared to organic farming system. Soil-plant transfer factors obtained from different soil extracts had contrasting results when organic and conventional systems were compared. Regardless of the scenario considered, children and adults in contact with lettuce leaves from the organic system had a lower health risk than individuals exposed to this vegetable conventionally produced.

Keywords: Heavy metals; Production system; Horticultural crops; BCF; Human health

### 2.1. Introduction

Vegetables have a high nutritional value, and their consumption is part of the human diet. Vegetables obtain water and nutrients from soil, but they can also absorb potentially toxic substances and elements (PTEs), such as heavy metals (Ovečka and Takáč, 2014; Xu et al., 2013). The consumption of contaminated vegetables is one of the main routes of human exposure to PTEs (Baraud and Leleyter, 2012; Xu et al., 2013). In recent years, the cultivation of vegetables in areas close to large cities and/or main roads has increased in Pernambuco state, Brazil, in

response to the high demand on food supplies. Therefore, many small farmers in the state have developed peri-urban or urban agriculture. However, such areas are susceptible to many sources of pollution linked to the urban environment (França et al., 2017).

The absorption of PTEs by vegetables depends on factors related to the cultivar and the management system. For example, fertilization and control of pests, diseases and weeds. Conventional and organic farming systems differ greatly, especially in the release rate of PTEs into the soil solution, which contributes directly to the crop's mineral composition. Therefore, the plant PTEs contents can be used as a fingerprint of the PTEs in a particular soil. Capuano et al. (2013) and Laursen et al. (2013) have shown that the concentration of elements such as Mn, Ca, Cu and Zn in crops grown under organic or conventional farming systems are markedly different.

The toxicity of a PTE to ecosystems and plants depends primarily on its phytoavailability (Pinto et al., 2014). An estimation of the metal uptake by plants can be achieved through chemical extractants such as calcium chloride or ammonium nitrate that extract the available fraction of the element (Gupta et al., 1996; Peijnenburg et al., 2007). Plant-soil transfer coefficients are used to characterize the intensity of absorption of elements by plants. These coefficients aid the understanding and the quantification of the polluting potential of a PTE for the environment and for humans. The bioconcentration coefficient ( $BC = [\text{element in the plant}] / [\text{element available in the soil}]$ ) reflects the crop's ability to absorb the available element from the soil (Inácio et al., 2013). Soil attributes such as pH, cationic exchange capacity, texture, organic material (OM) content, climatic conditions and agricultural practices influence absorption and long distance transport of these metals by plants (Chen et al., 2014). Thus, the evaluation of the plant-soil transfer coefficients for vegetables grown under either conventional or organic management systems allows the bioconcentration of PTEs in contrasting agricultural systems to be better understood.

High concentrations of PTEs in vegetables may be associated with risks to human health and these risks have been assessed by several methods (Abbasi et al., 2013; Dziubanek et al., 2015; Yang et al., 2014). In 2007, the Environmental Agency of São Paulo state – Brazil (CETESB) established a system to evaluate the human risks of exposure to contaminated areas, the so-called CETESB Spreadsheet for Risk Assessment. The use of this spreadsheet allowed the quantification of the maximum acceptable concentration of PTEs and organic contaminants to which humans should be exposed through vegetable consumption in a pre-established scenario (CETESB, 2014).

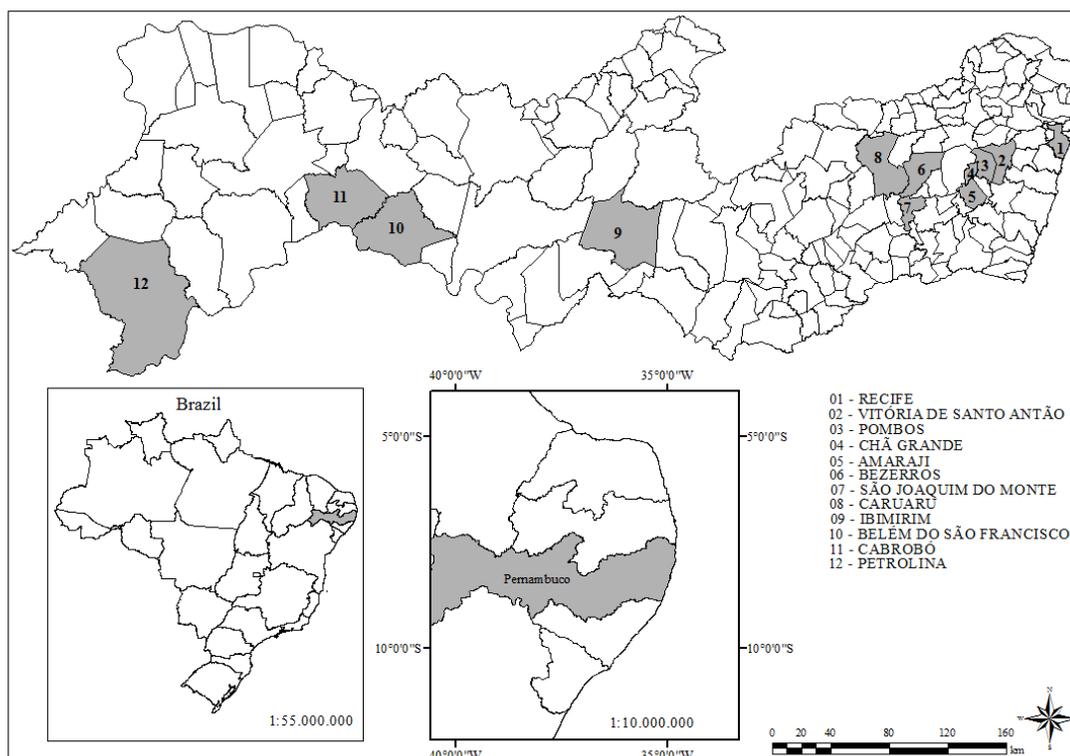
Taking into account the impact of different farming systems on the concentrations of toxic elements in soils and plants, this study was carried out to determine the levels of the PTEs Ba, Cu, Mn, Ni and Zn in soils and vegetables grown under organic and conventional farming systems in the State of Pernambuco, northeastern Brazil. In addition, we used soil-plant transfer factors to investigate the PTEs transfer to vegetables and estimate the human

health risks from the consumption of these vegetables as a function of the type of farming system used for their cultivation.

## 2.2. Materials and Methods

### 2.2.1. Sampling and pre-treatment of the samples

Since vegetables present different patterns of PTE absorption and translocation, we worked with the edible part of leafy (lettuce), bulb (onion) and fruit (bell pepper and tomato) vegetables. We selected farms under organic or conventional management systems that presented vegetables near harvesting (Figure 1). Lettuce (*Lactuca sativa*) samples were collected from eight farms using organic management systems and eight farms under conventional management systems. Onions (*Allium cepa*) samples were taken from three organic and eight conventional farms. Bell peppers (*Capsicum annuum*) grown were collected from one farm under an organic system and five farms under the conventional farming system. Tomato (*Solanum lycopersicum*) samples came from one organic and two conventional farming systems.



**Figure 1.** Map of study area in the state of Pernambuco (Brazil). Samples were obtained from the districts highlighted.

Ten units of each vegetable were collected randomly throughout the planted area of each farm along with the soil (0–20 cm layer) in contact with their roots. Samples of the organic fertilizers used in the farms were also taken. The producers answered a questionnaire about time of cultivation, use of insecticides, fungicides and herbicides, fertilization and the source of irrigation water. Any potential source of pollution near the farms was also noted.

Vegetable samples were washed in running water to remove any superficial impurities and attached soil particles. Samples were dried in an oven at  $55 \pm 5^\circ\text{C}$ , ground in a stainless-steel mill and stored in plastic bags. Organic composts and the soil samples were dried in the shade, crushed to break up the large lumps and passed through a 2 mm mesh sieve.

The properties labeled as organic farm were not certified, but they were part of Social Control Organizations (SCO). According to item VIII of 2nd Article of Decree No. 6323/07, SCO is defined as "a group, association, cooperative or consortium to which family farmers are affiliated, without the use of intermediates to sell their production. The SCO must be registered with the Brazilian Ministry of Agriculture, Livestock and Food Supply in order to generate credibility based on the interaction between people or organizations, based on participation, commitment, transparency, trust, and society recognition".

### **2.2.2. Analysis of the soil and vegetables**

The soil texture was obtained by the densimeter method (Gee and Or, 2002) and the pH was obtained by measuring the potential using a combined electrode immersed in a soil: water suspension (1:2.5). The potential acidity was obtained by extraction with 1 mol L<sup>-1</sup> calcium acetate (pH 7.0) and titration with NaOH using phenolphthalein as the indicator. To obtain the levels of exchangeable calcium, magnesium and aluminum, 1 mol L<sup>-1</sup> KCl was the extractant. Levels of available sodium, potassium and phosphorus were extracted with a double acid solution (Mehlich-1), following the protocol of Anderson and Ingram (1992).

Levels of well-crystallized ("free") iron and aluminum were extracted with sodium dithionite-citrate-bicarbonate (DCB) (Inda Junior and Kämpf, 2003; Mehra and Jackson, 1960), while amorphous Fe and Al were extracted with ammonium acid oxalate. Forms attached to organic matter in the soil were extracted with sodium pyrophosphate (McKeague and Day, 1966; Schwertmann, 1964).

The PTEs pseudototal concentrations in the soil were obtained by acid extraction in a microwave oven using the method EPA 3051a (1:3 HCl:HNO<sub>3</sub>, v/v) (USEPA, 1998). The available concentrations were extracted by diethylenetriaminepentaacetic acid (DTPA) (Abreu et al., 2001) and the reactive forms of the metals were extracted with 0.43 mol L<sup>-1</sup> HNO<sub>3</sub> (Römken et al., 2004). The total PTE concentration in the plants was obtained using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in a micro-wave assisted digestion (Araújo et al., 2002). The determinations were carried out using optical emission spectrometry (ICP-OES). Montana soil and tomato leaves used as the standard reference material were obtained from the National Institute of Standards and Technology (USA) and served as controls.

The organic carbon content of the soils was obtained by dry combustion in an elemental analysis apparatus. Laser induced fluorescence spectroscopy was used to obtain the degree of humification (HLIF) from the ratio between the area of the fluorescence emission spectrum and the total organic carbon concentration (HLIF = AFS/TOC) (Milorí et al., 2006).

### 2.2.3. Soil-plant transfer

In order to estimate the capacity of the plants to absorb and accumulate PTEs in its edible parts, transfer factors were estimated based on PTEs content from different soil fractions: pseudototal (Pinto et al., 2014; Xu et al., 2013); reactive (Rodrigues et al., 2010); and available (Inácio et al., 2013; Khaokaew and Landrot, 2014). The bioconcentration factor (BCF) utilized to quantify the soil-plant transfer is the result of the ratio between the metal content of the plant (mg kg<sup>-1</sup>, dry weight) and the pseudototal fraction in the soil (mg kg<sup>-1</sup>, dry weight) (BCF = [element in the plant]/[element pseudototal in the soil]). The BCFB is the transfer factor obtained by the relationship between the total content in the plant and the bioavailable content in the soil (BCFB = [element in the plant]/[bioavailable of the element in the soil]). The BCFR is obtained by the relationship [element in the plant]/[element in the soil extracted by HNO<sub>3</sub>].

### 2.2.4. Risk assessment

The BCF<sub>R</sub> was chosen for the risk concentration calculation. First of all, the final BCF<sub>R</sub> (BCF<sub>R</sub>') (1) was calculated by the geometric mean of the BCF<sub>R</sub> (GM(BCF<sub>R</sub>)) divided by the correction factor (Swartjes et al., 2007)(2).

$$BCF_{R'} = \frac{GM(BCF_R)}{CF} \quad (1)$$

$$CF = \frac{A + (B \times \%Clay) + (C \times \%SOM)}{A + (B \times \%Clay_{mean}) + (C \times \%SOM_{mean})} \quad (2)$$

Where: %clay = average clay content in the soil database (%); %SOM = average organic matter content in the soil database (%); %clay<sub>mean</sub> = average clay content; %SOM<sub>mean</sub> = average content of organic matter; A, B, C = variables specific to each metal as adopted by Swartjes et al. (2007). We used 2% SOM and 35% Clay following Boim et al. (2016).

The maximum acceptable concentration of the PTEs was obtained by the equation 3 based on Boim et al. (2016):

$$RC = QR \times \frac{RfDo}{TF} \times \left[ IRL \times \left( BCF_{Rf} + \frac{CD_p}{frb} \right) \right] \quad (3)$$

Where: RC = risk concentration (mg kg<sup>-1</sup> 10<sup>3</sup>); QR = non-carcinogenic risk quotient; RfDo = reference oral dose (mg kg<sup>-1</sup> d<sup>-1</sup>); TF = uptake factor (mg kg<sup>-1</sup> d<sup>-1</sup>); IRL = rate of ingestion of leafy cultures (kg d<sup>-1</sup>); BCF<sub>Rf</sub> = soil-plant transfer factor calculated using equation (1); CD<sub>p</sub> = concentration in plants from particles deposition (mg kg<sup>-1</sup> dry weight); frb = weight conversion factor from dry base to wet base for leafy cultures. The complete table with all the parameters and respective values is presented in Table S1 in Supplementary Information.

### 2.2.5. Data analysis

The statistical software Statistica (version 7, StatSoft Inc., OK, USA) was used to perform descriptive analysis, exploratory Principal Component Analysis (PCA) and linear correlation matrix plot.

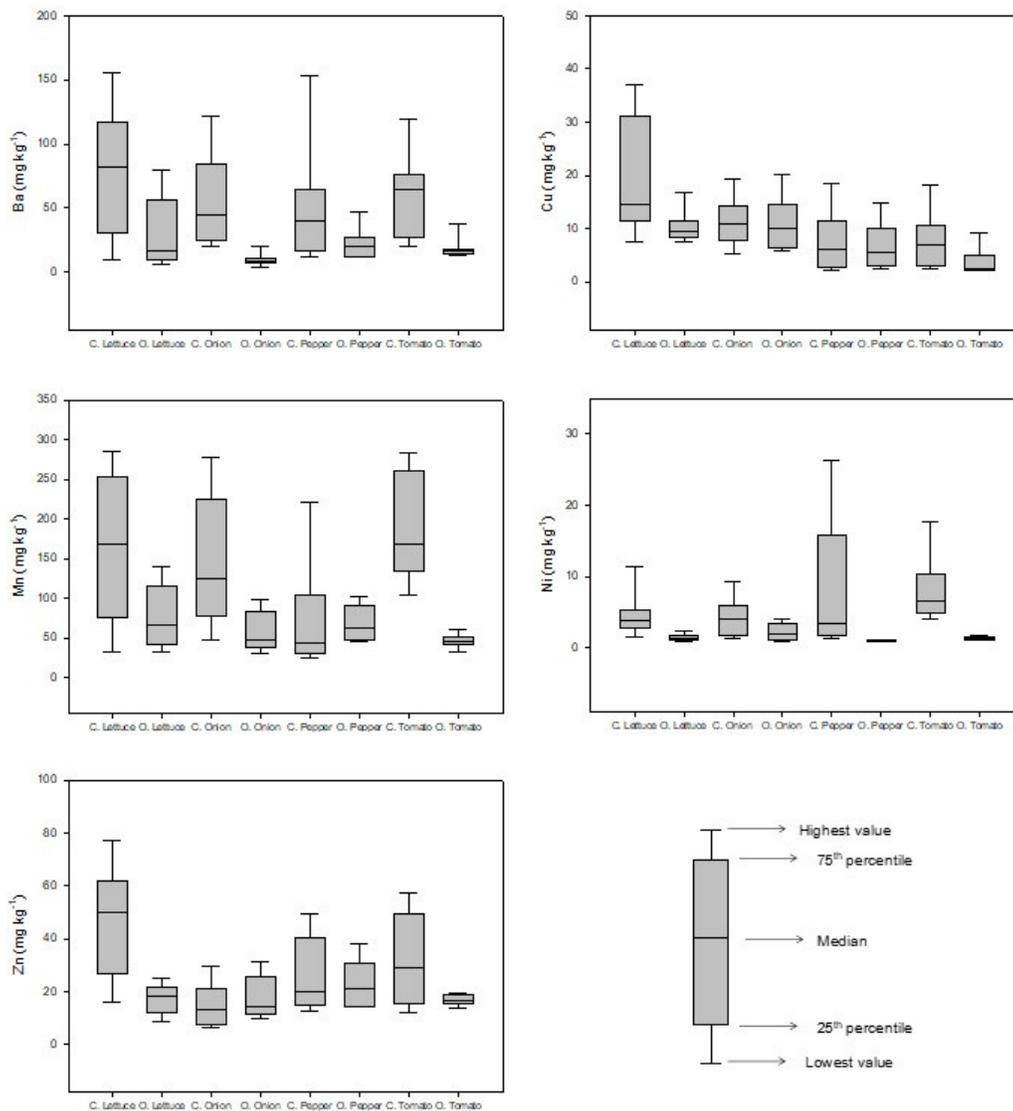
## 2.3. RESULTS AND DISCUSSION

The soils had pH values close to neutral and texture from sandy to clayey (Table 1). The high variability in the OM content suggests the diversity of organic fertilizers used. Based on field observations, it was possible to attest a high application of OM in lettuce fields, whether organic or conventional systems, in comparison to the other vegetable fields.

The National Environment Council of Brazil (CONAMA) established two values to monitor the soil pollution by PTEs in rural scenario: (i) prevention value (PV) is the concentration limit for the element in the soil; and (ii) investigation value (IV), is the concentration of the element above which there are potential (direct or indirect) risks to human health (CONAMA, 2012). For all the PTEs studied, the concentrations were below IV (300 mg kg<sup>-1</sup> for Ba, 200 mg kg<sup>-1</sup> for Cu, 70 mg kg<sup>-1</sup> for Ni, and 450 mg kg<sup>-1</sup> for Zn) (Figure 2). However, some sites presented Ba concentration higher than the PV (150 mg kg<sup>-1</sup>). The Brazilian legislation does not present threshold values for Mn. Biondi et al. (2011) analyzed 35 soils under natural conditions from Pernambuco and found that 70% of the samples presented pseudo totals for Mn content under 200 mg kg<sup>-1</sup>. Other authors, however, have reported values of up to 609 mg kg<sup>-1</sup> of Mn in moderately weathered soils. Considering that most of the samples presented values between 50 and 250 mg kg<sup>-1</sup> (Figure 2), it seems that the agricultural use of these soils has not lead to a substantial increase in Mn. Baraud and Leleyter (2012) found pseudo totals of Mn content above 558 mg kg<sup>-1</sup> in garden and rural French soils. On the other hand, Rodrigues et al. (2012) found a Mn range from 58 to 2,439 mg kg<sup>-1</sup> in 136 Portuguese soils.

**Table 1.** Descriptive analysis of physical and chemical properties of soil samples.

Variables	Mean	Min.	Max.	Median	Std. Deviation
pH <sub>H2O</sub>	6.3	4.4	7.9	6.4	0.8
pH <sub>KCl</sub>	5.7	3.8	7.1	5.7	0.8
$\Delta$ pH	-0.6	-1.3	-0.2	-0.6	0.4
P (mg kg <sup>-1</sup> )	202.4	7.1	980.2	155.6	202.9
mmol <sub>c</sub> kg <sup>-1</sup>					
K	3.7	0.2	11.8	3.1	3.4
Ca	3.4	0.6	10.6	2.6	2.4
Mg	1.7	0.1	4.6	1.3	1.1
Al	0.6	0.0	4.1	0.3	0.8
H+Al	14.2	0.2	55.4	11.9	11.9
CEC <sub>t</sub>	29.0	12.9	80.6	25.4	14.0
%					
V	52	11	99	44	30
m	6	0	47	2	10
g kg <sup>-1</sup>					
TOC	8.4	0.4	56.5	1.7	11.8
Sand	374.9	56.1	860.0	213.8	286.6
Silt	177.9	39.1	583.7	105.2	139.3
Clay	447.2	75.3	846.8	439.1	271.6
Fe <sub>2</sub> O <sub>3</sub> DCB	8.5	0.4	30.4	6.9	6.8
Al <sub>2</sub> O <sub>3</sub> DCB	4.5	0.8	16.0	3.9	2.9
Fe <sub>2</sub> O <sub>3</sub> OXA	1.0	0.1	3.8	0.7	0.8
Al <sub>2</sub> O <sub>3</sub> OXA	1.6	0.6	3.8	1.5	0.8
Fe <sub>2</sub> O <sub>3</sub> PIR	0.7	0.1	10.3	0.2	1.7
Al <sub>2</sub> O <sub>3</sub> PIR	3.9	1.3	6.5	4.0	1.7

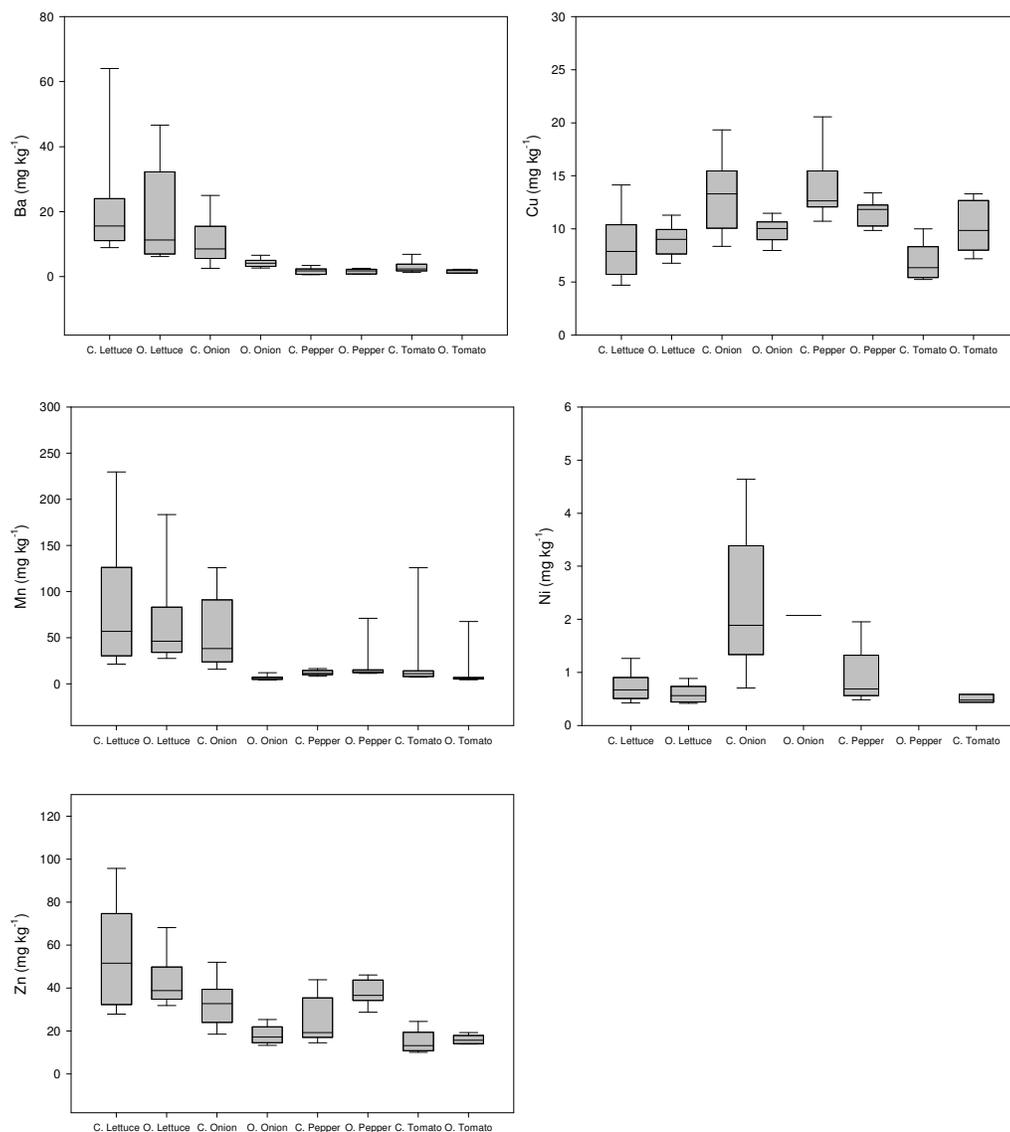


**Figure 2.** Boxplot of the pseudototal content (3051a) of Ba, Cu, Mn, Ni and Zn in the soil samples. Note: the outliers were excluded. Limit of detection of the matrix used: Ba – 0.8; Cu – 2; Mn – 2; Ni – 0.8; Zn – 0.8 mg kg<sup>-1</sup>.

Medians of the pseudo total concentrations of the PTEs in the soil samples from the conventional management farms were higher than those from organically grown vegetables (Figure 2). Probably, the use of mineral fertilizers contributed to the increase in the concentration of PTEs in the soil (da Silva et al., 2017). Using the average values of the pseudo total concentrations, the PTEs presented the following order of magnitude: Mn > Ba > Zn > Cu > Ni.

As a response to concentrations in soils, PTE content in the edible parts of the vegetables grown conventionally were higher than the concentration in the organically grown vegetables (Figure 3), except for Cu in organic tomato and Zn in organic bell pepper. Based on the average values, the PTEs were absorbed in the order highest to lowest: Mn > Zn > Ba > Cu > Ni. Ni content in organic bell pepper and tomato presented levels below the quantification limit for the equipment used (ICP-OES). Lettuce and onion samples had a high variability of

absorption, illustrated by the greater range of values of PTEs. Zhou et al. (2016) found a coefficient of variation for PTEs in vegetables that reached several hundred times and indicated that differences in PTE bioaccumulation existed among vegetables, highlighting that this difference exist even among cultivars and varieties within the same species.

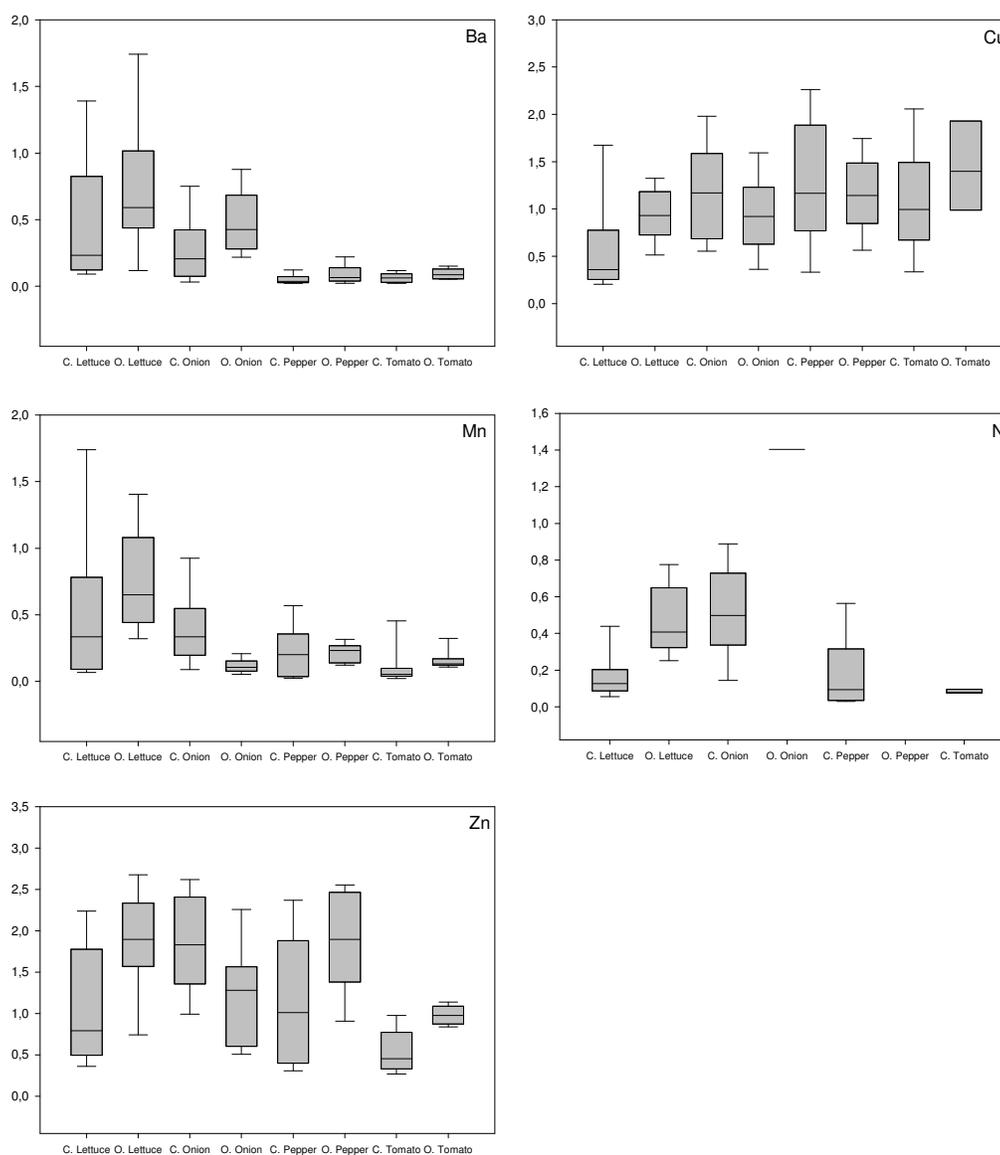


**Figure 3.** Boxplot of potentially toxic elements contents of plant samples. Ni content in samples from organic bell pepper and organic tomato presented values below the quantification limit. Quantification limits of the equipment: Ba – 0.4; Cu – 1; Mn – 1; Ni – 0.4; Zn – 0.4 mg kg<sup>-1</sup>.

Lettuce samples presented the highest values of Ba, Mn and Zn, while the onion samples presented the highest Ni content. Cu had the most variable distribution among all the samples. Zhou et al. (2016) evaluated the absorption of Pb, Cd, Zn, As and Cu by vegetables and observed that the concentration of Pb, Cd, Zn and As, but

not Cu, were higher in leaf vegetables than in other vegetables in the study (stalk, legume, root and melon vegetables). PTEs are unevenly distributed throughout the organs and tissues of plants. Usually, the accumulation of PTEs in reproductive organs is much lower than that in the vegetative parts of plants. PTE accumulation depends on the biological characteristics of the cultivar, the physiological role of the element, the amount of the element in the soil and its availability for plant uptake (Kuramshina et al., 2014). This can be clearly observed for Ba (a non-essential element) whose concentration in the edible parts of lettuce and onion were much higher than its concentration in bell pepper and tomato (reproductive organs). In addition, leafy vegetables usually exhibit higher PTE accumulation because they can absorb them through their broad leaves with larger surface area, which favors the accumulation of PTEs from contaminated soil particles on the leaf surface or from foliar intake during irrigation etc. (Garg et al., 2014).

In order to estimate the capability of vegetables to accumulate PTEs in their edible parts, transfer bioconcentration factors (BCF) for each of the metals were estimated (Figure 4). Using the average values, the BCFs followed this order of magnitude:  $Zn > Cu > Ni > Mn > Ba$ , showing that plants prefer absorb essential elements over non-essential ones (Ba). Inácio et al. (2013) studied the capacity of cabbage leaves (*Brassica oleracea* var. *capitata*) and tomato fruits to absorb As, Hg and Zn from Portuguese soils and observed that the uptake and transport of Zn to the studied edible tissues was more efficient than As and Hg absorption. Inácio et al. (2013) also observed that the capacity to accumulate Zn in the edible horticultural crop tissues was higher for cabbage than for tomato fruit.

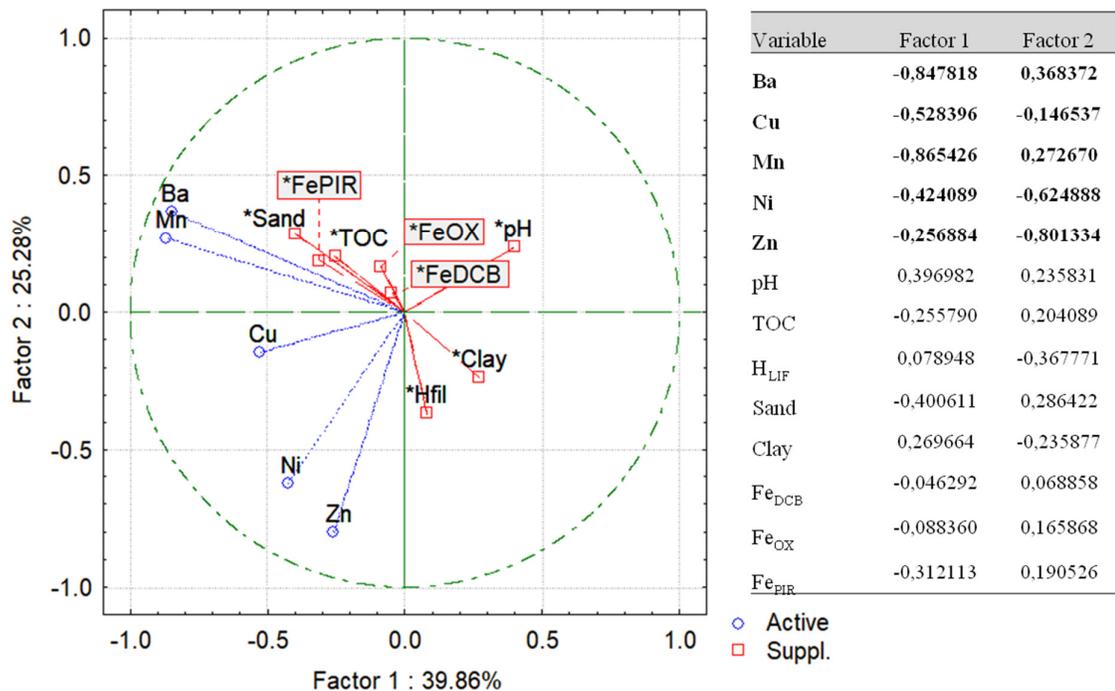


**Figure 4.** Boxplot of the BCF of potentially toxic elements contents in soils (3051a) and vegetable samples.

BCF boxplot had a distribution similar to the plant content results, but the BCFs in organic vegetables were higher than in conventionally produced plants. Inácio et al. (2013) used the following criteria to categorize the samples:  $BCF < 1$  = excluder;  $BCF: 1-10$  = accumulator, and  $BCF > 10$  = hyperaccumulator. Using the same classification, a set of samples that accumulated Zn and Cu, and were excluders of Ba, Mn and Ni (Figure 4). Soil-plant transference is an indicator of the tendency of an element to accumulate in plants, but it is highly dependent on the PTE studied, soil characteristics and plant genotype (Baraud and Leleyter, 2012). BCF tended to decrease with increasing pseudo total concentration of the PTEs in the soil (see Figure S1 in the supplementary materials), i.e., the proportion of the PTEs absorbed by the vegetables decreased as the pseudo total of the PTEs in the soil increased. This emphasizes that the pseudo total concentration of metals in soils may not be a good indicator of

phytoavailability, because of the different and complex distribution patterns of the PTEs in the soil matrix (Baraud and Leleyter, 2012). At higher concentrations, PTEs could be sorbed in more variable and complex forms, reducing their phytoavailability.

Principal component analysis was carried out to explore of the interdependence of some soil properties on the soil-plant transference (Figure 5). The BCF value for each PTE was used as the active variable, while the soil properties were classified as supplementary variables. The first two factors had an accumulated capacity to explain 65% of the variability of the samples. Looking at the composition of the Factor 1, the BCF for Ba and for Mn were the most important factors that explain the variability, while sand, pH and Fe extracted by pyrophosphate (Fe organically bound) were the most relevant among the supplementary variables. Factor 2 was mainly ruled by BCF for Zn and the humification index (HLIF). Taking the variable vectors into account, it is possible to observe that the BCF for Ba and Mn were closely associated with TOC and the sand content, and all the forms of Fe (free – DCB; low crystallized – OX; and binding to organic matter – PIR). The BCF for Cu had an opposite relationship to pH, and BCF for Ni and for Zn had a weak correlation with HLIF.

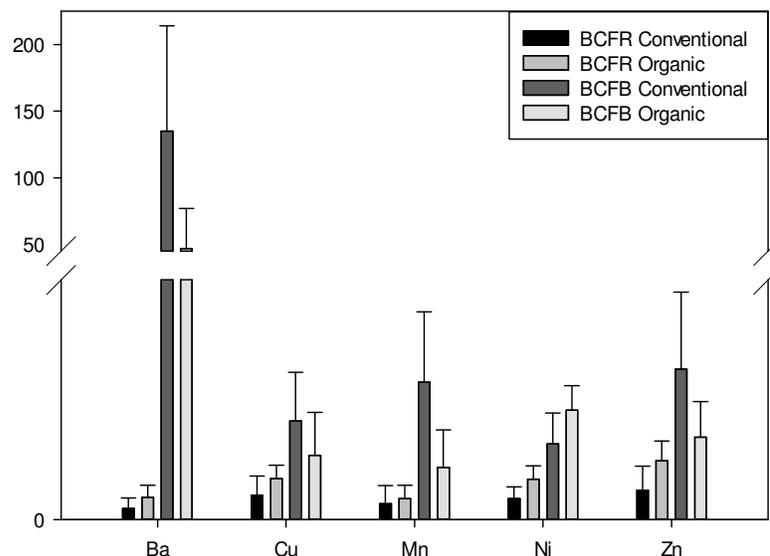


**Figure 5.** Principal component analysis of the variables related to chemical and physical characteristics (supplementary variables), Bioconcentration Factor of potentially toxic elements in soils of Pernambuco (active variables) and the factor coordinates of the variables, based on correlations.

The prediction of phytoavailability is important for the assessment of environmental quality and health risks (Adamo et al., 2014). However, phytoavailability is regulated by a complex system of chemical and biological processes and their interactions. The soil-plant transfer process is somehow influenced by soil properties such as pH, organic matter, soil texture, mineralogy etc. (Figure 5). Therefore, the pseudo total concentration of PTEs in soils (Figure 2) may not be a good indicator of phytoavailability because of the complex distribution patterns of metals among other chemical species or solid phases (Baraud and Leleyter, 2012). For this purpose, many chemical

extraction procedures have been proposed, and their use to predict phytoavailability has become a conventional approach. Single extraction procedures essentially fall into three categories depending on the nature of the extractant used: salts, dilute solutions of acids and complexing or reducing agents (Baraud and Leleyter, 2012).

As lettuce presented the highest number of samples in this study ( $n = 130$ ), soil samples collected in farms producing lettuce were used to calculate the PTE soil-plant factor using  $\text{HNO}_3$  (here named as BCFR) and DTPA (BCFB) (Figure 6). Since the PTE concentration in the plants were the same for both factors, the differences observed here were due to the different capacity of the acid and the complexing solution to extract the PTEs from the soil. DTPA, for example, had a low capacity to extract Ba, which lead to BCFB values much higher than using the  $\text{HNO}_3$  extraction ( $\text{BCFB} = 135 \pm 79$  for conventional system;  $\text{BCFB} = 47 \pm 30$  for organic system). Clay exchange sites are highly selective for  $\text{Ba}^{2+}$  over  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and Ba complexation by organic matter occurs to a limited extent. Therefore, Ba is relatively immobile in soils and occurs primarily in the residual fraction (Biondi et al., 2011a; Pichtel et al., 2000).



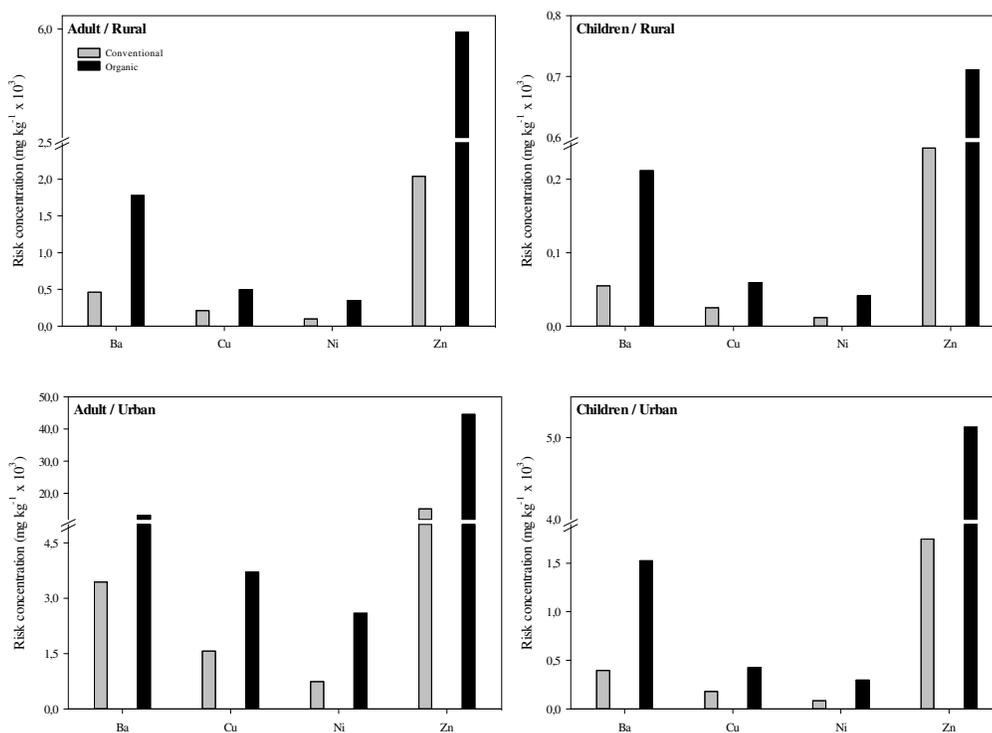
**Figure 6.** Bioconcentration Factor using reactive PTEs content in soil ( $\text{BCFR}$ ) and Bioconcentration Factor using bioavailable PTEs content ( $\text{BCFB}$ ) calculated for the lettuce samples based on the production system: conventional or organic.

The higher the BCFR or BCFB value, the easier the plant can absorb and accumulate the metal from the soil (Baraud and Leleyter, 2012). Based on the BCFR values, lettuce samples accumulated in the sequence  $\text{Zn} > \text{Cu} > \text{Ni} > \text{Mn} > \text{Ba}$  (Figure 6). This was the same sequence observed for the BCF values (Figure 4). For the BCFB values, however, the sequence was  $\text{Ba} \gg \text{Zn} > \text{Mn} > \text{Ni} > \text{Cu}$ . The different sequence observed for extractions with  $\text{HNO}_3$  and DTPA highlights the different capacities of the reagents to extract the PTEs. In contrast to the absolute value, changing the extractor also shifted the response profile of the samples: the EC values for organic samples were always higher than the conventional samples, but the BCF values had the opposite behavior, with the organic samples presenting the lowest levels (except for Ni). Evaluating the absorption of PTEs by radish (*Raphanus raphanistrum*), Baraud and Leleyter (2012) found that 0.05 mol L<sup>-1</sup> DTPA was less correlated than the 0.25 mol L<sup>-1</sup>

HCl to the phytoavailability of the elements. The acid extraction seemed to correctly predict the phytoavailability of Mn and Zn (Baraud and Leleyter, 2012).

PCA analysis carried out using the values of BCFR and BCFB as active variables and soil properties as supplementary variables (Figure S2 in Supplementary information) showed that BCFR and BCFB for Ni had completely distinct behavior. BCFR value for Ni was closely related to sand content, while the BCFB value for Ni was correlated with clay content, pH, FeOX and FeDCB. HNO<sub>3</sub> (used for BCFR determination) and DTPA (used to calculate BCFB values) have different capacities to extract PTEs from different pools of the soil matrix. While DTPA is an important complexing agent, HNO<sub>3</sub> is more commonly used to assess the geochemically reactive pools of PTEs. Therefore, the extractor can interact in different ways with the soils properties and PTEs, changing the elemental extraction (Rodrigues et al., 2010). The system (organic or conventional) presented a strong relationship only with the BCFR value for Ba. Since Ba uptake by leafy vegetables is not strongly related to the soil properties (Mcbride et al., 2014), the farm system could play a more important role in the BCFR based interpretations.

Lettuce was the most sampled vegetable and one of the most important horticultural crops in the region. Based on this, the BCFRs calculated for lettuce leaves were used to estimate the risk concentration (RC) for Ba, Cu, Ni and Zn under two scenarios (rural and urban) and for adults and children (Figure 7). Reference oral dose (RfDo) for Mn is not available in the literature used (CETESB, 2013; Swartjes et al., 2007).



**Figure 7.** Risk concentration for adult and children considering rural and urban scenario based on  $BCF_R$  calculated for lettuce leaves produced in Pernambuco (Brazil).

As the model used aims to calculate the maximum acceptable concentration for the PTE, the higher risk concentration values actually represent a lower risk to human health. In all the scenarios studied, the organic production of lettuce promoted a higher value for the risk concentration (Figure 7). Ba and Zn had the highest risk concentration, while Cu and Ni represented a risk concentration almost 15 times lower. The reference oral dose (RfDo) for Ba and Zn is one order higher than the RfDo for Cu and Ni, which contributes to a lower risk during the consumption of lettuce contaminated with Ba and Zn when compared to Cu and Ni. Boim et al. (2016) used data from the literature to derive the risk concentrations for Cu, Cr, Pb, Ni and Zn in humid tropical and temperate regions. Based on their results, the RC for children in the rural scenario in Pernambuco were 200, 50, and 116 times smaller than the RCs for Cu, Ni and Zn, respectively, in tropical regions, considering the same scenario. Risk concentrations were estimated using the BCF obtained with the pseudototal content of the PTEs in soil (Figure S3 in Supplementary Information) and presented the same pattern observed for the RC obtained based on the  $BCF_R$ , but with smaller values.

The low risk concentration values show that rural area for children is the most restrictive scenario, suggesting that this population is the most susceptible to soil and food contamination. On the other hand, adults in urban area are the less susceptible individuals. To the best of our knowledge, there has been no other paper published comparing the human risk associated with the consumption of vegetables, potentially contaminated with PTEs, based on the farming system.

## 2.4. Conclusions

- The average PTEs concentration in the soils were lower than the prevention value, and the vegetables sampled presented a capacity to accumulate Zn and Cu, and were excluder of Ba, Mn and Ni.
- The concentration of PTEs in soil and plants were higher in the conventional system than in the organic.
- The edible part of lettuce generally accumulated more PTEs than onion, bell pepper and tomato.
- The pseudototal content may not be a good indicator of pythoavailability. So, 0.43 mol L<sup>-1</sup> HNO<sub>3</sub> or DTPA as extractors could be a better way to study the PTEs transference from soil to the plant.
- Changing the extractor, the soil-plant factor value also change, what have to be considered in further interpretations.
- For the scenarios and conditions here studied, children and adults in contact to plants grown under organic system have a lower risk than individuals exposed to lettuce leaves conventionally produced.

### ACKNOWLEDGEMENTS

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## References

- Abbasi, A.M., Iqbal, J., Khan, M.A., Shah, M.H., 2013. Health risk assessment and multivariate apportionment of trace metals in wild leafy vegetables from Lesser Himalayas, Pakistan. *Ecotoxicol. Environ. Saf.* 92, 237–44. doi:10.1016/j.ecoenv.2013.02.011
- ABREU, C.A., ABREU, M.F., ANDRADE, J.C., 2001. Determinação de cobre, ferro, manganês, zinco, cádmio, cromo, níquel e chumbo em solos usando a solução de DTPA pH 7,3, in: RAIJ, B. van., ANDRADE, J.C., CANTARELLA, H., QUAGGIO, J.A. (Eds.), *Análise Química Para Avaliação Da Fertilidade de Solos Tropicais*. Instituto Agronômico, Campinas, pp. 240–250.
- Adamo, P., Iavazzo, P., Albanese, S., Agrelli, D., De Vivo, B., Lima, A., 2014. Bioavailability and soil-to-plant transfer factors as indicators of potentially toxic element contamination in agricultural soils. *Sci. Total Environ.* 500–501, 11–22. doi:10.1016/j.scitotenv.2014.08.085
- Anderson, J.M., Ingram, J.S.I., 1992. *Tropical soil biology and fertility: a handbook of methods*. Wallingford: CAB International, Berlin.
- Araújo, G.C., Gonzalez, M.H., Ferreira, A.G., Nogueira, A.R.A., Nóbrega, J.A., 2002. Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials. *Spectrochim. Acta Part B At. Spectrosc.* 57, 2121–2132. doi:10.1016/S0584-8547(02)00164-7
- Baraud, F., Leleyter, L., 2012. Prediction of phytoavailability of trace metals to plants: Comparison between chemical extractions and soil-grown radish. *Comptes Rendus Geosci.* 344, 385–395. doi:10.1016/j.crte.2012.07.003
- Biondi, C.M., do Nascimento, C.W.A., de Brito Fabricio Neta, A., 2011a. Teores naturais de bário em solos de referência do estado de pernambuco. *Rev. Bras. Cienc. do Solo* 35, 1819–1826. doi:10.1590/S0100-06832011000500036

- Biondi, C.M., Nascimento, C.W.A., Neta, A. de B.F., Ribeiro, M.R., 2011b. Teores de Fe, Mn, Zn, Cu, Ni e Co em solos de referência de Pernambuco. *Rev. Bras. Ciência do Solo* 35, 1057–1066. doi:10.1590/S0100-06832011000300039
- Boim, A.G.F., Melo, L.C.A., Moreno, F.N., Alleoni, L.R.F., 2016. Bioconcentration factors and the risk concentrations of potentially toxic elements in garden soils. *J. Environ. Manage.* 170, 21–27. doi:10.1016/j.jenvman.2016.01.006
- Capuano, E., Boerrigter-Eenling, R., van der Veer, G., van Ruth, S.M., 2013. Analytical authentication of organic products: An overview of markers. *J. Sci. Food Agric.* 93, 12–28. doi:10.1002/jsfa.5914
- CETESB, (Environmental Agency of São Paulo State), 2014. Board Decision 045/2014/E/C/I, from February 20, 2014. Provides for the Adoption of the Guiding Values for Soil and Groundwater in the State of São Paulo – 2014, Replacing the Guiding Values of 2005 and Other Provisions. Official Gazette of the State of S. Brazil.
- CETESB, (Environmental Agency of São Paulo State), 2013. Planilha CETESB de Avaliação de Risco à Saúde Humana [WWW Document]. URL <http://cetesb.sp.gov.br/areas-contaminadas/planilhas-para-avaliacao/> (accessed 1.1.17).
- Chen, Z., Ai, Y., Fang, C., Wang, K., Li, W., Liu, S., Li, C., Xiao, J., Huang, Z., 2014. Distribution and phytoavailability of heavy metal chemical fractions in artificial soil on rock cut slopes alongside railways. *J. Hazard. Mater.* 273, 165–173. doi:10.1016/j.jhazmat.2014.03.042
- CONAMA, 2012. Current Conama Resolutions published between September 1984 and January 2012 1126.
- da Silva, F., do Nascimento, C., Araujo, P., 2017. Environmental risk of trace elements in P-containing fertilizers marketed in Brazil. *J. Soil Sci. Plant Nutr.* 17, 635–647. doi:10.4067/S0718-95162017000300007
- Dziubanek, G., Piekut, A., Rusin, M., Baranowska, R., Hajok, I., 2015. Ecotoxicology and Environmental Safety Contamination of food crops grown on soils with elevated heavy metals content. *Ecotoxicol. Environ. Saf.* 118, 183–189. doi:10.1016/j.ecoenv.2015.04.032
- França, F.C.S.S., Albuerque, A.M.A., Almeida, A.C., Silveira, P.B., Filho, C.A., Hazin, C.A., Honorato, E. V., 2017. Heavy metals deposited in the culture of lettuce (*Lactuca sativa* L.) by the influence of vehicular traffic in Pernambuco, Brazil. *Food Chem.* 215, 171–176. doi:10.1016/j.foodchem.2016.07.168
- Garg, V.K., Yadav, P., Mor, S., Singh, B., Pulhani, V., 2014. Heavy metals bioconcentration from soil to vegetables and assessment of health risk caused by their ingestion. *Biol. Trace Elem. Res.* 157, 256–265. doi:10.1007/s12011-014-9892-z
- GEE, G.W., OR, D., 2002. Methods of soil analysis. Physical methods., in: DANE, J.H., TOPP, G.C. (Eds.), . Soil Science Society of America Journal, Madison.
- Gupta, S.K., Vollmer, M.K., Krebs, R., 1996. The importance of mobile, mobilisable and pseudo total heavy metal fractions in soil for three-level risk assessment and risk management 178, 11–20.
- Inácio, M., Neves, O., Pereira, V., Ferreira da Silva, E., 2013. Levels of selected potential harmful elements (PHEs) in soils and vegetables used in diet of the population living in the surroundings of the Estarreja Chemical Complex (Portugal). *Appl. Geochemistry* 44, 38–44. doi:10.1016/j.apgeochem.2013.07.017
- INDA JUNIOR, A. V., KÄMPF, N., 2003. Avaliação de procedimentos de extração dos óxidos de ferro pedogênicos com ditionito-citrato-bicarbonato de sódio. *Rev. Bras. ciência do solo* 27, 1139–1147. doi:doi.org/10.1590/S0100-06832003000600018

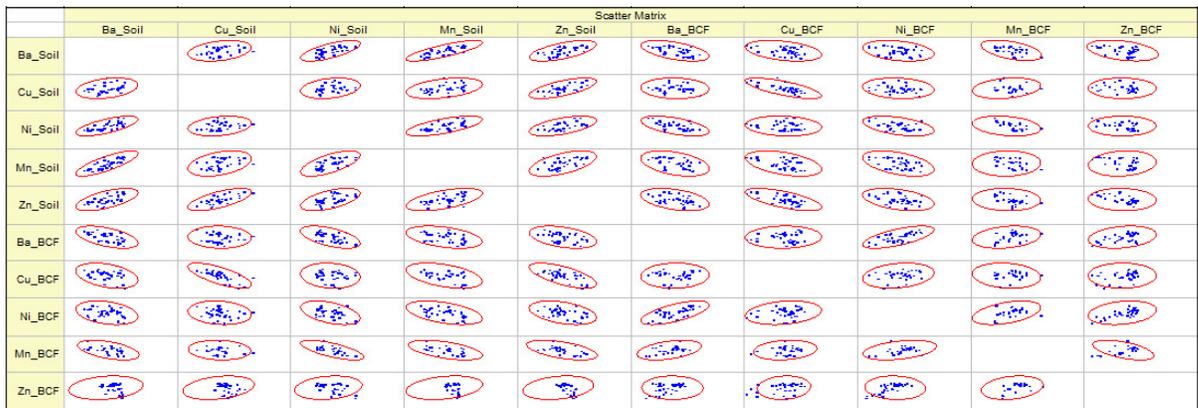
- Khaokaew, S., Landrot, G., 2014. A field-scale study of cadmium phytoremediation in a contaminated agricultural soil at Mae Sot District, Tak Province, Thailand: (1) Determination of Cd-hyperaccumulating plants. *Chemosphere* 1–5. doi:10.1016/j.chemosphere.2014.09.108
- Kuramshina, N.G., Kuramshin, E.M., Nikolaeva, S. V., Imashev, Y.B., 2014. The biogeochemical characteristics of the content of heavy metals in soil, plants and animals in different natural areas of Bashkortostan. *J. Geochemical Explor.* 144, 237–240. doi:10.1016/j.gexplo.2014.01.027
- Laursen, K.H., Mihailova, a., Kelly, S.D., Epov, V.N., Berail, S., Schjoerring, J.K., Donard, O.F.X., Larsen, E.H., Pedentchouk, N., Marca-Bell, a. D., Halekoh, U., Olesen, J.E., Husted, S., 2013. Is it really organic? - Multi-isotopic analysis as a tool to discriminate between organic and conventional plants. *Food Chem.* 141, 2812–2820. doi:10.1016/j.foodchem.2013.05.068
- Mcbride, M.B., Shayler, H. a, Spliethoff, H.M., Mitchell, R.G., Marquez-bravo, L.G., Ferenz, G.S., Russell-anelli, J.M., Casey, L., Bachman, S., 2014. Concentrations of lead , cadmium and barium in urban garden-grown vegetables : The impact of soil variables. *Environ. Pollut.* 194, 254–261. doi:10.1016/j.envpol.2014.07.036
- McKeague, J.A., Day, J.H., 1966. DITHIONITE- AND OXALATE-EXTRACTABLE Fe AND Al AS AIDS IN DIFFERENTIATING VARIOUS CLASSES OF SOILS. *Can. J. Soil Sci.* 46, 13–22. doi:10.4141/cjss66-003
- MEHRA, J.P., JACKSON, M.L., 1960. Iron oxides removal from soils and clays by a dithionite-citrate-bicarbonate system buffered with bicarbonate sodium. *Clay Clay Miner.* 7, 317–327. doi:10.1016/B978-0-08-009235-5.50026-7.
- Milori, D.M.B.P., Galeti, H.V.A., Martin-Neto, L., Dieckow, J., González-Pérez, M., Bayer, C., Salton, J., 2006. Organic Matter Study of Whole Soil Samples Using Laser-Induced Fluorescence Spectroscopy. *Soil Sci. Soc. Am. J.* 70, 57. doi:10.2136/sssaj2004.0270
- Ovečka, M., Takáč, T., 2014. Managing heavy metal toxicity stress in plants: Biological and biotechnological tools. *Biotechnol. Adv.* 32, 73–86. doi:10.1016/j.biotechadv.2013.11.011
- Peijnenburg, W.J.G.M., Zablotskaja, M., Vijver, M.G., 2007. Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicol. Environ. Saf.* 67, 163–79. doi:10.1016/j.ecoenv.2007.02.008
- Pichtel, J., Kuroiwa, K., Sawyerr, H.T., 2000. Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. *Environ. Pollut.* 110, 171–178.
- Pinto, E., Almeida, A. a., Aguiar, A. a R.M., Ferreira, I.M.P.L.V.O., 2014. Changes in macrominerals, trace elements and pigments content during lettuce (*Lactuca sativa* L.) growth: Influence of soil composition. *Food Chem.* 152, 603–611. doi:10.1016/j.foodchem.2013.12.023
- Rodrigues, S.M., Henriques, B., da Silva, E.F., Pereira, M.E., Duarte, A.C., Groenenberg, J.E., Römkens, P.F.A.M., 2010. Evaluation of an approach for the characterization of reactive and available pools of 20 potentially toxic elements in soils: Part II - Solid-solution partition relationships and ion activity in soil solutions. *Chemosphere* 81, 1560–1570. doi:10.1016/j.chemosphere.2010.09.042
- Rodrigues, S.M., Pereira, M.E., Duarte, a. C., Römkens, P.F. a M., 2012. Soil-plant-animal transfer models to improve soil protection guidelines: A case study from Portugal. *Environ. Int.* 39, 27–37. doi:10.1016/j.envint.2011.09.005
- Römkens, P.F.A.M., Groenenberg, J.E., Bonten, L.T.C., Vries, W. de, Bril, J., 2004. Derivation of partition relationships to calculate Cd, Cu, Ni, Pb, Zn solubility and activity in soil solutions. Wageningen.

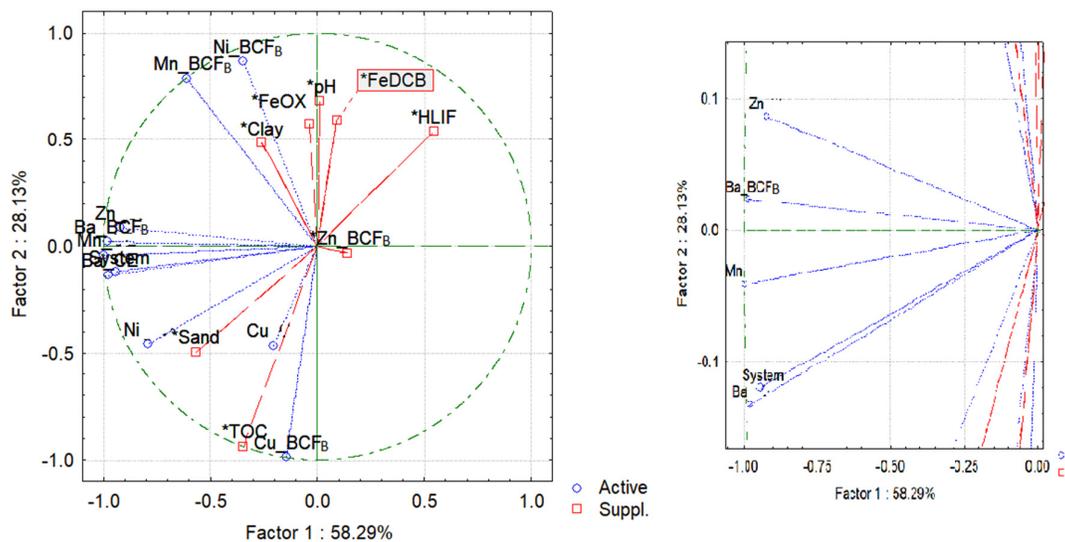
- SCHWERTMANN, U., 1964. Differenzierung der eisen oxide des bodens durch extraction unit saurer ammoniumoxalat-losung. *Pflanzenernaehr* 105, 194–202.
- Swartjes, F.A., Breemen, E.M., Otte, P.F., Beelen, P., Rikken, M.G.J., Tuinstra, J., Spijker, J., Lijzen, J.P.A., van Breemen, E., van Beelen, P., 2007. Human health risks due to consumption of vegetables from contaminated sites. Bilthoven.
- USEPA, U.S.E.P.A.-, 1998. Method 3051a – Microwave assisted acid digestion of sediments, sludges, soils, and oils [WWW Document]. URL <https://www.epa.gov/hw-sw846/sw-846-test-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-soils-and-oils> (accessed 9.10.15).
- Xu, D., Zhou, P., Zhan, J., Gao, Y., Dou, C., Sun, Q., 2013. Assessment of trace metal bioavailability in garden soils and health risks via consumption of vegetables in the vicinity of Tongling mining area, China. *Ecotoxicol. Environ. Saf.* 90, 103–11. doi:10.1016/j.ecoenv.2012.12.018
- Yang, L., Huang, B., Hu, W., Chen, Y., Mao, M., Yao, L., 2014. The impact of greenhouse vegetable farming duration and soil types on phytoavailability of heavy metals and their health risk in eastern China. *Chemosphere*.
- Zhou, H., Yang, W.T., Zhou, X., Liu, L., Gu, J.F., Wang, W.L., Zou, J.L., Tian, T., Peng, P.Q., Liao, B.H., 2016. Accumulation of heavy metals in vegetable species planted in contaminated soils and the health risk assessment. *Int. J. Environ. Res. Public Health* 13. doi:10.3390/ijerph13030289

## Supplementary information

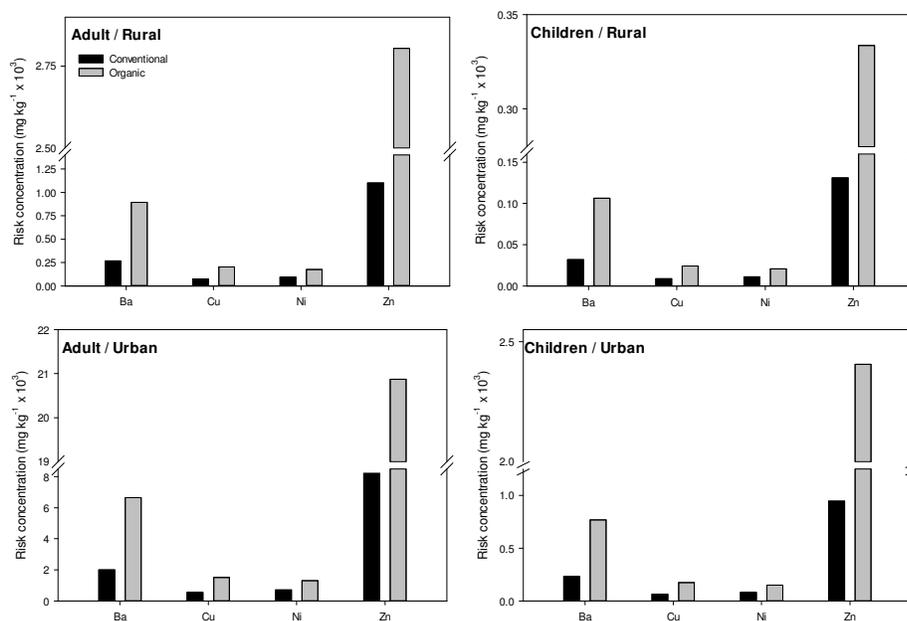
**Table S1.** Parameters used in the calculation of the risk concentration for the consumption of lettuce leaves for standard rural and urban scenarios.

Parameter	Description	Unit	Value			
			Children		Adult	
			Rural	Urban	Rural	Urban
QR	non-carcinogenic risk	---	1		1	
TF	uptake factor	mg kg <sup>-1</sup> day <sup>-1</sup>	0,0211	0,00256	0,00502	0,000548
IRL	rate of ingestion of leafy	kg day <sup>-1</sup>	0,008	0,007	0,016	0,013
CD <sub>p</sub>	concentration in plants from particle deposition	mg kg <sup>-1</sup> (dry weight)	0,01		0,01	
frb	weight conversion factor from dry base to wet base cultures leafy	---	10,2		10,2	
RfDo	reference oral dose	mg kg <sup>-1</sup> day <sup>-1</sup>	Ba	Cu	Ni	Zn
			0,2	0,04	0,02	0,3

**Figure S1.** Matrix plot of the linear correlation between pseudototal PTEs content in soil and the BCF.



**Figure S2.** Principal component analysis of the values of  $BCF_R$  and  $BCF_B$  as active variables and soil properties as supplementary variables. For better representation, the initials “ $BCF_R$ ” were suppressed.



**Figure S3.** Risk concentration for adult and children considering rural and urban scenario based on  $BCF$  calculated for lettuce leaves produced in Pernambuco (Brazil).



### 3. EFFECT OF SOIL ORGANIC MATTER ON THE KINETIC DESORPTION OF COPPER IN GARDEN SOILS

#### ABSTRACT

Kinetic studies can help to elucidate factors related to copper (Cu) desorption, once that is a key phenomenon affecting the metal bioavailability. Furthermore, reaction time, contact time and the content of soil organic matter- OM (and its multiple interactions with the soil mineral fraction) are closely related to the processes of sorption and solubilization of Cu in the soil. Our aim is to assess the effect of aging and OM on Cu desorption in garden soils. Kinetic experiments were carried out using rates of organic composts added to Cu-spiked soils. A batch experiment and a stirred-flow chamber system were used, with extracts being collected at fixed intervals. Percentage of Cu desorption against time, the total Cu desorbed, and the desorption rate were calculated to study the effect of time and OM application. The soils presented a high capacity to retain the Cu applied, represented by low desorption rate and accumulated release. The addition of OM promoted greater Cu desorption, while aging lead to a reduction in the Cu release.

Keywords: Amendment; Metal release; Stirred-flow; Non-equilibrium system; Aging effect

#### 3.1. INTRODUCTION

In agricultural production and arable soils, copper (Cu) is one of the most toxic heavy metals with potential effect to organisms (Strobel et al., 2001; Tella et al., 2016). Intensive cultivation of arable soils includes the frequent application of inorganic and organic fertilizer, sewage sludge and pesticides what has contributed to Cu accumulation in topsoil (Sayen et al., 2009; Strobel et al., 2001). Besides, even if the total Cu content is important to evaluate a soil contamination, it gives insufficient information about the potential mobility, bioavailability and toxicity of this metallic pollutant, which are governed by its speciation in soils (Sayen et al., 2009). Therefore, elucidating the dynamics of Cu in acid soils with a long history of agricultural use is important to predict possible environmental problems (Nóvoa-Muñoz et al., 2008).

The organic matter (OM) is probably the most important ligand in the environment that affects the Cu fate (Shi et al., 2016). The effect of composted OM on Cu mobility, reactivity and bioavailability in the soil depends on the rate and the frequency of application (Cambier et al., 2014) once Cu forms strong complexes with organic ligands in soils and soil solutions (Sayen et al., 2009). The percentage of Cd and Cu sorbed onto the soil decreases as the organic acids' (from a less humified OM) concentration increases, i.e., high organic acids concentrations promoted a high desorption of these elements (Najafi and Jalali, 2015). Under conditions of constant OM addition, as found in garden soils, the interaction between organic matter composts and the mineral soil fraction could increase Cu bioavailability.

The soil OM has different binding characteristics with metals depending on the environment and on the type of soil (Gustafsson et al., 2003). Iron and aluminum oxides, for example, play an important role on the reactivity of the mineral fraction of tropical and subtropical highly weathered soils. In these soils, Cu dynamics may be influenced not only by OM, but also by the mineral constituents of the soil. The organic components generally form stable metal-organic complexes with potentially toxic elements (PTEs) like Cu, while clay minerals and oxides concentrate heavy metal ions through surface ion exchange and metal-complex surface adsorption (Hernandez-Soriano et al., 2013; Sipos et al., 2008). Diagboya and Olu-owolabi (2015) evaluated retention patterns of Pb, Cd and Cu before and after soil OM and iron oxides removal and showed that, in the short term, soils containing a high level

of OM ( $62 \text{ g kg}^{-1}$ ) retained more metals than soils containing a high iron oxide level ( $2.5 \text{ g kg}^{-1}$ ). However, in the long term, the opposite occurred: soils rich in oxides had more retention capacity and a delayed desorption compared to soils with a high OM content. It is very difficult to establish a standard behavior, once it depends, mainly, on organic matter content, and the amount and the quality of soil oxides.

Another important parameter to consider for the prediction of copper transport is the contact time between the soil and the pollutant (aging effect). Generally, the bioavailability and mobility of heavy metals in soils decrease with the increase of residence time. Thus, the reactions occurring during the aging modify the Cu availability and toxicity over time and consequently they should be considered for risk assessment and soil remediation strategies. It is therefore important to understand the behavior of Cu in soils over time in order to predict its long-term fate and transport in environment (Sayen et al., 2009).

Kinetics studies are a very useful tool to understand the bindings of Cu in soil samples under different conditions like pH, time (Jalali and Khanlari, 2008), soil particle size (Liu et al., 2015), and OM content (Shi et al., 2013). Fernández-Calviño et al. (2017) evaluated alternatives for mine soil remediation and verified that aging and pine bark application reduce the desorption of potentially toxic elements. However, the aging changes the speciation of the metals more evidently in no-amended soils. This kind of study also could be more important in garden soils that are intensively tilled and receive large amounts of organic material over the years, thus affecting PTE dynamics in the soil and their availability to plants (Xu et al., 2013).

We hope that the findings of this work would improve understanding of Cu desorption mechanisms from amended variable charge soils in order to provide information for evaluation of Cu potential toxicity and ecological risk in vegetable production areas. So, our aim is to determine the effect of organic matter and aging on Cu desorption in contaminated and no-contaminated garden soils with different particle sizes.

## **3.2. MATERIAL AND METHODS**

### **3.2.1. Soil and organic composts samples**

Samples were collected from “garden soils” with production of lettuce (*Lactuca sativa*) and onion (*Allium cepa*) under organic (sample LO) and conventional (samples LC and OC) production system in the state of Pernambuco (Northeast of Brazil). All soil samples were collected from the 0 – 20 cm layer (soil in contact with the roots of the plants). Then, they were air-dried and sieved (2 mm screen). Three of the four samples selected presented pseudototal Cu content [using the standard method EPA 3051a (1:3 HCl:HNO<sub>3</sub>, v/v) (USEPA, 1998)] under the concentrations established by Brazilian legislation as prevention values for rural scenario ( $60 \text{ mg kg}^{-1}$ ). One of the samples had a level of pseudototal Cu between the prevention value and the investigative value, i.e. the metal concentration above which there are risks to human health:  $200 \text{ mg kg}^{-1}$  (CONAMA, 2012). So, this sample is under suspicion of contamination (sample SC). The organic compost used in those garden soils was also collected, air-dried and sieved (0.4 mm screen).

**Table 1.** Characterization of the garden soils sampled in Pernambuco (Brazil).

ID	pH <sub>(water)</sub>	Sand	Silt	Clay	TOC	Fe <sub>DCB</sub>	Cu
		----- g kg <sup>-1</sup> -----					
LC*	5.4	451	100	448	56	30	2
LO	7.2	859	65	75	9	3	10
OC	5.5	291	584	126	1	17	12
SC	7,1	799	76	126	13	7	162

\*LC = lettuce conventional; LO = lettuce organic; OC = onion conventional; SC = suspicion of contamination

### 3.2.2. Sample Pretreatment

#### 3.2.2.1. Compost incubation

To simulate the addition of compost in an agricultural soil, the samples were submitted to three treatments: 5%, 10% and 25% (w/w) of compost addition and control. After weighted and mixed, distilled water was slowly added until reach a friable point. An aliquot was taken to assess the humidity (estimated in 25%). The mixtures were kept in the dark and under room temperature (25°C ±2). Daily, the mixtures were shaken, and the humidity adjusted. After one week, the mixtures were air-dried and sieved (2 mm).

#### 3.2.2.2. Spiking

To evaluate the effect of time on the Cu desorption, the samples with pseudototal Cu content under the threshold value were kept in contact with the metal for 1 and 30 days. After incubated with the organic compost, the samples were submitted to solution containing Cu(NO<sub>3</sub>)<sub>2</sub> corresponding to 150 mg kg<sup>-1</sup> of Cu [75% of the investigative value established by CONAMA (2012)] and 0.01 mol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> (1:20 soil solution w/w). Preliminary kinetic experiments (data not shown) indicated that the Cu sorption had minimal change after 24 h, so the suspensions were shaken for 24 h on an end-over-end shaker at 10 rpm. For the 30 days treatment, after the initial 24h of agitation, the suspensions were kept in contact with the Cu solution for more 29 days, in the dark and under room temperature (25°C ±2). After the incubation time (1 and 30 days) the suspensions were centrifuged at 3,000 rpm for 10 min. The supernatant was stored for posterior Cu quantification. The residual solid samples were washed twice with 18 MΩ de-ionized water to extract eventual no sorbed Cu trapped into the sample and then dried, sieved (2 mm) and stored until the desorption experiments.

#### 3.2.3. Batch experiment

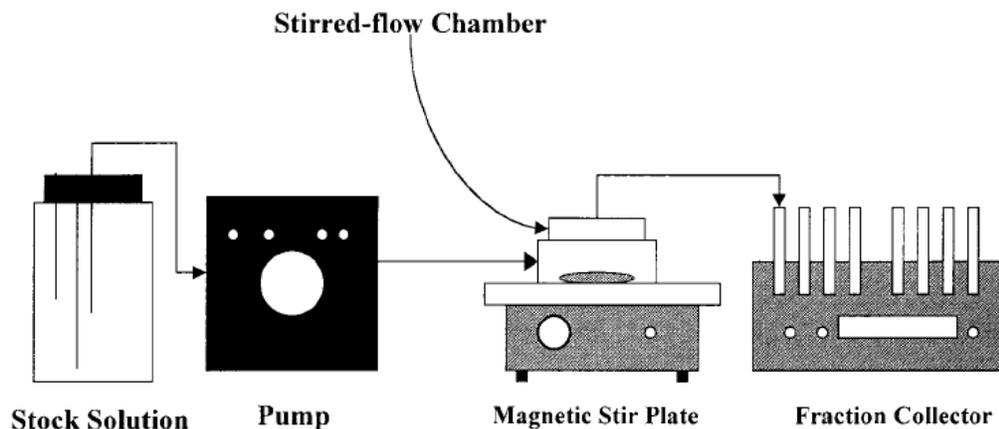
To gain information about the desorption behavior of the samples, a batch experiment was carried out in triplicate with all the treatments (three organic compost rates, plus control and two spiking times, totalizing 26 treatments) before the stirred-flow study. One gram of pretreated samples was submitted to 20 mL 0.01 mol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> in 50 mL polypropylene tube. The suspensions were shaken for 0.5, 1, 2, 5, 16 and 24h (accumulated

time). After each shake time, the suspension was centrifuged (3,000 rpm for 10 min), the supernatant was stored for Cu quantification, and the residual sample resuspended with 20 mL of 0.01 mol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> until complete 24h of desorption. The pH of the extracts was measured during the sorption and in each point of the desorption.

### 3.2.4. Stirred-flow experiment

Based on the results from the batch experiment, the treatments with addition of 5% and 25% of compost, 1 and 30 days, were selected for the stirred-flow experiment. 0.4 g of pretreated samples and a teflon-coated magnetic stir bar were placed into the reaction cell with approximately 8 mL of volume. A syringe was used to rapidly full fill the chamber with 0.01 mol L<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> and desorption was evaluated by allowing the solution to flow for 3 h at 1.3 mL min<sup>-1</sup>. A 0.45 µm membrane (mixed cellulose - Merck Millipore Ltd., Tullagreen, Ireland) was used to keep the soil in the chamber. Samples of the effluent were collected using a fraction collector at fixed time intervals (5 min). The fraction collector was set to start as soon as the first drop exit the outflow tube. The flow was monitored over all the experiments and fluctuate less the 3%. To attest if the desorption is rate limited, the flow was stopped for 30 min (Shi et al., 2013; Strawn and Sparks, 2000). This experiment was carried out in duplicates.

The Cu contents in effluents collected in the batch and stirred-flow experiments were determined using microwave induced plasma analytical spectrometry (MIP-MS). When light particles of the organic compost were detected, the extracts were filtered in 0.45 µm membrane (mixed cellulose - Merck Millipore Ltd., Tullagreen, Ireland) to avoid interference in the MIP functionality. Pretest showed no effect of filtration on the Cu concentration (data not shown).



**Figura 1.** Schematic illustrating the experimental set up of the stirred-flow experiment [modified from Strawn and Sparks (2000)].

### 3.2.5. Data analysis

Graphs showing the percentage of Cu desorbed versus time were created for visual comparison of the effect of the treatments. Cumulative Cu desorption was plotted in function of time, and a linear model was used to interpret desorption rate (Strobel et al., 2001). The distribution coefficient (kg L<sup>-1</sup>) was obtained by  $K_d = S/C$ , where  $S$  is the concentration of metal retained by the solid sample (mg kg<sup>-1</sup>); and  $C$  is the concentration of the metal in the equilibrium solution (mg L<sup>-1</sup>) (Sposito, 1984).

The statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to compare means using the least square means tests with measures repeated on time ( $p < 0.05$ ). When the interaction among the factors were observed ( $p < 0.05$ ), the main unfolding were presented. Covariance analysis were carried out to assess the possible effect of pH change on the Cu desorption.

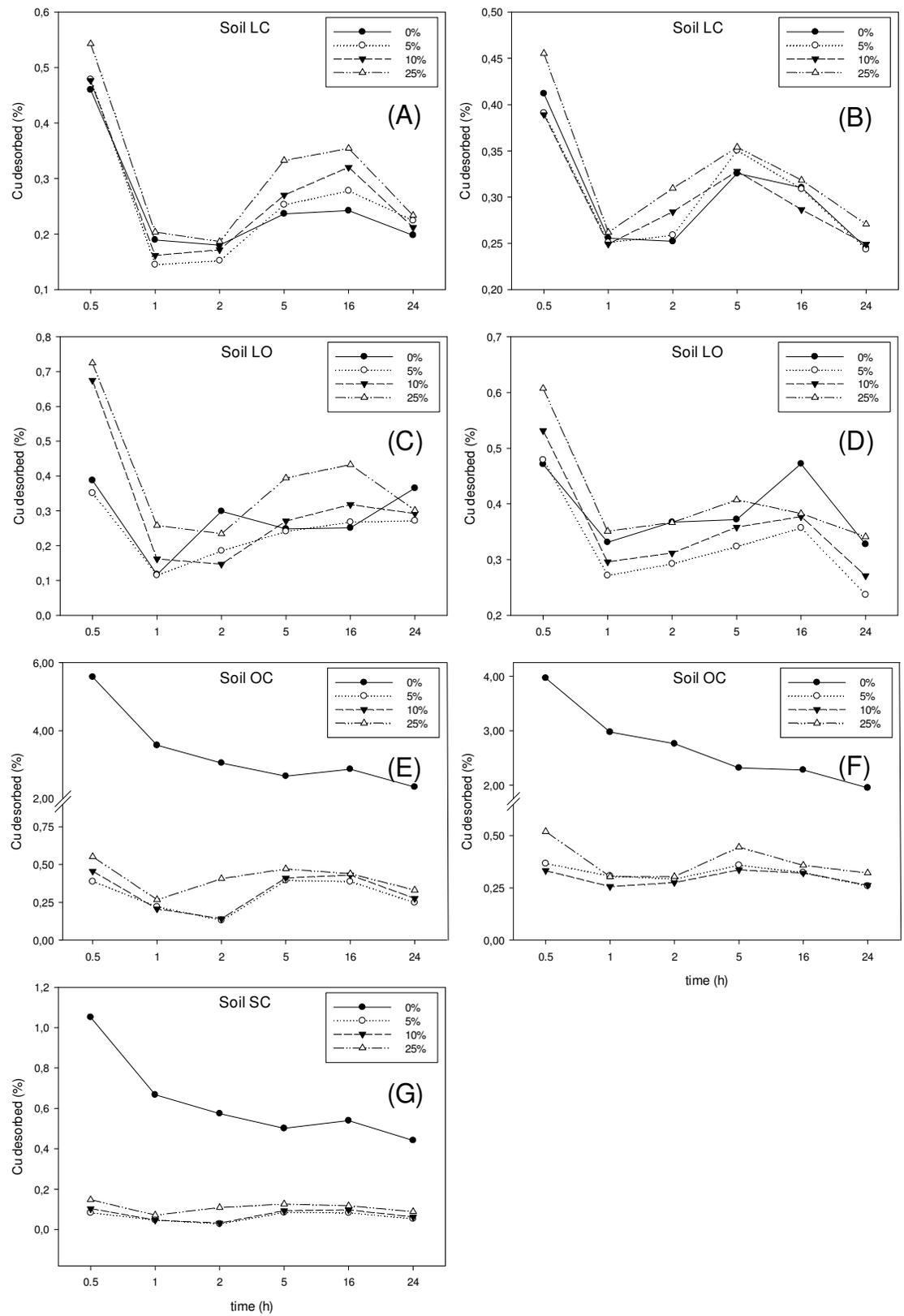
Horizontal Analysis can be used to interpret the results of the least square means tests with measures repeated on time. The horizontal analysis consists in answer five questions: i) is the interaction between treatment and time null? (i. e. are the curves parallel?); ii) if the curves are parallel, are they coincident? (i.e. is the treatment effect null?); iii) if the curves are parallel, are they horizontal? (i.e., is the time effect null?); iv) if the curves are not parallel, is the time effect null into each one of the treatments?; v) if the curves are not parallel, is the treatment effect null into each one of the time?

### **3.3. RESULTS AND DISCUSSION**

#### **3.3.1. Batch experiment**

Regardless of the incubation time, the curves of desorption presented a similar behavior (Figures 2). However, in the LO soil incubated for one day (Figure 2 C) the treatments presented a more variable profile. For the samples SC and OC (in both incubation time) the addition of compost reduced the desorption of Cu. On the other hand, LC and LO soils had more desorption after addition of 25% of compost. Increasing the incubation time resulted in reduction of Cu desorption in all treatments. However, in all treatments was observed a low Cu desorption, representing less than 10% of the metal applied.

The variance of pH has no effect on the variation on the amount of Cu desorbed ( $p > 0.05$ ) (data not shown), what means that the effect of time and treatment is more important for the desorption than the pH for or study. Based on this conclusion, a least square means test with measures repeated on time was made to assess the effect of the organic compost addition in each soil on the desorption of Cu over time ( $p < 0.05$ ) (Table 2).



**Figure 2.** Percentage of Cu desorbed from the samples LC, LO and OC under three organic compost rate and control after 1 day (A, C, and E) and 30 days (B, D and F) in contact with the metal; and the percentage of Cu desorbed from the sample SC (G).

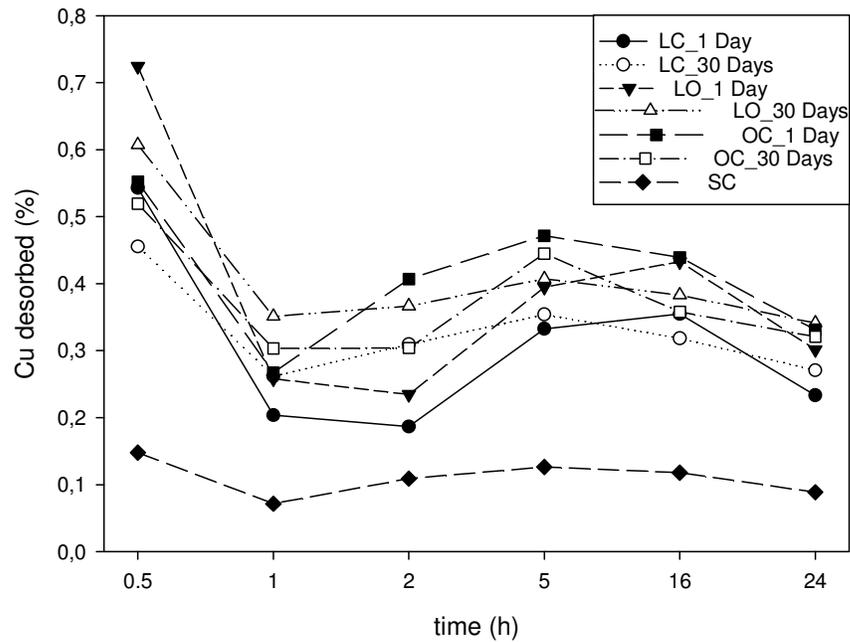
**Table 2.** Result for least square means tests with measures repeated on time (six points of desorption).

Soil sample	Compost rate (%)	Desorption after 1 day						Desorption after 30 days					
		1	2	3	4	5	6	1	2	3	4	5	6
LC	0	Aa*	Ba	Ba	Bb	Bb	Ba	Aab	Ca	Cb	Ba	Ba	Ca
	5	Aa	Cb	Cb	Bab	Bab	Ba	Ab	Da	Db	Ba	Ca	Da
	10	Aa	Ca	Ca	Bcab	Bab	Ca	Ab	Da	Ca	Ba	Ca	Da
	25	Aa	Ca	Ca	Ba	Ba	Ca	Aa	Da	Ca	Ba	BCa	Da
LO	0	Ab	Cb	Aa	ABb	Bb	Aa	Ab	Cab	Ba	Bab	Ab	Ca
	5	Ab	Cb	Bb	Bb	ABb	ABa	Ab	Cb	BCb	Bb	Bb	Db
	10	Aa	Cb	Cb	Bb	Bab	Ba	Ab	Cb	Cb	Bb	Bb	Cb
	25	Aa	Ca	Ca	BCa	Ba	Ca	Aa	Ca	BCa	Ba	Ba	Aa
OC	0	Aa	Ba	BCa	BCa	BCa	Ca	Aa	Ba	Ba	Ca	Ca	Da
	5	Ac	Bb	Cb	Ab	Ab	Bb	Ac	Bb	Bb	Ac	ABb	Cc
	10	Abc	Bb	Cc	Ab	Ab	Bb	Ac	Bc	Bb	Ac	Ab	Bc
	25	Ab	Cb	Bc	AB b	AB b	BCb	Ab	Cb	Cb	Bb	Cb	Cb

\*Different capital letters represent difference in the row; different lower cases represent difference in the column ( $p < 0.05$ ).

Following the script for Horizontal Analysis, the curves (Figure 2; Table 2) were classified as no parallel, once there was interaction between treatments and time also was observed effect of time in all treatments. It was also observed the effect of the treatments in each point of desorption, except for the soil LC, especially after 30 days of incubation, where the effect of treatment was null in most of the desorption points.

To better compare the Cu desorption from the soils, the treatment with 25% of organic compost was chosen (Figure 3). All the samples, in both incubation times, had a similar behavior, characterized by a rapid desorption in the 1st hour, followed by a lower desorption rate until five hours, and after that, the desorption increased again. Increasing the incubation time from 1 day to 30 days lead to a decrease in the initial desorption in all treatments. The reduction of the amounts of Cu released with aging may be attributed to changes in fractionation, causing a decrease in the most labile fraction (soluble fraction) with time (Fernández-Calviño et al., 2017). However, the treatments submitted to 30 days of incubation had a more constant desorption, resulting in a higher accumulated desorption (Table 3). This phenomenon may suggest that after 30 days of incubation, just the most labile Cu fraction changed to a less labile form.



**Figure 3.** Percentage of Cu desorbed from the samples submitted to 25% of organic compost addition after 1 and 30 days in contact with the metal.

All the samples presented a low rate of desorption (Table 3), and the addition of compost seemed to increase the desorption of Cu, except for the soils OC and SC, where the highest percentage of desorption occurred in the control treatment. This treatment also had the smallest  $K_d$  value (Table 3) what may explain the relatively high desorption.

In general, all the samples presented high values of  $K_d$ , and the increasing in the incubation time promoted higher affinity for the metal. The increase of Cu sorption capacity with time is reported by several authors (Sayen et al., 2009; Arias-Estévez et al., 2007). The rate of sorption can be governed by the transport rate of metal ions from outer surface (exterior) to the inner side (interior) of the adsorbent (Mishra et al., 2017). In addition to being transported in soil, Cu fractions change gradually with time from soluble and exchangeable Cu (exchangeable Cu and organically bound Cu) to less labile fractions (Cu bound to crystalline and amorphous materials) (Arias-Estévez et al., 2007). Besides that, the way Cu was distributed into the different fractions varied with the amount of Cu added and also with soil type (Nóvoa-Muñoz et al., 2008).

**Table 3.**  $K_d$  of the spiked samples and the total Cu desorption observed in the batch experiment.

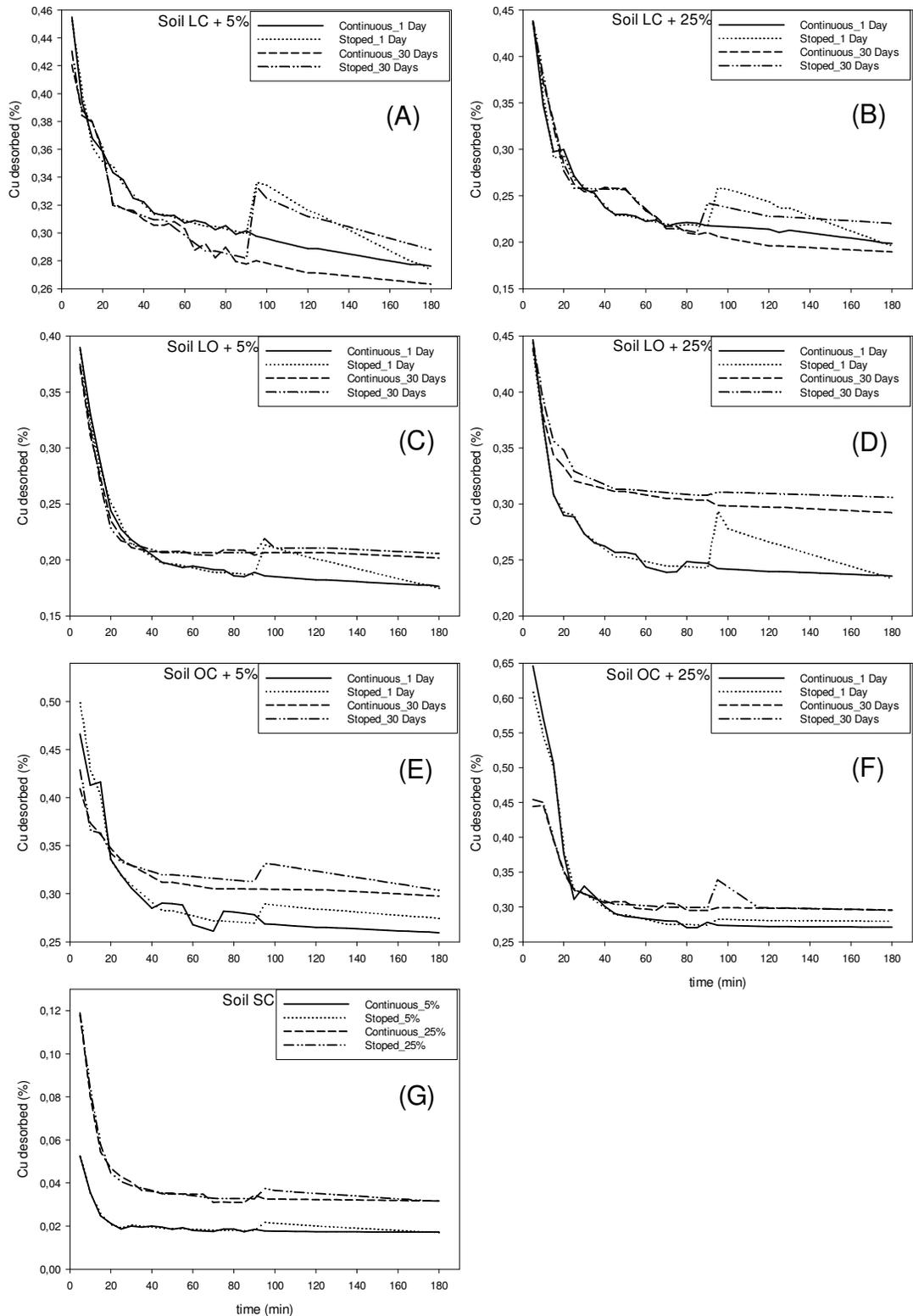
Soil	Treatment	$K_d$ (kg L <sup>-1</sup> )		total Cu desorption (%)	
		1 Day	30 Days	1 Day	30 Days
LC	0%	19.67	19.76	1.51	1.80
	5%	19.59	19.71	1.53	1.80
	10%	19.56	19.71	1.61	1.79
	25%	19.38	19.58	1.85	1.97
LO	0%	19.81	19.86	1.67	2.34
	5%	19.69	19.73	1.43	1.96
	10%	19.49	19.59	1.86	2.15
	25%	19.07	19.30	2.35	2.46
OC	0%	17.96	18.56	20.07	16.24
	5%	19.50	19.58	1.77	1.91
	10%	19.52	19.59	1.92	1.78
	25%	19.24	19.37	2.47	2.25
SC	0%			3.78	
	5%			0.38	
	10%			0.44	
	25%			0.66	

The mineralogy of the soil could play an important role on the EPT's dynamic. López-Periago et al. (2008) observed that amphibolite soils can take more Cu from the exchange sites to form slowly reversible bonds than can granite soils. The amphibolite soils contain more reactive surfaces forming complexes with Cu, presumably inner sphere complexes, than do the granite soils. On the other hand, the granite soils contain more permanently charged clay mineral where Cu is preferably bound to silanol groups of the lattice face, resulting in weak bonds. Instead, in amphibolite soils with dominant variable charge, Cu mainly interact with aluminol groups forming stronger bonds. Once the soil OC (sampled in an area of Planossol occurrence) presumably has more minerals permanently charged, Cu could form weaker bindings to this soil, what explain the greater desorption in the non-amended treatment. In this case, the addition of organic compost (5, 10 and 25% treatments) created additional bounds site to the Cu, reducing the desorption.

### 3.3.2. Stirred-flow experiment

Based on the results from the batch experiment, the treatments with 5 and 25% of compost were chosen for the stirred-flow experiment (Figure 4). Among all the samples, the soil LO presented a clearer change in the Cu desorption when the incubation time was increased. It is possible to see a more intense desorption in the first 25 to 35 min., followed by a slower desorption rate, indicating that the Cu desorption is ruled by distinct processes: a more readily extractable Cu fraction; and a second one corresponding to a Cu fraction with slower extraction kinetics. This

“two-step” release process is generally observed with natural adsorbent and was reported by Baranimotlagh and Gholami (2013) working with Zn desorption.



**Figure 4.** Percentage of Cu desorbed from the samples submitted to 5 and 25% of organic compost addition in stirred-flow experiment with continuous flow and 30 min stopped flow (stopped).

The bioavailability, toxicity and mobility of heavy metals in soils are largely determined by their kinetic process between solution and solid phase. The rates of metal release from the soil phase provide the basic information for describing the kinetic process (Wang et al., 2009). In general, the increasing of amendment application and incubation time resulted in a higher desorption rate and accumulated Cu desorption (%) and the proportional changes in the desorption rate and accumulated desorption followed the same tendency in all treatments (Table 4). Tella et al. (2016) observed that Cu and Zn availability were both increased in organic waste amended soil and Cu speciation in organic waste was dominated by organic species. Cu release through the mineralization of organic carbon from organic waste was responsible for increase in Cu availability.

The soil LC presented the contrary behavior. Looking the shape of the desorption curves, it is noticeable that the soil LC treated with 5% of compost (Figure 4 A) presented an initial desorption less intense than the same soil treated with 25% of compost (Figure 4 B), but the second step of release (less intense) was reached faster and in a higher concentration. This behavior suggests that, for the soil LC, the addition of organic compost, changed the Cu dynamic more than the incubation time. The soil LC presented the higher TOC content (56.5 mg kg<sup>-1</sup>) and lower pH (5.4) before the experiment (Table 1). Studying the effect of pine bark addition and aging on the Cu release capacity of a Spolic Technosol (sandy loam texture), Fernández-Calviño et al. (2017) concluded that the ageing effects were lower in the pine-bark amended than in the non-amended soil samples, thus suggesting that diffusive controlled reactions were less important in the pine bark than in the bulk soil.

**Table 4.** Desorption rate and accumulated desorption observed in the stirred-flow experiment.

Soil	Treatment	Desorption rate (mg g <sup>-1</sup> min <sup>-1</sup> )		Accumulated desorption (%)	
		1 Day	30 Days	1 Day	30 Days
LC	5%	0.0601	0.0571	11.11	10.60
	25%	0.0443	0.0432	8.40	8.25
LO	5%	0.0381	0.0416	7.29	7.80
	25%	0.0494	0.0606	9.28	11.15
OC	5%	0.0551	0.0616	10.37	11.31
	25%	0.0566	0.0604	11.05	11.25
SC	5%	0.0036		0.71	
	25%	0.0067		1.36	

Although presenting the same behavior of the other samples, the soil SC had a Cu release capacity much lower (Figure 4; Table 4). Sayen et al. (2009) found that the application of Cu by means of several additions, as in field conditions, increases the retention capacity of the soil relative to a single application of the metal (as proceeded with the samples LC, LO and OC in this experiment). The aging effect is responsible for this phenomenon, since it increases the amount of adsorbed Cu. With time, sites initially occupied are liberated, which provides more site for further Cu retention. Aging the soil reduces the amount of available Cu during a Ca(NO<sub>3</sub>)<sub>2</sub> leaching, what suggests a redistribution of a weakly-bound Cu fraction (exchangeable sites, for example) to a more strongly-bound fraction (thermodynamically more stable sites or into soil aggregates) (Sayen et al., 2009). For Sayen et al. (2009), the kinetic of Cu redistribution would be slower than the time scale considered in their experiment (until 60 days), probably

because of that, it was not possible to verify a drastic change in the desorption capacity between 1 and 30 days incubation time in our experiment (Figure 4).

Tella et al. (2016) and Liu (2016) observed that the Cu-availability coefficient was positively correlated with the C-mineralization coefficient. This suggests that organic carbon mineralization triggered the release of Cu. Organic waste mineralization could explain the increase in Cu availability during incubation. So, if part of the organic compost applied in the experiment was degraded over the 30 days of incubation time, this could explain the increasing in the accumulated release of Cu in the soils LO and OC (Figure 4).

The Cu release kinetics seem more complicated. Strobel et al. (2001), modeling the Cu release kinetics as depending on dissolved organic carbon and pH, observed that the Cu release rate depends on the interaction of both parameters. The addition of dissolved organic carbon might have affected the release kinetics slightly by increasing the ionic strength of the solution, because the ionic strength in the influent solutions was kept low to simulate forest soil solution conditions (Strobel et al., 2001).

Overall, the accumulated desorption using the stirred-flow system (Table 4) was greater than the accumulated desorption observed in the batch experiment (Table 3). During the desorption, in the stirred-flow system, the metal released into the liquid phase is removed from the chamber, which accelerates further desorption, especially if the flow rate is fast enough to avoid readsorption of the released metal (López-Periago et al., 2008). To determine if the desorption reaction was rate limited, the flow was stopped during the experiment for 30 min. If the reaction was not at equilibrium when the flow was stopped, the desorption would continue, shown by a peak in the desorption curve. There is a clear shift in the desorption in the sample LC treated with 5% and 25% of organic compost (Figure 4 A, B). In the sample LO+5%, the change only happened with one day of incubation and the same occurred in the LO+25% treatment (Figure 4 C, D). For the samples OC and SC, the interruption in the flow apparently had a minor influence. Thus, the reactions in soil LC are highly rate limited.

### 3.4. CONCLUSIONS

The low accumulated Cu desorption observed in all treatments suggest that the soils (amended or not) have a high capacity to retain the concentration of Cu applied. The Cu desorption occurs in two steps: one fast (from the most labile/exchangeable forms), followed by slower desorption (Cu bound to thermodynamically more stable sites). Increasing the contact time had a small effect in the desorption capacity. The mineralogy seems to play a role as important as the organic matter in the Cu sorption, once soils with high Fe oxides and clay content presented a higher retention capacity. In soils with initial low TOC content, the addition of organic compost lead to a greater Cu desorption rate and accumulated release. Our results suggest that the organic composts in garden soils could increase the Cu desorption, what may represent a potential risk, especially in more acid or sandy soils.

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## References

- Arias-Estévez, M., Nóvoa-Muñoz, J.C., Pateiro, M., López-Periago, E., 2007. Influence of aging on copper fractionation in an acid soil. *Soil Sci.* 172, 225–232.
- Baranimotlagh, M., Gholami, M., 2013. Time-Dependent Zinc Desorption in Some Calcareous Soils of Iran. *Pedosphere* 23, 185–193. doi:10.1016/S1002-0160(13)60006-5
- Cambier, P., Pot, V., Mercier, V., Michaud, A., Benoit, P., Revallier, A., Houot, S., 2014. Impact of long-term organic residue recycling in agriculture on soil solution composition and trace metal leaching in soils. *Sci. Total Environ.* 499, 560–573. doi:10.1016/j.scitotenv.2014.06.105
- CONAMA, 2012. Current Conama Resolutions published between September 1984 and January 2012 1126.
- Diagboya, P.N., Olu-owolabi, B.I., 2015. Effects of time , soil organic matter , and iron oxides on the relative retention and redistribution of lead , cadmium , and copper on soils 10331–10339. doi:10.1007/s11356-015-4241-0
- Fernández-Calviño, D., Cutillas-Barreiro, L., Paradelo-Núñez, R., Nóvoa-Muñoz, J.C., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., Arias-Estévez, M., 2017. Heavy metals fractionation and desorption in pine bark amended mine soils. *J. Environ. Manage.* 192, 79–88. doi:10.1016/j.jenvman.2017.01.042
- Gustafsson, J.P., Pechová, P., Berggren, D., 2003. Modeling metal binding to soils: the role of natural organic matter. *Environ. Sci. Technol.* 37, 2767–2774. doi:10.1021/es026249t
- Hernandez-Soriano, M.C., Peña, A., Mingorance, M.D., 2013. Soluble metal pool as affected by soil addition with organic inputs. *Environ. Toxicol. Chem.* 32, 1027–1032. doi:10.1002/etc.2159
- Jalali, M., Khanlari, Z. V., 2008. Effect of aging process on the fractionation of heavy metals in some calcareous soils of Iran. *Geoderma* 143, 26–40. doi:10.1016/j.geoderma.2007.10.002
- Liu, H., 2016. Relationship between organic matter humification and bioavailability of sludge-borne copper and cadmium during long-term sludge amendment to soil. *Sci. Total Environ.* 566–567, 8–14. doi:10.1016/j.scitotenv.2016.05.023
- Liu, P.Y., Wen, Q.L., Li, Y.J., Dong, C.X., Pan, G.X., 2015. Kinetics of Specific and Non-Specific Copper Sorption on Aggregates of an Acidic Paddy Soil from the Taihu Lake Region in East China. *Pedosphere* 25, 37–45. doi:10.1016/S1002-0160(14)60074-6
- López-Periago, J.E., Arias-Estévez, M., Nóvoa-Muñoz, J.C., Fernández-Calviño, D., Soto, B., Pérez-Novo, C., Simal-Gándara, J., 2008. Copper Retention Kinetics in Acid Soils. *Soil Sci. Soc. Am. J.* 72, 63. doi:10.2136/sssaj2006.0079
- Mishra, S.R., Chandra, R., Kaila A., J., Darshi B., S., 2017. Kinetics and isotherm studies for the adsorption of metal ions onto two soil types. *Environ. Technol. Innov.* 7, 87–101. doi:10.1016/j.eti.2016.12.006
- Najafi, S., Jalali, M., 2015. Effects of organic acids on cadmium and copper sorption and desorption by two calcareous soils. doi:10.1007/s10661-015-4804-z
- Nóvoa-Muñoz, J.C., Arias-Estévez, M., Pérez-Novo, C., López-Periago, J.E., 2008. Diffusion-induced changes on exchangeable and organic bound copper fractions in acid soil samples enriched with copper. *Geoderma* 148, 85–90. doi:10.1016/j.geoderma.2008.09.009
- Sayen, S., Mallet, J., Guillon, E., 2009. Aging effect on the copper sorption on a vineyard soil: Column studies and SEM-EDS analysis. *J. Colloid Interface Sci.* 331, 47–54. doi:10.1016/j.jcis.2008.11.049

- Shi, Z., Di Toro, D.M., Allen, H.E., Sparks, D.L., 2013. A general model for kinetics of heavy metal adsorption and desorption on soils. *Environ. Sci. Technol.* 47, 3761–7. doi:10.1021/es304524p
- Shi, Z., Wang, P., Peng, L., Lin, Z., Dang, Z., 2016. Kinetics of Heavy Metal Dissociation from Natural Organic Matter: Roles of the Carboxylic and Phenolic Sites. *Environ. Sci. Technol.* 50, 10476–10484. doi:10.1021/acs.est.6b01809
- Sipos, P., Németh, T., Kis, V.K., Mohai, I., 2008. Sorption of copper, zinc and lead on soil mineral phases. *Chemosphere* 73, 461–469. doi:10.1016/j.chemosphere.2008.06.046
- Sposito, G., 1984. *The chemistry of soils*. Oxford, New York. 277p.
- Strawn, D., Sparks, D., 2000. Effects of soil organic matter on the kinetics and mechanisms of Pb (II) sorption and desorption in soil. *Soil Sci. Soc. Am. J.* 64, 144–156. doi:10.2136/sssaj2000.641144x
- Strobel, B.W., Hansen, H.C.B., Borggaard, O.K., Andersen, M.K., Raulund-Rasmussen, K., 2001. Cadmium and copper release kinetics in relation to afforestation of cultivated soil. *Geochim. Cosmochim. Acta* 65, 1233–1242. doi:10.1016/S0016-7037(00)00602-5
- Tella, M., Bravin, M.N., Thuriès, L., Cazevaille, P., Chevassus-Rosset, C., Collin, B., Chaurand, P., Legros, S., Doelsch, E., 2016. Increased zinc and copper availability in organic waste amended soil potentially involving distinct release mechanisms. *Environ. Pollut.* 212, 299–306. doi:10.1016/j.envpol.2016.01.077
- USEPA, U.S.E.P.A.-, 1998. Method 3051a – Microwave assisted acid digestion of sediments, sludges, soils, and oils [WWW Document]. URL <https://www.epa.gov/hw-sw846/sw-846-test-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-soils-and-oils> (accessed 9.10.15).
- Wang, D.Z., Jiang, X., Rao, W., He, J.Z., 2009. Kinetics of soil cadmium desorption under simulated acid rain. *Ecol. Complex.* 6, 432–437. doi:10.1016/j.ecocom.2009.03.010
- Xu, D., Zhou, P., Zhan, J., Gao, Y., Dou, C., Sun, Q., 2013. Assessment of trace metal bioavailability in garden soils and health risks via consumption of vegetables in the vicinity of Tongling mining area, China. *Ecotoxicol. Environ. Saf.* 90, 103–111. doi:10.1016/j.ecoenv.2012.12.018

## 4. ASSOCIATION BETWEEN DISSOLVED COPPER AND DISSOLVED ORGANIC MATTER IN DAIRY-MANURE AMENDED SOILS

### Highlights

- Application of dairy manure to soil increase soil Cu concentration, impacting environmental quality.
- Dissolved organic matter (DOM) in soil from manure application facilitates Cu mobility through dissolved Cu-organic complexes.
- Water extraction of manure-amended soils showed correlations between DOC, Cu, Fe, and P.
- UV/Vis and FTIR spectral analysis suggest that DOM characteristics are important in Cu-binding to DOM.

### ABSTRACT

Dairy manure often has elevated concentrations of copper (Cu), and when applied to soil causes an increase in soil Cu concentration that is detrimental to soil microbial processes and poses risks for contamination of surface and ground water. Dissolved organic matter (DOM) concentrations also increase in manure-amended soils, which may facilitate Cu mobility. The goal of this study was to determine how manure application to soils alters water soluble Cu concentrations. Samples were collected from 0 – 30 cm and 30 – 60 cm depths in three successive years following annual or biennial field applications of dairy manure. Dissolved organic carbon (DOC), Cu, Fe, and P concentrations were measured in water extracts from the samples. Ultraviolet/visible spectroscopy (UV/Vis) and Fourier-transform infrared (FTIR) spectra were used to assess the DOM characteristics. After 3 years of dairy manure application, water extractable Cu concentration was greater in the surface and subsurface layers of manure-amended soils as compared to non-amended control soils and fertilizer-amended soils. The extractable Cu concentration was greatest in the plots that had the highest manure amendment rates (35 t ha<sup>-1</sup> and 52 t ha<sup>-1</sup>, dry weight). The UV/Vis parameters SUVA<sub>254</sub> and E<sub>2</sub>/E<sub>3</sub> correlated with Cu concentration in the extracts, suggesting that DOM characteristics are important in Cu-binding. FTIR spectra showed that carboxylic groups in the extracts from the manure-amended soils increased, Carboxylic acid groups on DOM will complex Cu, enhancing its mobility.

Keywords: DOC; DOM; Manure amendment; Cu contamination; Organic complexation of Cu

### 4.1. INTRODUCTION

Copper (Cu) is used in dairy hoof baths at concentrations of 5 to 10% to control the incidence of hoof disorders (Osorio et al., 2016). Commonly, the hoof baths are washed out of the barns and end up mingling with manure (Ippolito et al., 2013). Copper is also a micronutrient fed to dairy cows, of which a majority is excreted by the cows in their manure (Hristov et al., 2006). Subsequent application of manure to soils to increase organic matter content and promote nutrient cycling creates concern for the fate of Cu in soils and the environment.

In addition to increase in Cu from manure application to soils, organic matter and nutrient concentrations also increase. Increases in soil organic matter causes increases in dissolved organic matter (DOM), which can complex Cu, changing its mobility and availability. Ashworth and Alloway (2004) applied sewage sludge to a sandy loam soil column and observed a strong relationship between DOC and Cu that facilitated mobilization through the soil column. Application of P has also been shown to promote leaching of Cu and Cd through the soil profile (Zhang and Zhang, 2010), which was proposed to result from the competitive sorption between dissolved organic

matter and P for soil sorption sites, thus mobilizing the DOM and associated metals that are complexed to the DOM (Zhang and Zhang, 2010).

Dissolved organic carbon is a product of organic matter decomposition, which plays an important role in many biogeochemical processes (Antoniadis and Alloway, 2002; Hagedorn et al., 2015). Knowledge of the chemical and physical properties of DOM is useful to infer reactivity and mobility of soil organic materials, as well as its reactivity with other chemicals within the soil. To evaluate DOM characteristics, spectroscopic techniques are used to probe molecular composition and reactivity (Chen et al., 2016). Ultraviolet/visible (UV/Vis) spectroscopy is a rapid, high sensitivity technique that allows for inferences on the composition of natural organic matter (Hassouna et al., 2010). Weishaar et al. (2003) showed the specific UV absorbance determined at 254 nm ( $SUVA_{254}$ ) is strongly correlated with the amount of aromaticity as verified by  $^{13}C$  NMR. Using Fourier-transform infrared spectra (FTIR) and UV/Vis absorbance together can provide information on the chemical characteristics of DOM, including information on aromaticity, molecular weight and size, and functional groups (He et al., 2011).

Mobilization of DOM through the soil profile could promote metal mobilization. At typical soil pH values, DOM has a net negative charge, which promotes its mobility through the soil profile (Ashworth and Alloway, 2004), and attracts metal cations. However, DOM is subject to biodegradation, and can adsorb or precipitate with soil mineral constituents. Hagedorn et al. (2015) used autoradiographs to monitor DOM migration through a soil profile; they showed that dissolved organic  $C^{14}$  ( $DO^{14}C$ ) was retained in the upper 3-cm of the soil columns, but in deeper soil, the  $^{14}C$  was concentrated along soil pores and textural discontinuities. These results suggest that in well-structured soils, DOM is transported through preferential flow pathways. Hagedorn et al. (2015) also showed that leaching of  $DO^{14}C$  correlated negatively with oxalate-extractable Al, Fe and Mn, suggesting that soil oxides can immobilize DOM.

The aim of this study is to quantify extractable Cu and DOC released from soils receiving dairy manure applications, and characterize the DOM using UV and FTIR spectroscopy. We hypothesize that association of Cu and DOM promotes the transport of Cu through the soil profiles in alkali soils under high manure application and P fertilization rates.

## 4.2. MATERIALS AND METHODS

### 4.2.1. Sample characterization

The study plots are located in a long-term manure application experiment in Kimberly, Idaho (United States). The soil was Portneuf silt loam, a coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid. The experiment is designed in randomized blocks with four replicates, with each block consisting of all treatments. The manure is applied at two application frequencies (annual and biennial), and three yearly application rates (17 t ha<sup>-1</sup>; 35 t ha<sup>-1</sup>; 52 t ha<sup>-1</sup> of dairy manure, on a dry weight basis). Annual treatments received manure application in the fall of 2012, 2013, and 2014; biennial treatments received manure applications in the fall of 2012 and 2014. A fertilizer treatment (N, P, and K chemical fertilizers applied at recommended rates) and control plot (no nutrients applied) were also established. The applied manure had average pH of 8.8 ( $\pm 0.17$ ); 291.2 ( $\pm 62.90$ ) g kg<sup>-1</sup> of total organic carbon; 63.9 ( $\pm 12.12$ ) mg kg<sup>-1</sup> of total P; and 89.3 ( $\pm 31.95$ ) mg kg<sup>-1</sup> of total Cu. Plots from three blocks were used for the laboratory experiments. The soils were sampled at 0-30 cm and 30-60 cm depths before planting of wheat (*Triticum aestivum* L.) in 2013; potato (*Solanum tuberosum* L.) in 2014; and barley (*Hordeum vulgare* L.) in 2015. Average

pH,  $P_{\text{NaHCO}_3}$ , DTPA extractable Cu, and total organic matter content are provided for the unamended soil plots in Table S1.

#### 4.2.2. DOM Extraction and Filtration

DOM was extracted from the soil and manure samples using 18 M $\Omega$  de-ionized water with a solid to solution ratio of 1:5 (g:ml). Preliminary kinetic experiments (data not shown) showed that desorbed Cu concentrations did not change after 2 h, suggesting equilibrium. The suspensions were shaken for 2 h on an end-over-end shaker at 10 rpm. Following mixing, the suspensions were centrifuged at 1500 rpm for 7 min, and then filtered through a 45-mm diameter, 0.45- $\mu\text{m}$  mixed cellulose membrane filter (Merck Millipore Ltd., Tullagreen, Ireland). After filtration, the extracts were stored and kept at 4 °C until they were analyzed.

#### 4.2.3. Extract Characterization

The DOC content was determined by TOC analyzer (model TOC-L, Shimadzu, Maryland) for total organic carbon determination after acidification to remove carbonates. The content of water extractable Ca, Cu, Fe, and P were determined by atomic emission spectrometry (ICP-AES, model Thermo-iCAP 6000, Waltham, Massachusetts), which was standardized using NIST traceable standards (Specpure Inc., Ward Hill, Massachusetts). Dissolved reactive P (DRP) was determined by molybdate blue colorimetric test on the water extracts (Pote and Daniel, 2009), with solution absorption measured on a Thermo Scientific Genesys 10S UV-Vis spectrophotometer (Waltham, Massachusetts).

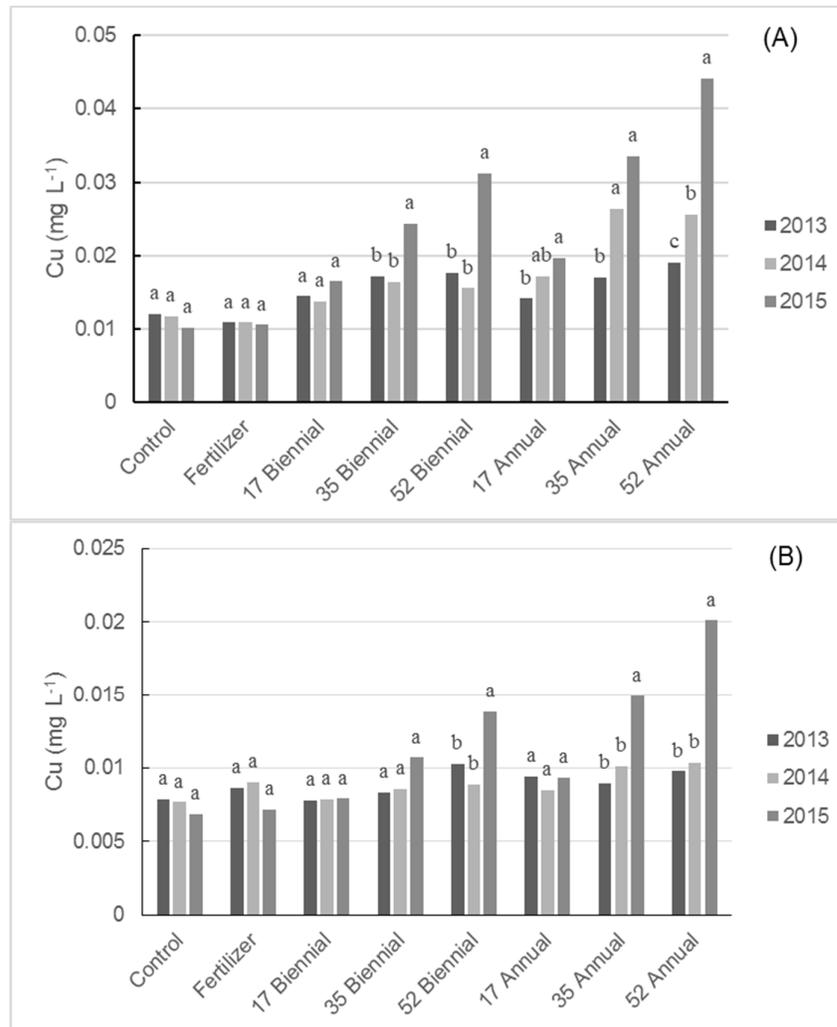
UV spectra were measured on a Thermo Scientific Genesys 10S UV-Vis spectrophotometer using a 1-cm path-length disposable plastic cuvettes (Brandtech Inc., Essex, Connecticut) in the wavelength range of 200-700 nm. The specific UV absorbance at 254 nm ( $\text{SUVA}_{254}$  ( $\text{L mg}^{-1} \text{m}^{-1}$ )) was used as an aromaticity index by normalizing the absorbance at 254 nm of each sample by the respective DOC concentration ( $\text{mg L}^{-1}$ ) (Mouloubou et al., 2016). The  $E_2/E_3$  ratio (the absorbance at 250 nm divided by the absorbance at 365 nm) was calculated to assess the molecular size index (Thomsen et al., 2002). Selected DOC extract samples were analyzed by FTIR analysis after freeze-drying and mixing with KBr (1:33 by weight). The FTIR spectra were collected between 400 and 4000  $\text{cm}^{-1}$  using a Perkin Elmer 2000 FTIR (Waltham, Massachusetts) and a diffuse reflection infrared Fourier transform (DRIFT) sample accessory.

#### 4.2.4. Data Analysis

The statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to compare means using the least square means tests with measures repeated on time ( $p < 0.05$ ). Pearson's Correlation tests were performed to investigate correlations between variables.

### 4.3. RESULTS AND DISCUSSION

In surface soils, annual application of manure for three years caused an increase with time ( $p < 0.05$ ) in water extractable Cu concentrations in all the manure treatments, except for the 17 t ha<sup>-1</sup> biennial application (Figure 1). In the subsurface soil samples, however, an increase in extractable Cu occurred only after three years of the two highest manure application rates (52 and 35 t ha<sup>-1</sup> annual and 52 t ha<sup>-1</sup> biennial).



**Figure 1.** Mean Cu concentration in soil extracts from the soil samples collected from 0-30 cm (A) and 30-60 cm (B) in 2013, 2014, and 2015. Numbers preceding the x-axis labels indicate application rate in t ha<sup>-1</sup>. Different letters represent difference by least square means tests with measures repeated on time ( $p < 0.05$ ) among the year for each treatment.

After three years of manure amendment, extractable Cu concentrations in the two highest manure application rates were about four times higher than Cu concentrations in the control and fertilizer plots. In the first year, the 17 t ha<sup>-1</sup> annual and biennial applications had 1.5 times less extractable Cu concentration than in the 52 t ha<sup>-1</sup> manure application, but two times higher than the extractable Cu concentration of the control samples. Water extractable Cu concentration from the manure itself averaged 2.85 ( $\pm 1.24$ ) mg L<sup>-1</sup>, suggesting that manure is the source for increased extractable Cu in the soils. Sun et al. (2017) studied agricultural soils under long-term pig

manure application, and observed that increases in Cu concentration in the soil caused increased bioavailability in the form of elevated Cu concentrations in wheat (*Triticum* spp.) straw and corn (*Zea mays* L.) stalks.

In the 35 and 52 t ha<sup>-1</sup> samples, annual application of manure led to greater extractable Cu concentrations at the 0-30 cm soil depth than the other treatments (Figure 1 and SI Table S2). After 3 years, the 52 t ha<sup>-1</sup> biennial application samples had similar extractable Cu concentrations as extracts from the 35 t ha<sup>-1</sup> annual application, suggesting that multiple high application rates of manure cause extractable Cu concentrations to dramatically increase, even if applied only biennially.

Water extracted Cu concentrations are a good measure of concentrations of the element in soil pore-water, and thus bioavailability. Moreno-Jiménez et al. (2011) compared the phytotoxicity of metals in soil solution extracted from soil pore water and de-ionized water soil extractions (1:20 soil:solution ratio) and found that both solutions reduced germination of *Lolium perenne* seeds. In addition to phytotoxicity, elevated Cu concentrations in soil pore-water pose potential risks to water quality. While there are no listed limits for soil water, typical freshwater concentrations of Cu are 0.003 mg L<sup>-1</sup> (Bowen, 1979), and the maximum contaminant level goal for Cu in drinking water is 1.3 mg L<sup>-1</sup> (EPA, 2009). Thus, Cu concentrations in soil extracts as reported here are approximately 10 times higher than present in uncontaminated freshwater ecosystems. An accurate estimate of environmental risk would require Cu speciation in the extract to be known. However, a 10-fold increase in extractable Cu concentration after only three years of manure application shows the risks of high manure application rates, which may affect plant growth (Rooney et al., 2006). The potential risks of elevated Cu concentrations to aquatic ecosystems by way of leaching or runoff is also likely to increase.

Extractable DOC concentrations increased with manure application rates and number of applications (Table 1). DOC concentration decreased with soil depth in all treatments (Table 1). In the 35 t ha<sup>-1</sup> and 52 t ha<sup>-1</sup> annual manure application rate treatments, subsurface DOC content was less than half of surface DOC content. Elevated DOC concentrations in upper soil layers are related to both increased input from plant residues (Hassouna et al., 2010), organic amendments such as manure, and their respective degradation by-products. DOC in the deeper layers is proposed to be composed of aged compounds, lixiviated organic compounds, or resynthesized carbon compounds from microbial activity (Kaiser and Kalbitz, 2012; von Lutzow et al., 2006).

**Table 1.** DOC (mg L<sup>-1</sup>) content for the soil extracts in 0-30 cm and 30-60 cm layers in 2013, 2014, and 2015.

Treatment	2013	2014	2015	2013	2014	2015
	0 – 30 cm			30 – 60 cm		
Control	29.1aC*	28.9aC	22.8bD	19.7aA	20.4aA	17.8aB
Fertilizer	25.4aC	29.8aC	27.3aD	19.0bA	23.9aA	18.9bA
17 t ha <sup>-1</sup> - Biennial	30.1aB	31.9aC	30.3aC	18.1aA	21.3aA	20.0aB
35 t ha <sup>-1</sup> - Biennial	38.1aAB	38.0aBC	43.9aB	20.5aA	21.4aA	23.7aB
52 t ha <sup>-1</sup> - Biennial	42.2aA	41.2aB	52.0aA	22.9aA	22.3aA	27.1aA
17 t ha <sup>-1</sup> - Annual	32.8aB	42.0aB	36.8aB	21.2aA	23.8aA	21.9aB
35 t ha <sup>-1</sup> - Annual	37.5bAB	59.2aA	55.1aA	21.1bA	24.6abA	27.6aA
52 t ha <sup>-1</sup> - Annual	44.8bA	60.2aA	66.4aA	22.2bA	23.9aA	30.8aA

\* Different lower case represents difference in row, and different capital letters represent difference in column by least square means tests with measures repeated on time ( $p < 0.05$ ).

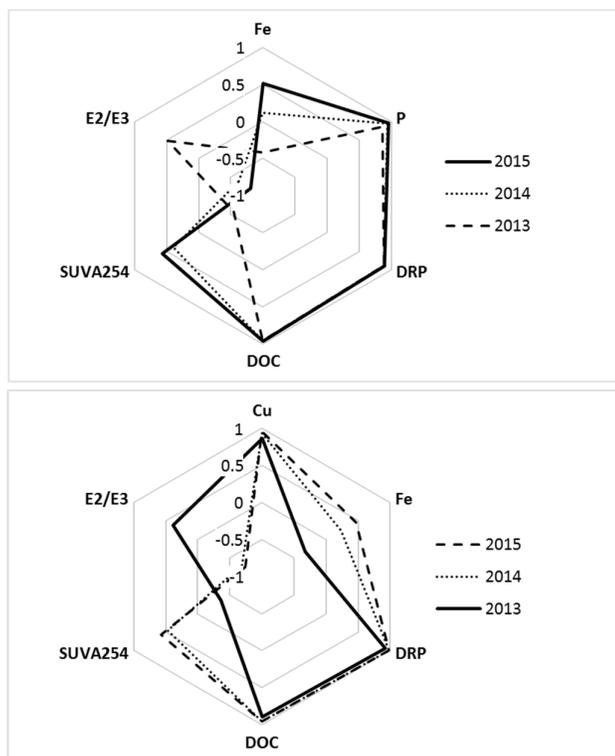
The aromaticity index (SUVA<sub>254</sub>) of DOM decreased with soil depth (Table 2), suggesting either preferential flow of structurally simple molecules to deeper soil horizons, or retention of more complex and aromatic DOM molecules by mineral components of deeper soil horizons that prevents them from being extracted with water (Corvasce et al., 2006). Hassouna et al. (2010) proposed that several seasons may be needed to observe significant changes in SUVA<sub>254</sub> in soils with manure application. However, increases in the SUVA<sub>254</sub> values in our study were observed after only three years (Table 2), providing evidence that high manure application rates accelerate enrichment of distinct organic matter compounds in the subsurface.

**Table 2.** SUVA<sub>254</sub> (L mg<sup>-1</sup> m<sup>-1</sup>) values for the soil extracts in 0-30 cm and 30-60 cm layers in 2013, 2014, and 2015.

Treatment	2013	2014	2015	2013	2014	2015
	0 – 30 cm			30 – 60 cm		
Control	0.0236bA	0.0251bAB	0.0301aB	0.0271aA	0.0224aA	0.0275aA
Fertilizer	0.0253aA	0.0245aB	0.0261aC	0.0283aA	0.0222aA	0.0226aA
17 t ha <sup>-1</sup> - Biennial	0.0256bA	0.0259bB	0.0325aA	0.0335aA	0.0187aA	0.0251aA
35 t ha <sup>-1</sup> - Biennial	0.0227bA	0.0283bA	0.0284aB	0.0264aA	0.0224aA	0.0234aA
52 t ha <sup>-1</sup> - Biennial	0.0224bA	0.0274aA	0.0304aB	0.0239aA	0.0220aA	0.0236aA
17 t ha <sup>-1</sup> - Annual	0.0228bA	0.0221bB	0.0325aA	0.0261aA	0.0112bB	0.0247aA
35 t ha <sup>-1</sup> - Annual	0.0229bA	0.0236bB	0.0331aA	0.0223aA	0.0198aA	0.0269aA
52 t ha <sup>-1</sup> - Annual	0.0223cA	0.0257bA	0.0346aA	0.0217aA	0.0222aA	0.0330aA

\* Different lower case represents difference in row, and different capital letters represent difference in column by least square means tests with measures repeated on time ( $p < 0.05$ ).

Correlation of manure application rate, extractable DOC concentrations, and extractable Cu concentrations (Figure 2) suggest that soluble metal-DOM complexes are enhancing Cu mobility in soil (Zhang et al., 2016). The correlation between Cu and SUVA<sub>254</sub> became more positive after each successive annual application (Figure 2). E<sub>2</sub>/E<sub>3</sub> is used to track changes in the relative size of organic matter molecules; as the E<sub>2</sub>/E<sub>3</sub> ratio decreases, the aromaticity (SUVA<sub>254</sub>) increases (Mouloubou et al., 2016; Thomsen et al., 2002). The correlation of extractable Cu concentrations with E<sub>2</sub>/E<sub>3</sub> became more negative with time, indicating that DOM associated with extractable Cu has more aromatic components and larger molecular weights (Figure 2). Chen et al. (2013) found that high molecular weight (HMW) DOM from riverine samples generally showed stronger binding affinities to Cu<sup>2+</sup> and Hg<sup>2+</sup> than low molecular weight (LMW) DOM. Chen et al. (2013) proposed that the HMW DOM has structural (e.g., aromatic functional groups) or compositional advantages such as a diversity of functional groups that favor the formation of strong metal binding.



**Figure 2.** Pearson's correlations observed for water extractable Cu (top panel) and total dissolved P (bottom panel) in 2013, 2014 and 2015, 0-30 cm soil depth.

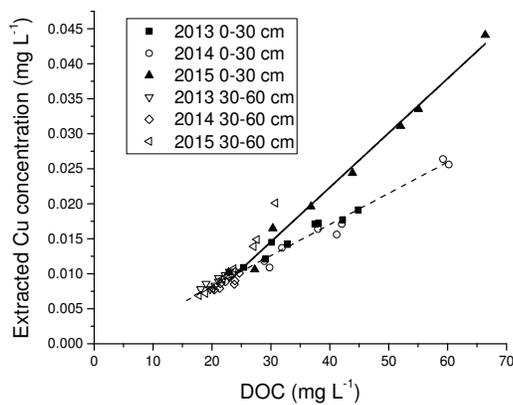
The absorbance of DOM at a specific UV/Vis wavelength (SUVA<sub>254</sub>) is used to study reactivity of DOM (Corvasce et al., 2006; He et al., 2016; Kikuchi et al., 2017). DOM aromaticity indices have been related to increases in complexation of Cd, Ni, and Zn (Baken et al., 2011; Cornu et al., 2011). Gangloff et al. (2014) observed similar relationships for rare earth elements. Amery et al. (2010) showed that Cu affinity for DOM at low Cu<sup>2+</sup> activity in wastewater, soil, and soil amendments (pig manure and sewage sludge) was linked to aromaticity as determined by SUVA and DOM concentration. Thus, increased aromatic DOM components appear to facilitate strong bonds with Cu, which will allow Cu to be transported through the soil profile bound to functional groups on the DOM. The high correlation between Cu and SUVA<sub>254</sub> (Figure 2) suggests that the relatively high water extractable Cu in subsurface soils (Figure 1) is due to increased mobilization of Cu bound to high molecular weight DOM.

There was a strong correlation between Cu, total dissolved P concentrations, and DRP in the water extracts (Figure 2). These correlations become slightly stronger with time, probably because total loading of P and Cu are increasing with continued manure application. Both P and DOM are negatively charged molecules, and interact in soils in a variety of ways that potentially influence their adsorption reactions. Zhang and Zhang (2010) evaluated the effect of P application on DOM and Cu, Cd, and Zn leaching in soils and showed that at high P application rates (500 mg kg<sup>-1</sup> P), DOM leaching increased by 145%. The effect of P on DOM and metals leaching was greater in sandy soils than in loamy soils. It is likely that sorption of P increases the negative charge on the soil surface, making it more difficult for sorption of DOM, thus making DOM more mobile. The interaction of P with DOM-metal complexes creates a potential risk for metal mobilization, especially in agricultural soils treated with high organic matter inputs or high fertilization rates.

The correlation between extracted P concentration and DOC concentration was strongly positive, approaching one by the third year of application (Figure 2). The correlation between extracted P concentrations and  $E_2/E_3$  trended more negative with time, and the association with  $SUVA_{254}$  became more positive. These correlations indicate a relationship between DOC amount and its molecular properties and water extractable soil P. Working with a loam soil from agricultural land in Australia, Sharma et al. (2017) found that soluble organic carbon increased significantly with P application rates, and that the soluble forms of both elements were linearly correlated ( $R^2 = 0.79$ ). This suggests that some phosphate forms are sorbed on organic compounds, effectively increasing their mobility through pore water in the soil profile. In addition to affecting DOM adsorption to soil particles, available P can facilitate soil organic matter degradation by stimulating microbial activity (Mao et al., 2017), which could either increase or decrease the concentration of DOM, depending on the net result of carbon mineralization and immobilization. Song et al. (2011) observed that after one year of amendment, P application stimulated microbial growth and consequently, increased DOC concentration in soil pore water.

The Cu/DOC ratio (SI Figure S1) can be used as an indicator of Cu-DOM binding because it allows a relative comparison of the affinity and association between the dissolved metal and organic matter (Nierop et al., 2002). Figure 3 shows extractable Cu concentrations versus DOM for all the plots and years. There is an overall positive linear correlation for the combined data ( $r^2 = 0.925$ ,  $p < 0.01$ ); however, the presence of two distinct relationships suggests that extractable Cu-DOC interactions differ chemically (Figure 3). The year 2015 0-30 cm samples have a greater Cu/DOC ratio than the remaining data, which indicates that the highest rate of manure input caused an increase in extractable Cu concentration without a corresponding increase in extractable DOC concentration.

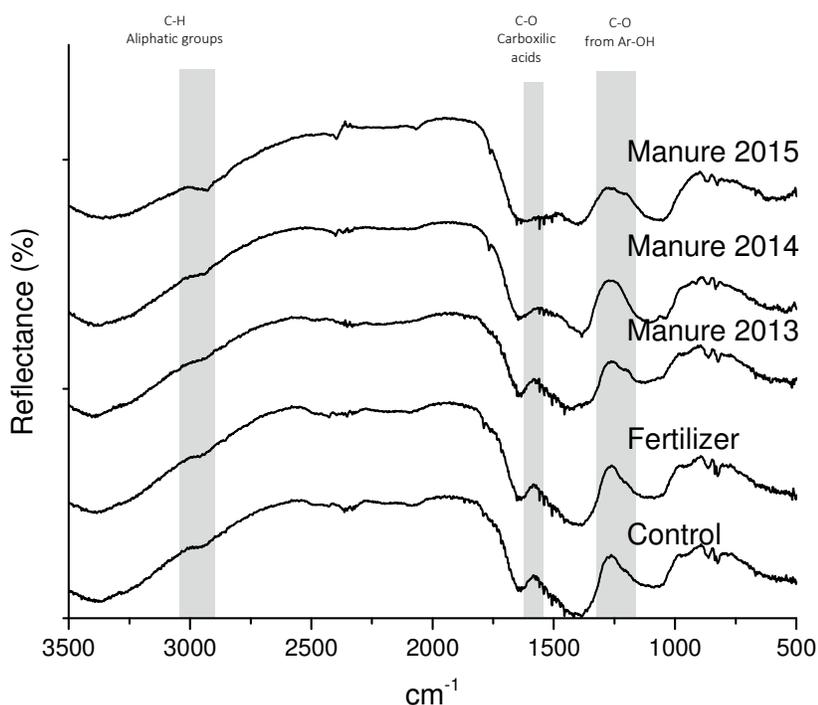
In subsurface soils, the three greatest manure input rates caused changes in the Cu/DOC only after 3 years of application (SI Figure S1); those points also appear to have a unique slope (Figure 3). At low  $Cu^{2+}$  activity, high affinity sites on DOM are preferentially occupied by Cu (Amery et al., 2010). As  $Cu^{2+}$  activity increases, low affinity sites become occupied. Thus, high Cu/DOC ratios suggest that Cu is occupying high and low affinity sites. In the  $52 \text{ t ha}^{-1}$  annual manure input soil extracts, the Cu/DOC ratios are similar in samples from both depths (SI Figure S1), suggesting that at high manure application rates a strong Cu-DOC association occurs even in lower soil layers. If a high Cu/DOC ratio is indicative of greater saturation of DOC sites, then increased manure application is promoting Cu-DOC complexation and consequently an important mechanism for increased Cu mobility.



**Figure 3.** Water extractable Cu concentrations vs DOC concentrations in soil extracts for all plots, years and depths.

Soil pH is an important factor in binding of Cu by DOM. Nierop et al. (2002) showed that more Cu is bound to DOM at pH 4.5 than pH 3.5 at a given Cu/C ratio. In soils with pH greater than  $\sim 6$ , such as the ones used in this study, functional groups on the DOM molecules are deprotonated, resulting in decreased proton competition and higher affinity of DOM to bind metals.

DOM is a complex mixture of organic compounds (carboxyl, phenol, quinonyl, amino, and other functional groups) formed by decomposition of plant and animal residues. In FTIR spectra, absorption bands and intensity indicate the presence and quantity of functional groups with known chemical compositions (Bantle et al., 2014). Figure 4 shows the DRIFT-FTIR spectra of samples from the control and fertilizer plots collected in 2013, and plots of the treatment with  $52 \text{ t ha}^{-1}$  applied in 2013, 2014, and 2015. Differences in the spectral region between  $3040 \text{ cm}^{-1}$  and  $2890 \text{ cm}^{-1}$  are present; this region is related to the stretching of C-H of aliphatic groups. Changes in the shapes of the peaks between  $1625 \text{ cm}^{-1}$  and  $1560 \text{ cm}^{-1}$  correspond to the presence of carboxylic groups. Changes in the peaks between  $1340 \text{ cm}^{-1}$  and  $1180 \text{ cm}^{-1}$  are related to binding of the C-O from carboxylic acids and C-O from Ar-OH (Abdulla et al., 2010; Ellerbrock and Kaiser, 2005; Zhang et al., 2017).



**Figure 4.** DRIFT-FTIR spectra of the soil extracts: Control 2013, fertilizer 2013; and  $52 \text{ t ha}^{-1}$  annual application sampled in 2013, 2014 and 2015 in surface (0 – 30 cm). Gray bars highlight regions discussed in text.

Hassouna et al. (2010) monitored DOM from a soil under maize production and concluded that the alteration of DOM upon increasing soil depth was associated with variations of organic matter production, elimination, and stabilization mechanisms operating within the soil column. They also concluded that DOM production in the surface soil layer was apparently influenced by plant precursors, as indicated by the higher contribution of hydrophobic and aromatic structures presumably derived from oxidative degradation of lignin. In deeper soil layers, Housouna et al. (2010) observed that DOM was enriched in aliphatic, hydrophilic, and more

humified structures, most likely derived from highly altered SOM. In the current study, plots with 3 years of manure application had a relative decrease in C-O from Ar-OH at  $1230 (\pm 30) \text{ cm}^{-1}$  typically associated with lignin groups (Abdulla et al., 2010; Pandey, 1999). Lignin in soils is indicative of a lower degree of oxidation or mineralization because the lignin may be accumulated in the form of relatively intact particulate plant-derived debris (Peltre et al., 2017). Thus, the decrease in peaks at  $1230 \text{ cm}^{-1}$  in the manure-amended soil suggest that that lignin-type compounds in the DOM are decreasing in the soil, and that the continuous manure application is leading to a more intense degradation process is occurring in these soils compared to the NPK and control plots. Peltre et al. (2017) proposed that different types of organic amendments promoted formation of different types of carbon compounds within the soils because of varying microbial activities.

The FTIR peaks in the  $52 \text{ T ha}^{-1}$  manure application rate soils indicate that the proportion of aliphatic and carboxylic functional groups increased compared to the other samples (Figure 4). The association of Cu with DOM compounds depends on the hydrophobic/hydrophilic nature of the DOM (Hur and Lee, 2011). Chen et al. (2015) used two-dimensional correlation spectroscopy and synchronous fluorescence and infrared absorption spectroscopy to explore the binding process of Cu and humic acid (HA) and concluded that the Cu binding to HA involved functional groups with an affinity as follows: carboxyl, C-O of polysaccharides, phenolic groups, aryl, amide, aliphatic groups.

Phenolic and aromatic carboxylic groups may form highly stable ring structures with Cu, with a relatively high stability constant (Chen et al., 2015; Manceau and Matynia, 2010). Fuentes et al. (2013) studied Cu binding by several humic acid materials and proposed that Cu ions selectively complex to phenolic functional groups and O-alkyl functional groups on aromatic molecules. Wen et al. (2014) studied the effect of short- (3 years) and long- (22 years) term fertilization (NPK and NPK + swine manure) on Al-DOM bonds with soils and observed that fertilization changed the Al-binding characteristics as well as the aliphatic groups within the DOM. Complexes between DOM and Cu dissociate very slowly because of the high affinity of Cu with the functional groups, facilitating Cu leaching to groundwater in the form of Cu-DOM complexes (Amery et al., 2010). Therefore, increase in the carboxylic acid functional groups, shown by the peaks between  $1625 \text{ cm}^{-1}$  and  $1560 \text{ cm}^{-1}$  (Figure 4), suggests that Cu-DOM is an important species in the manure-amended soils, which would cause increased Cu mobility in the soil profile.

Chen et al. (2015) proposed that the relevant aspects of metal-organic interactions remain unclear because the metal binding functionalities in DOM are not uniform and the availability of models to predict DOM-Cu binding are limited. Results from research such as presented in this paper complement the knowledge about DOM dynamics under agricultural soil conditions, facilitating the development of more accurate models.

#### 4.4. CONCLUSIONS

After three years of manure application at  $35 \text{ t ha}^{-1}$  and  $52 \text{ t ha}^{-1}$ , concentrations of water extractable Cu in the surface and subsurface soils increased. Correlation analysis confirmed the association between DOC and Cu, and also suggested that the DOM properties are important factors for binding of Cu to the DOM. Spectroscopic evaluation showed that functional groups known for having strong bonds with Cu (aliphatic and carboxylic) increased in DOM due to the accumulated effect of high manure amendment amounts. Once highly stable Cu complexes form with the DOM, Cu will have increased mobility and greater potential for transport deeper in the soil profile. As a result of enhanced Cu-mobility, soil health and water quality may be negatively impacted by manure

application to soils. Thus, monitoring is required in manure-amended soils to ensure that Cu concentrations do not exceed a critical threshold.

#### ACKNOWLEDGMENT

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#### REFERENCES

- Abdulla HAN, Minor EC, Dias RF, Hatcher PG. Changes in the compound classes of dissolved organic matter along an estuarine transect: A study using FTIR and C-13 NMR. *Geochimica et Cosmochimica Acta* 2010; 74: 3815-3838.
- Amery F, Degryse F, Van Moorleghem C, Duyck M, Smolders E. The dissociation kinetics of Cu-dissolved organic matter complexes from soil and soil amendments. *Analytica Chimica Acta* 2010; 670: 24-32.
- Antoniadis V, Alloway BJ. The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils. *Environmental Pollution* 2002; 117: 515-521.
- Ashworth DJ, Alloway BJ. Soil mobility of sewage sludge-derived dissolved organic matter, copper, nickel and zinc. *Environmental Pollution* 2004; 127: 137-144.
- Baken S, Degryse F, Verheyen L, Merckx R, Smolders E. Metal Complexation Properties of Freshwater Dissolved Organic Matter Are Explained by Its Aromaticity and by Anthropogenic Ligands. *Environmental Science & Technology* 2011; 45: 2584-2590.
- Bantle A, Borken W, Ellerbrock RH, Schulze ED, Weisser WW, Matzner E. Quantity and quality of dissolved organic carbon released from coarse woody debris of different tree species in the early phase of decomposition. *Forest Ecology and Management* 2014; 329: 287-294.
- Bowen HJM. *Environmental chemistry of the elements*. London ; New York: Academic Press, 1979.
- Chen TC, Hseu ZY, Jean JS, Chou ML. Association between arsenic and different-sized dissolved organic matter in the groundwater of black-foot disease area, Taiwan. *Chemosphere* 2016; 159: 214-220.
- Chen W, Habibul N, Liu XY, Sheng GP, Yu HQ. FTIR and Synchronous Fluorescence Heterospectral Two-Dimensional Correlation Analyses on the Binding Characteristics of Copper onto Dissolved Organic Matter. *Environmental Science & Technology* 2015; 49: 2052-2058.
- Chen WB, Smith DS, Gueguen C. Influence of water chemistry and dissolved organic matter (DOM) molecular size on copper and mercury binding determined by multiresponse fluorescence quenching. *Chemosphere* 2013; 92: 351-359.
- Cornu JY, Schneider A, Jezequel K, Denaix L. Modelling the complexation of Cd in soil solution at different temperatures using the UV-absorbance of dissolved organic matter. *Geoderma* 2011; 162: 65-70.
- Corvasce M, Zsolnay A, D'Orazio V, Lopez R, Miano TM. Characterization of water extractable organic matter in a deep soil profile. *Chemosphere* 2006; 62: 1583-1590.
- Ellerbrock RH, Kaiser M. Stability and composition of different soluble soil organic matter fractions - evidence from delta C-13 and FTIR signatures. *Geoderma* 2005; 128: 28-37.
- EPA. National Primary Drinking Water Regulations. In: Agency USEP, editor, Washington, DC, 2009.

- Fuentes M, Olaetxea M, Baigorri R, Zamarreno AM, Etienne P, Laine P, et al. Main binding sites involved in Fe(III) and Cu(II) complexation in humic-based structures. *Journal of Geochemical Exploration* 2013; 129: 14-17.
- Gangloff S, Stille P, Pierret MC, Weber T, Chabaux F. Characterization and evolution of dissolved organic matter in acidic forest soil and its impact on the mobility of major and trace elements (case of the Strengbach watershed). *Geochimica et Cosmochimica Acta* 2014; 130: 21-41.
- Hagedorn F, Bruderhofer N, Ferrari A, Niklaus PA. Tracking litter-derived dissolved organic matter along a soil chronosequence using C-14 imaging: Biodegradation, physico-chemical retention or preferential flow? *Soil Biology & Biochemistry* 2015; 88: 333-343.
- Hassouna M, Massiani C, Dudal Y, Pech N, Theraulaz F. Changes in water extractable organic matter (WEOM) in a calcareous soil under field conditions with time and soil depth. *Geoderma* 2010; 155: 75-85.
- He W, Lee JH, Hur J. Anthropogenic signature of sediment organic matter probed by UV-Visible and fluorescence spectroscopy and the association with heavy metal enrichment. *Chemosphere* 2016; 150: 184-193.
- He XS, Xi BD, Wei ZM, Jiang YH, Geng CM, Yang Y, et al. Physicochemical and spectroscopic characteristics of dissolved organic matter extracted from municipal solid waste (MSW) and their influence on the landfill biological stability. *Bioresource Technology* 2011; 102: 2322-2327.
- Hristov AN, Hazen W, Ellsworth JW. Efficiency of use of imported nitrogen, phosphorus, and potassium and potential for reducing phosphorus imports on Idaho dairy farms. *Journal of Dairy Science* 2006; 89: 3702-3712.
- Hur J, Lee BM. Characterization of binding site heterogeneity for copper within dissolved organic matter fractions using two-dimensional correlation fluorescence spectroscopy. *Chemosphere* 2011; 83: 1603-1611.
- Ippolito JA, Strawn DG, Scheckel KG. Investigation of Copper Sorption by Sugar Beet Processing Lime Waste. *Journal of Environmental Quality* 2013; 42: 919-924.
- Kaiser K, Kalbitz K. Cycling downwards - dissolved organic matter in soils. *Soil Biology & Biochemistry* 2012; 52: 29-32.
- Kikuchi T, Fujii M, Terao K, Jiwei R, Lee YP, Yoshimura C. Correlations between aromaticity of dissolved organic matter and trace metal concentrations in natural and effluent waters: A case study in the Sagami River Basin, Japan. *Science of the Total Environment* 2017; 576: 36-45.
- Manceau A, Matynia A. The nature of Cu bonding to natural organic matter. *Geochimica et Cosmochimica Acta* 2010; 74: 2556-2580.
- Mao R, Li SY, Zhang XH, Wang XW, Song CC. Effect of long-term phosphorus addition on the quantity and quality of dissolved organic carbon in a freshwater wetland of Northeast China. *Science of the Total Environment* 2017; 586: 1032-1037.
- Moreno-Jimenez E, Beesley L, Lepp NW, Dickinson NM, Hartley W, Clemente R. Field sampling of soil pore water to evaluate trace element mobility and associated environmental risk. *Environmental Pollution* 2011; 159: 3078-3085.
- Mouloubou OR, Prudent P, Mounier S, Boudenne JL, Abaker MG, Theraulaz F. An adapted sequential chemical fractionation coupled with UV and fluorescence spectroscopy for calcareous soil organic matter study after compost amendment. *Microchemical Journal* 2016; 124: 139-148.
- Nierop KGJ, Jansen B, Vrugt JA, Verstraten JM. Copper complexation by dissolved organic matter and uncertainty assessment of their stability constants. *Chemosphere* 2002; 49: 1191-1200.
- Osorio JS, Batistel F, Garrett EF, Elhanafy MM, Tariq MR, Socha MT, et al. Corium molecular biomarkers reveal a beneficial effect on hoof transcriptomics in periparturient dairy cows supplemented with zinc, manganese, and

- copper from amino acid complexes and cobalt from cobalt glucoheptonate. *Journal of Dairy Science* 2016; 99: 9974-9982.
- Pandey KK. A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy. *Journal of Applied Polymer Science* 1999; 71: 1969-1975.
- Peltre C, Gregorich EG, Bruun S, Jensen LS, Magid J. Repeated application of organic waste affects soil organic matter composition: Evidence from thermal analysis, FTIR-PAS, amino sugars and lignin biomarkers. *Soil Biology & Biochemistry* 2017; 104: 117-127.
- Pote DH, Daniel TC. Dissolved Phosphorus in Water Samples. In: Kovar J, Pierzynski G, editors. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. SERA-IEG 17, A USDA-CSREES Regional Committee, 2009, pp. 110-112.
- Rooney CP, Zhao FJ, McGrath SP. Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils. *Environmental Toxicology and Chemistry* 2006; 25: 726-732.
- Sharma R, Bella RW, Wong MTF. Dissolved reactive phosphorus played a limited role in phosphorus transport via runoff, throughflow and leaching on contrasting cropping soils from southwest Australia. *Science of the Total Environment* 2017; 577: 33-44.
- Song CC, Liu DY, Song YY, Yang GS, Wan ZM, Li YC, et al. Effect of exogenous phosphorus addition on soil respiration in *Calamagrostis angustifolia* freshwater marshes of Northeast China. *Atmospheric Environment* 2011; 45: 1402-1406.
- Sun FS, Li YQ, Wang X, Chi ZL, Yu GH. Using new hetero-spectral two-dimensional correlation analyses and synchrotron-radiation-based spectromicroscopy to characterize binding of Cu to soil dissolved organic matter. *Environmental Pollution* 2017; 223: 457-465.
- Thomsen M, Lassen P, Dobel S, Hansen PE, Carlsen L, Mogensen BB. Characterisation of humic materials of different origin: A multivariate approach for quantifying the latent properties of dissolved organic matter. *Chemosphere* 2002; 49: 1327-1337.
- von Lutzow M, Kogel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, et al. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science* 2006; 57: 426-445.
- Weishaar JL, Aiken GR, Bergamaschi BA, Fram MS, Fujii R, Mopper K. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science & Technology* 2003; 37: 4702-4708.
- Wen YL, Li H, Xiao J, Wang C, Shen QR, Ran W, et al. Insights into complexation of dissolved organic matter and Al(III) and nanominerals formation in soils under contrasting fertilizations using two-dimensional correlation spectroscopy and high resolution-transmission electron microscopy techniques. *Chemosphere* 2014; 111: 441-449.
- Zhang J, Chen LP, Yin HL, Jin S, Liu F, Chen HH. Mechanism study of humic acid functional groups for Cr(VI) retention: Two-dimensional FTIR and C-13 CP/MAS NMR correlation spectroscopic analysis. *Environmental Pollution* 2017; 225: 86-92.
- Zhang J, Hua P, Krebs P. The influences of dissolved organic matter and surfactant on the desorption of Cu and Zn from road-deposited sediment. *Chemosphere* 2016; 150: 63-70.
- Zhang MK, Zhang HM. Co-transport of dissolved organic matter and heavy metals in soils induced by excessive phosphorus applications. *Journal of Environmental Sciences* 2010; 22: 598-606.

### SUPPLEMENTARY INFORMATION

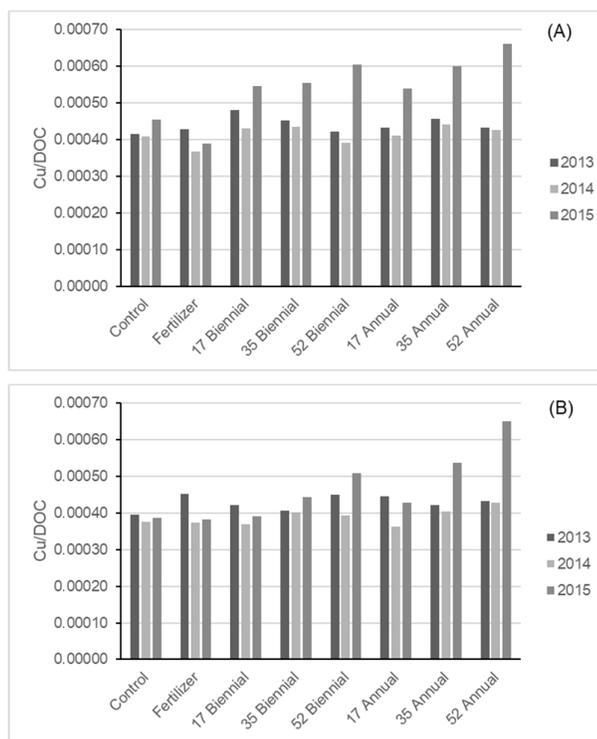
**Table S1.** Average organic matter, sodium bicarbonate extractable phosphorus (P<sub>NaHCO<sub>3</sub></sub>), pH and DTPA extractable Cu concentration from soils samples collected from three control plots (no amendment) in years 2013-2016. Values in parenthesis are standard deviations.

Depth (cm)	Organic Matter (%)	P <sub>NaHCO<sub>3</sub></sub> (mg kg <sup>-1</sup> )	pH	DTPA Cu (mg kg <sup>-1</sup> )
0-30	1.40 (0.11)	13.2 (1.6)	7.84 (0.074)	1.11 (0.14)
30-60	0.83 (0.067)	3.10 (0.51)	7.8 (0.12)	1.18 (0.23)

**Table S2.** Extractable Cu concentrations for the soil extracts in 0-30 cm and 30-60 cm layers in 2013, 2014, and 2015.

Treatment	Cu (µg L <sup>-1</sup> )					
	2013	2014	2015	2013	2014	2015
	0 – 30 cm			30 – 60 cm		
Control	12.1aBC	011.8aC	10.2aE	7.8aA	7.7aA	6.9aC
Fertilizer	10.9aC	10.9aC	10.6aED	8.6aA	9.0aA	7.2aC
17 t ha <sup>-1</sup> - Biennial	14.5Ab	13.7aBC	16.5aD	7.8aA	7.9aA	7.9aC
35 t ha <sup>-1</sup> - Biennial	17.2Ba	16.4bB	24.4aD	8.3aA	8.6aA	10.7aB
52 t ha <sup>-1</sup> - Biennial	17.7Ba	15.6bB	31.1aC	10.3bA	8.9bA	13.9aB
17 t ha <sup>-1</sup> - Annual	14.2Bb	17.1abB	19.6aD	9.4aA	8.5aA	9.3aBC
35 t ha <sup>-1</sup> - Annual	17.1Ba	26.4aA	33.5aB	8.9bA	10.1bA	14.9aA
52 t ha <sup>-1</sup> - Annual	19.1Ca	25.6bA	44.1aA	9.8bA	10.3bA	20.1aA

\* Different lower case represents difference in row, and different capital letters represent difference in column (p<0.05).



**Figure S1.** Cu/DOC ratios in soil extracts in 0-30 cm (A) and 30-60 cm (B) in 2013, 2014, and 2015. 17 Biennial = 17 t ha<sup>-1</sup> Biennial application; 35 Biennial = 35 t ha<sup>-1</sup> Biennial application; 52 Biennial = 52 t ha<sup>-1</sup> Biennial application; 17 Annual = 17 t ha<sup>-1</sup> Annual application; 35 Annual = 35 t ha<sup>-1</sup> Annual application; 52 Annual = 52 t ha<sup>-1</sup> Annual application.



## 5. FINGERPRINTING AND MACHINE LEARNING TECHNIQUES TO EVALUATE THE AUTHENTICITY OF ORGANIC AND CONVENTIONAL VEGETABLES

### Highlights

- Support Vector Machine is a powerful tool to vegetal classification based on elemental composition
- The crop systems promoted an elemental differentiation in vegetables which can be used for classification
- Using few variables to testify the authenticity made the process easier and cheaper
- Machine learning techniques can be used for inspection agencies for food quality control

### Abstract

Concern for the consumption of organic vegetables is growing throughout the world. We verified the efficiency of machine learning techniques in the classification of vegetables produced under both organic and conventional systems in the state of Pernambuco, Brazil. The contents of 25 elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn) were determined in 359 vegetables samples. Data mining techniques such as feature selection (F-score and Chi-squared) combined with classification algorithms (Support Vector Machine - SVM, Multilayer Perceptron - MLP, and Random Forest - RF) were applied to these samples. SVM, combined with chi-squared, outperformed the other algorithms obtaining accuracy of 100% in bell pepper samples (*Capsicum annuum*) and onion (*Allium cepa*), 97% in tomato (*Solanum Lycopersicum*) samples, of which 95% was the hit rate in organic samples. For lettuce (*Lactuca sativa*) samples the accuracy obtained was 92%, with a 90% hit rate of samples grown in the organic system. This high assertiveness rate highlights the potential of using elemental quantification and algorithms as support techniques in the process of authenticity and inspection of organic products.

Keywords: Classification; ICP-OES; Organic, conventional; Data mining; Support vector machine

### 5.1. Introduction

When evaluating food quality, researchers are often concerned with the use of pesticides, antibiotics and hormones in agriculture along with genetic modification and additives in food processing (Aschemann-witzel, Maroscheck, & Hamm, 2013). The number of people preferring organic food products is increasing, and this strong growing demand has led to an increase in the number of farmers committing to organic farming (Bigot, Meile, Kapitan, & Montet, 2015).

The intensification of the organic sector, driven by the growing demand and high prices of organic products could stimulate the occurrence of fraud (Laursen et al., 2013). For example, a conventional vegetable could easily be erroneously labeled as organic given that there are no visual differences between these products. Because of this, food authentication is of interest to many scientific communities, regulatory bodies and, principally, consumers. Food authentication methods must be scientifically proven, accurate and reliable so that, genuine products can be protected and distinguished from illegal alterations (Charlebois, Schwab, Henn, & Huck, 2016; Danezis, Tsagkaris, Brusic, & Georgiou, 2016)

In Brazil, organic farming that does not have an official organic certification can be part of a Social Control Organization (SCO), defined as a “group, association, cooperative or syndicate to which the family farmer

binds himself in direct sales, previously registered with the Ministry of Agriculture, Livestock and Supply, gaining a credible, organized process from the interaction of people or organizations, sustained participation, commitment, transparency and trust, recognized by society” (Brasil, 2007). In this kind of business, the consumer trades directly with the producer and has to trust in the product quality. A situation like this lends importance to the authentication process to ensure the organic origin of products.

The organic farming system differs primarily from the conventional one in its prohibition of the use of pesticides and synthetically produced fertilizers. This significant difference in agricultural practice and nutrient sources may be reflected in the chemical composition of plants (Laursen et al., 2013). Laursen et al. (2011) evaluated the applicability of elemental fingerprinting to the discriminating of organically and conventionally grown wheat (*Triticum aestivum* L. cv. Tommi), spring barley (*Hordeum vulgare* L. cv. Mixture of Simba/Smilla/Power), faba bean (*Vicia faba* L. cv. Columbo) and potato (*Solanum tuberosum* L. cv. Sava) in Denmark. Laursen et al. (2011) concluded that the chemical composition of plant tissue reflected the geographical origin as well as the cultivation system. However, they also stated that multi-elemental analysis alone did not contain sufficient discriminative power to enable accurate authentication.

Statistical and mathematical methods, such as multivariate statistics (Gundersen, Bechmann, Behrens, & Sturup, 2000) or machine learning models (Maione, Batista, Campiglia, Barbosa, & Barbosa, 2016) can be used to improve the interpretation of the chemical fingerprinting of products. In the latter, algorithms are used to analyze and classify results, and then recognize patterns. In other words, machine learning models are a powerful predictor instrument (Barbosa et al., 2015) and (Kotsiantis, Zaharakis, & Pintelas, 2006). Our aim in this study was to apply the machine learning model to classify organic and conventional vegetable samples based on their chemical composition.

## 5.2. Material and Methods

### 5.2.1. Vegetable samples

We collected edible parts from 158 lettuce (*Lactuca sativa*) from eight conventionally and eight organically cultivated sites (making a total of 80 and 78 samples, respectively); 106 samples of onions (*Allium cepa* Hysam) from eight conventionally and three organically cultivated sites (making a total of 76 and 78 samples, respectively); 56 samples of bell pepper (*Capsicum annuum*) from five conventionally and one organically cultivated sites (making a total of 46 and 10 samples, respectively); and 29 more samples of tomato (*Solanum lycopersicum*) from two conventionally (totalizing 19 samples) and one organically (totalizing ten samples) cultivated sites (making a total of 19 and 10 samples, respectively) in the state of Pernambuco, a state responsible for 20% of the vegetable production in the Northeast of Brazil (IBGE, 2006). Ten samples of certified organic lettuce and five of certified organic bell pepper from supermarkets in Recife (capital of the state of Pernambuco) were also collected. All these samples originated from farms in Pernambuco to avoid differences due to geographical variation. The samples considered “organic” were obtained from non-certified organic farms (SCO).

### 5.2.2. Chemical determination

The samples were dried (at 45°C) and milled (<1.0 mm). Digestion followed the method described by Araujo, Nogueira, & Nobrega (2002), using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in micro-wave assisted digestion. The contents of Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Ti, V, Zn were determined by inductively coupled plasma / optical emission spectroscopy (ICP-OES). Quality control of data was obtained using NIST Standard Reference material (spinach leaf) from the National Institute of Standards and Technology (NIST, USA).

### 5.2.3. Feature selection

When the number of features of a dataset is large, many of these features cannot strongly contribute to a meaningful data analysis. Thus, feature selection is a useful tool for obtaining models with a low computational cost (Tang, Alelyani, & Liu, 2014) and (Guyon & Elisseeff, 2003). Two feature selection methods were used in our study: F-score (Chen & Lin, 2006) and chi-squared (Pearson, 1992). The higher the F-score, the more likely the feature is more discriminative. However, a disadvantage of F-score is that it does not reveal mutual information common to other features because each feature is considered individually. The compute of F-score of the  $i$ th feature is shown in (Eq. 1):

$$F(i) = \frac{\left(\bar{x}_i^{(+)} - \bar{x}_i\right)^2 + \left(\bar{x}_i^{(-)} - \bar{x}_i\right)^2}{\frac{1}{n_+ - 1} \sum_{k=1}^{n_+} \left(\bar{x}_{k,i}^{(+)} - \bar{x}_i^{(+)}\right)^2 + \frac{1}{n_- - 1} \sum_{k=1}^{n_-} \left(\bar{x}_{k,i}^{(-)} - \bar{x}_i^{(-)}\right)^2} \quad (1)$$

where  $\bar{x}_i$ ,  $\bar{x}_i^{(+)}$  and  $\bar{x}_i^{(-)}$  represent the average of the total  $i$ th feature, the average of the positive  $i$ th feature and the average of the negative  $i$ th feature, respectively;  $n_+$  e  $n_-$  indicate the number of positive and negative instances, respectively;  $\bar{x}_{k,i}^{(+)}$  and  $\bar{x}_{k,i}^{(-)}$  indicate the  $i$ th feature of  $k$ -positive and  $k$ -negative instances, correspondingly.

Chi-squared (Pearson, 1992) is one statistical hypothesis test which is intended to find a dispersion value for two nominal features, evaluating the association between qualitative features. In our study, the FSelector package was used in the chi-squared test. In this test, the scores are obtained by Cramer coefficient between source attributes and destination attribute (Romanski, Kotthoff, & Kotthoff, 2016). Cramer's V is a measure of association between two nominal features, based on Pearson's chi-squared statistic, assigning values to each feature between 0 and 1 (inclusive) (Kochkina, 2015). This measure is computed by using (Eq 2).

$$V = \sqrt{\frac{\phi^2}{\min(r - 1, c - 1)}} \quad (2)$$

where  $c$  is the row number and  $r$  the column number (Bergsma, 2013).  $V$  is a symmetrical matrix where each element represents the intercorrelation of two discrete features, which have two or several levels. Cramér's  $V$  assigns values from 0 (no association) to 1 (total association) and reaches 1 only when the features are equal to each other (Kochkina, 2015; Pearson, 1992).

After computing the relative importance of the features, we generated the features' subsets which would be used to build the classification model. Each subset was generated using those variables that achieved the top  $N$

score values, where  $N$  indicates the number of features of each data set. Subset 1 had the top-rated feature; subset 2 had the two top rated features and so forth.

#### 5.2.4. Classification Algorithms

Classification is a useful tool in data mining. In our study, classification algorithms were used to build a model and predict the class of label in the testing of data.

#### 5.2.5. Support Vector Machines

Support Vector Machine (SVM) (Cortes & Vapnik, 1995) represents a number of novel learning techniques that have been introduced in the framework of structural risk minimization (SRM) and the theory of Vapnik-Chervonenkis (VC) bounds. Compared to other methods, SVM has shown outstanding performance in pattern recognition tasks. SVM is a binary classifier which aims to find a hyperplane with a maximum margin to represent the decision boundary and has as its main characteristic excellent generalization capacity, (Smola, Bartlett, Scholkopf, & Schuurmans, 1999). Given a dataset and labels that are represented by  $(x_i, y_i)$ , where  $x_i \in \mathbb{R}^n$  and  $y_i \in \{-1, 1\}$ . SVM seeks the solution of the following optimization problem:

$$\min_{w, b, \xi_i} \frac{1}{2} w' w + C \left( \sum_{i=1}^l \xi_i \right) \quad (3)$$

$$s. a. \quad y_i (w' \phi(x_i) + b) \geq 1 - \xi_i \quad e \quad \xi_i \geq 0 \quad (4)$$

where  $C > 0$  is the penalty parameter provided by the user (Kohavi & Provost, 1998) and  $\xi_i$  the penalty parameter of samples incorrectly classified. The training vector  $x_i$  is mapped from the input space into a higher-dimensional feature space via a mapping function  $\phi$ ,  $w' w$  represents the inner product between the transpose of vector  $w$ , which we denoted by  $w'$ , and  $w$ . This higher-dimensional feature space can be infinite (Chih-Wei Hsu, Chih-Chung Chang, 2008). SVM seeks to find a hyperplane separator, in this high dimensional space, with maximum margin (Cristianini & Shawe-Taylor, 2000). (Eq. 5) is called the kernel function (Haykin, 2008)

$$K(x_i, x_j) = \phi(x_i)' \phi(x_j) \quad (5)$$

In this study, we used the Gaussian Radial Basis Function (RBF) kernel for the SVM (Smola & Schölkopf, 1998) as defined by:

$$K(x_i, x_j) = \exp\left(-\frac{\|x_i - x_j\|^2}{2\sigma^2}\right) \quad (6)$$

#### 5.2.6. Artificial Neural Networks

Computational models inspired by the human nervous system are called Artificial Neural Networks (ANN) (Pang-Ning, Steinbach, & Kumar, 2006) whose aim is to create a model that correctly maps the input to the output using historical data. Thus, the model can then be used to produce the output when the desired output is

unknown (Notton, Paoli, & Diaf, 2013). Multilayer networks termed multilayer perceptron (MLP) are defined by an input layer, one or more hidden layers consisting of neurons and an output layer (Haykin, 2008). Networks of the MLP type belong to the class of feedforward networks (Hornik, Stinchcombe, & White, 1989) since these networks do not contain epochs (iterations over the entire training set) and the output depends only on the input instances.

Backpropagation algorithm (Rumelhart, Hinton, & Williams, 1986), result in optimization based on the gradient descent of the error function. Using learning rules, it is possible to obtain the best fit by evaluating the error and making adjustments. Backpropagation algorithm uses two passes, the first known as forward pass, or propagation, in which the initial synaptic weights are randomly defined and remain unchanged at the epoch. Second pass, known as backward pass or backpropagation, is characterized as the movement initiated in the output layer returning to the hidden layers, in which the local gradient of each neuron is calculated as a function of the error (Eq. 7), and the synaptic weights are all adjusted in accordance with an error-correction rule (Haykin, 2008). This process is repeated until the chosen convergence criterion is reached, the criterion generally being the minimizing of the quadratic error or the maximizing of the number of epochs.

$$E(w) = \frac{1}{2} \sum_{i=1}^N (y_i - \hat{y}_i)^2 \quad (7)$$

### 5.2.7. Random Forest

Random forest (Breiman, 2001) is a method that can be used for either classification or regression which constructs a large number of decision trees. For each tree in a set the random forest method works as follows, and draws a bootstrapped sample from the training data. For each sample, a decision tree is grown, and at each node of the tree a subset of  $m_{try}$  features from the  $p$  total features that are available is randomly drawn; in this set the best feature and the best split from this set of  $m_{try}$  features are selected and continue until the tree is fully grown. The final ensemble of trees is then bagged to make random forest predictions (Zumel & Mount, 2014).

### 5.2.8. Validation methods

Leave-one out cross-validation (LOOCV) (Kohavi, 1995) is another version of  $K$ -fold cross-validation, wherein  $K$  is equal to the number of samples, the process consists of using  $K - 1$  samples for training and the rest is used for the test. This process is undertaken until all the samples are tested (Chandrashekar & Sahin, 2014).

### 5.2.9. Performance assessment

Table 1 introduces the confusion matrix (Kohavi & Provost, 1998) which reports information about current classification and obtained classification, analyzed by classification algorithm. TP and TN are positive and negative samples, respectively, that were correctly classified, whereas FP and FN are positive and negative samples, respectively, that were wrongly classified.

Using confusion matrix elements it is possible to compute the values of accuracy  $\left(\frac{TP+TN}{TP+FP+FN+TN}\right)$ , sensitivity  $\left(\frac{TP}{TP+FN}\right)$  and specificity  $\left(\frac{TN}{FP+TN}\right)$ .

**Table 1.** Confusion matrix representation.

Actual	Predicted	
	Positive	Negative
Positive	TP	FN
Negative	FP	TN

Our analysis was conducted using free software termed the R Project for Statistical Computing program (R Development Core Team, 2014) which provides a wide variety of packages to carry out statistical analysis, including classification, feature selection, and other data mining functions. In our study, the XLConnect package (Mirai, 2016) was used for reading data, and we implemented a score algorithm to feature selection according to (Chen & Lin, 2006). The FSelector package (Romanski et al., 2016) was used for chi-squared testing. SVM, MLP, and RF functions were taken from the caret package (Kuhn, 2016) for data classification. Graphics for visualizing a segment of the results were constructed using the Matlab program, version 2013b (Mathworks, 2013).

On initial observation, the data were balanced to provide proportional quantities of samples. The synthetic minority over-sampling technique (Chawla, Bowyer, Hall, & Kegelmeyer, 2002) was used to increase the quantity of samples when necessary, while undersampling was used to reduce the quantity of samples to maintain the same proportions between samples grown in both conventional and organic systems.

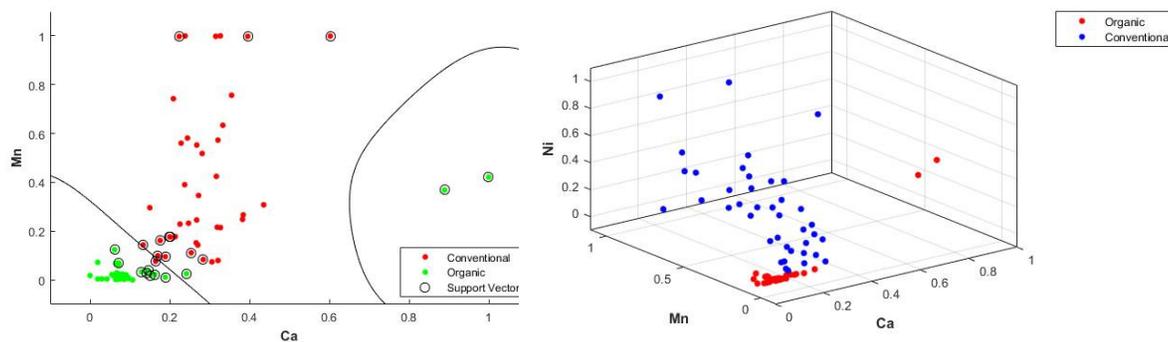
The samples were standardized between  $[0 - 1]$  since there was a very wide variation between the measurements taken. The next step, was to use feature selection applied to the classification algorithm (SVM, MLP e RF). We used the (RBF) kernel for SVM, defined by (Eq. 6). Parameters  $(C, \sigma)$  for tuneGrid were constructed using the following values:  $C \in \{2^{-5}, 2^{-3}, 2^{-1}, \dots, 2^{13}, 2^{15}\}$  and  $\sigma \in \{2^{-15}, 2^{-13}, 2^{-11}, \dots, 2, 2^3\}$  as suggested by (Kohavi & Provost, 1998). For MLP and RF we used tuneLength parameter equal to 10. This way, we conducted the training using leave-one-out cross validation (LOOCV).

### 5.3. Results

Regarding the tomato samples, SVM combined with chi-squared to obtain an accuracy rate of 97% using just two elements (Si, and Ca); pepper samples reached 100% with six elements selected by chi-squared (Si, Ca, B, Mg, P, and Na). Onion samples required seven elements (Mn, Ca, Fe, Ni Na, Zn, and Cu), selected by chi-squared, and also reached a 100% accuracy rate, whilst lettuce samples achieved an accuracy rate of 92% with six elements selected by chi-squared (Si, B, S, Na, Cu, and Ba) (Table 4).

(Figure 1a) shows the behavior of onion samples using just two elements selected by chi-squared (Mn and Ca), and achieved a 98% accuracy rate using SVM with the RBF kernel. When we add one more feature (Ni), the accuracy rate improves by more than 1%, achieving a 99% accuracy rate, and the separation between organic and

conventional samples becomes more distinct (Figure 1b). Thus, this technique that combines feature selection with classification algorithm (SVM) showed impressive classification capability.



**Figure 1:** SVM combined with two chemical elements (a) (Ca-Mn) and (b) three chemical element (Ca-Mn-Ni)

**Table 2.** Minimum and maximum, average  $\pm$  standard deviation (mg kg<sup>-1</sup>) of tomato, bell pepper, onion and lettuce samples grown in organic and conventional systems (continue)

Tomato	Organic	Element	Al	B	Ba	Ca	Cu	Fe	K	Mg
		Mín-max	0 – 43.7	0 – 36.2	0 – 0.9	448.1 – 3268	6.7 – 14.8	28.1 – 80.9	24940 – 41700	885.5 – 3737
		Mean $\pm$ SD	6.2 $\pm$ 12.6	19.5 $\pm$ 12.7	2.9 $\pm$ 0.6	823.5 $\pm$ 685.1	11.1 $\pm$ 2.5	43.7 $\pm$ 16.6	34070 $\pm$ 5651.7	1475 $\pm$ 700.2
		Element	Mn	Mo	Na	Ni	P	S	Se	Si
		Mín-max	4.0 – 155.9	0 – 3.9	102.1 – 964.3	0	5363 – 7428	1116 – 4119	0 – 9.1	11.4 – 42.5
		Mean $\pm$ SD	28.5 $\pm$ 47.1	1.1 $\pm$ 1.2	461.1 $\pm$ 262.5	0	6499 $\pm$ 504.4	2716 $\pm$ 1121.8	1.4 $\pm$ 2.6	30 $\pm$ 8.3
		Element	Zn	--	--	--	--	--	--	--
		Mín-max	13.78 – 22.21	--	--	--	--	--	--	--
		Mean $\pm$ SD	17 $\pm$ 2.4	--	--	--	--	--	--	--
	Conventional	Element	Al	B	Ba	Ca	Cu	Fe	K	Mg
		Mín-max	0 – 86.0	0 – 15.4	0.7 – 6.9	832.5 – 2459	4.9 – 10.7	7.8 – 89.9	24311 – 37415	1183 – 3716
		Mean $\pm$ SD	7.7 $\pm$ 19.7	1.3 $\pm$ 3.6	3.1 $\pm$ 2.0	1445 $\pm$ 454.4	6.9 $\pm$ 1.8	50.1 $\pm$ 18.9	29604 $\pm$ 3382	2293 $\pm$ 609.3
		Element	Mn	Mo	Na	Ni	P	S	Se	Si
		Mín-max	5.9 – 126.2	0 – 1.6	330.6 – 1477	0 – 0.6	3668 – 64431	1321 – 2587	0 – 6.9	6.6 – 19.9
Mean $\pm$ SD		28.6 $\pm$ 43.4	0.4 $\pm$ 0.5	797.6 $\pm$ 261.4	0.08 $\pm$ 0.2	8256 $\pm$ 13650	1621 $\pm$ 307.7	3.92.4	10.7 $\pm$ 3.6	
Element		Zn	---	---	---	---	---	---	---	
Mín-max	9.2 – 34.3	--	--	--	--	--	--	--		
Mean $\pm$ SD	15.6 $\pm$ 6.6	--	--	--	--	--	--	--		

**Table 6.** Minimum and maximum, average  $\pm$  standard deviation (mg kg<sup>-1</sup>) of tomato, bell pepper, onion and lettuce samples grown in organic and conventional systems (continuation).

Bell pepper	Organic	Element	Al	B	Ba	Ca	Cd	Co	Cr	Cu
		Mín-max	0 – 212.3	0 – 46.1	0.5 – 2.7	88.5 – 2945	0 – 1.1	0	0	3.0 – 14.6
		Mean $\pm$ SD	35.1 $\pm$ 46.6	4.8 $\pm$ 10.2	1.7 $\pm$ 0.7	1791 $\pm$ 753	0.04 $\pm$ 0.2	0	0	11.4 $\pm$ 2.7
		Element	Fe	Hg	K	Mg	Mn	Mo	Na	Ni
		Mín-max	14.1 – 207.8	0 – 2.1	136.8 – 58635	25.9 – 5400	10.1 – 215.1	0 – 2.7	42.3 – 484	0
		Mean $\pm$ SD	89.2 $\pm$ 36.3	0.3 $\pm$ 0.7	24704 $\pm$ 12464	3398 $\pm$ 1362	25.1 $\pm$ 45.7	0.7 $\pm$ 0.8	306.7 $\pm$ 112.2	0
		Element	P	S	Se	Si	Zn	---	---	---
		Mín-max	11.4 – 9111	91.34 – 3973	0 – 11.1	2.5 – 74.1	1.6 – 58.3	--	--	--
	Mean $\pm$ SD	5899.3 $\pm$ 2241	2514.2 – 880	3.9 $\pm$ 3.2	32.4 $\pm$ 14.6	33.0 $\pm$ 12.8	--	--	--	
	Conventional	Element	Al	B	Ba	Ca	Cd	Co	Cr	Cu
		Mín-max	0 – 268.6	0 – 15.2	0.4 – 6.5	291.4 – 13139	0 – 3.1	0 – 0.5	0 – 1.3	10.5 – 39.2
		Mean $\pm$ SD	29.4 $\pm$ 55.7	1.2 $\pm$ 3.6	1.9 $\pm$ 1.6	1527 $\pm$ 2465	0.17 $\pm$ 0.7	0.01 $\pm$ 0.1	0.05 $\pm$ 0.3	15.6 $\pm$ 6.7
		Element	Fe	Hg	K	Mg	Mn	Mo	Na	Ni
		Mín-max	34.8 – 256.4	0 – 2.8	12891 – 34904	1509 – 14698	6.0 – 60.7	0 – 2.1	94.4 – 15531	0 – 7.9
Mean $\pm$ SD		87 $\pm$ 41.7	0.2 $\pm$ 0.7	21509 $\pm$ 5442	3278 $\pm$ 2445	14.8 $\pm$ 10.1	0.3 $\pm$ 0.7	969 $\pm$ 3042	0.8 $\pm$ 1.6	
Element		P	S	Se	Si	Zn	---	---	---	
Mín-max		3616 – 9327	1387 – 4327	0 – 9.8	4.8 – 246	14.1 – 69.6	--	--	--	
Mean $\pm$ SD	5462 $\pm$ 1407	2291 $\pm$ 603.5	2.7 $\pm$ 2.5	22.0 $\pm$ 47.0	28.7 $\pm$ 15.4	--	--	--		

**Table 2.** Minimum and maximum, average  $\pm$  standard deviation (mg kg<sup>-1</sup>) of tomato, bell pepper, onion and lettuce samples grown in organic and conventional systems (continuation).

Onion	Organic	Element	Al	B	Ba	Ca	Co	Cr	Cu	Fe
		Mín-max	0 – 196.7	9 – 51.6	1.8 – 10.1	541.6 – 12134	0	0	7.0 – 22.3	18.8 – 210
		Mean $\pm$ SD	9.2 $\pm$ 40.7	30.5 $\pm$ 6.6	4.8 $\pm$ 1.9	2023.7 $\pm$ 268	0	0	10.4 $\pm$ 2.8	33.6 $\pm$ 38.4
		Element	Hg	K	Mg	Mn	Mo	Na	Ni	P
		Mín-max	0 – 2.9	1471 – 29880	872 – 13244	3.8 – 55.4	0 – 1.1	112.0 – 10963	0 – 2.4	3562 – 6431
		Mean $\pm$ SD	0.3 $\pm$ 0.7	13663 $\pm$ 4845	1656 $\pm$ 2525	9.0 $\pm$ 10.5	0.08 $\pm$ 0.3	917 $\pm$ 2185	0.1 $\pm$ 0.5	4699 $\pm$ 755.5
		Element	Pb	S	Se	Si	Zn	---	---	---
		Mín-max	0 – 38.6228	3010 – 15638	0 – 14.9	4.7 – 29.1	12.7 – 80.62	--	--	--
		Mean $\pm$ SD	0.9 $\pm$ 6.1	9336 $\pm$ 2180	3.2 $\pm$ 3.8	13.9 $\pm$ 4.2	21.6 $\pm$ 13.4	--	--	--
	Conventional	Element	Al	B	Ba	Ca	Co	Cr	Cu	Fe
		Mín-max	0 – 67.7	9.0 – 39.8	1.8 – 37.1	2085 – 7527	0 – 0.6	0 – 0.4	7.4 – 21.5	7.2 – 207
		Mean $\pm$ SD	4.6 $\pm$ 12.4	25.8 $\pm$ 7.7	10.7 $\pm$ 7.1	3745 $\pm$ 1024.8	0.01 $\pm$ 0.09	0.01 $\pm$ 0.07	13.1 $\pm$ 3.7	52.8 $\pm$ 36.5
		Element	Hg	K	Mg	Mn	Mo	Na	Ni	P
		Mín-max	0 – 3.3	5908 – 25140	807.1 – 3387	12.9 – 126.2	0 – 0.8	40.71 – 1436	0 – 7.8	2689 – 7232
Mean $\pm$ SD		0.3 $\pm$ 0.9	14945 $\pm$ 3416	1360.6 $\pm$ 656	53.0 $\pm$ 38.6	0.06 $\pm$ 0.2	215.2 $\pm$ 263.1	2.0 $\pm$ 2.0	4792 $\pm$ 1217	
Element		Pb	S	Se	Si	Zn	---	---	---	
Mín-max	0	3265 – 17952	0 – 15.6	3.3 – 43.4	13.9 – 71.7	--	--	--		
Mean $\pm$ SD	0	9459 $\pm$ 4105	6.8 $\pm$ 3.5	15.5 $\pm$ 6.7	33.3 $\pm$ 11.1	--	--	--		

**Table 2.** Minimum and maximum, average  $\pm$  standard deviation (mg kg<sup>-1</sup>) of tomato, bell pepper, onion and lettuce samples grown in organic and conventional systems (conclusion).

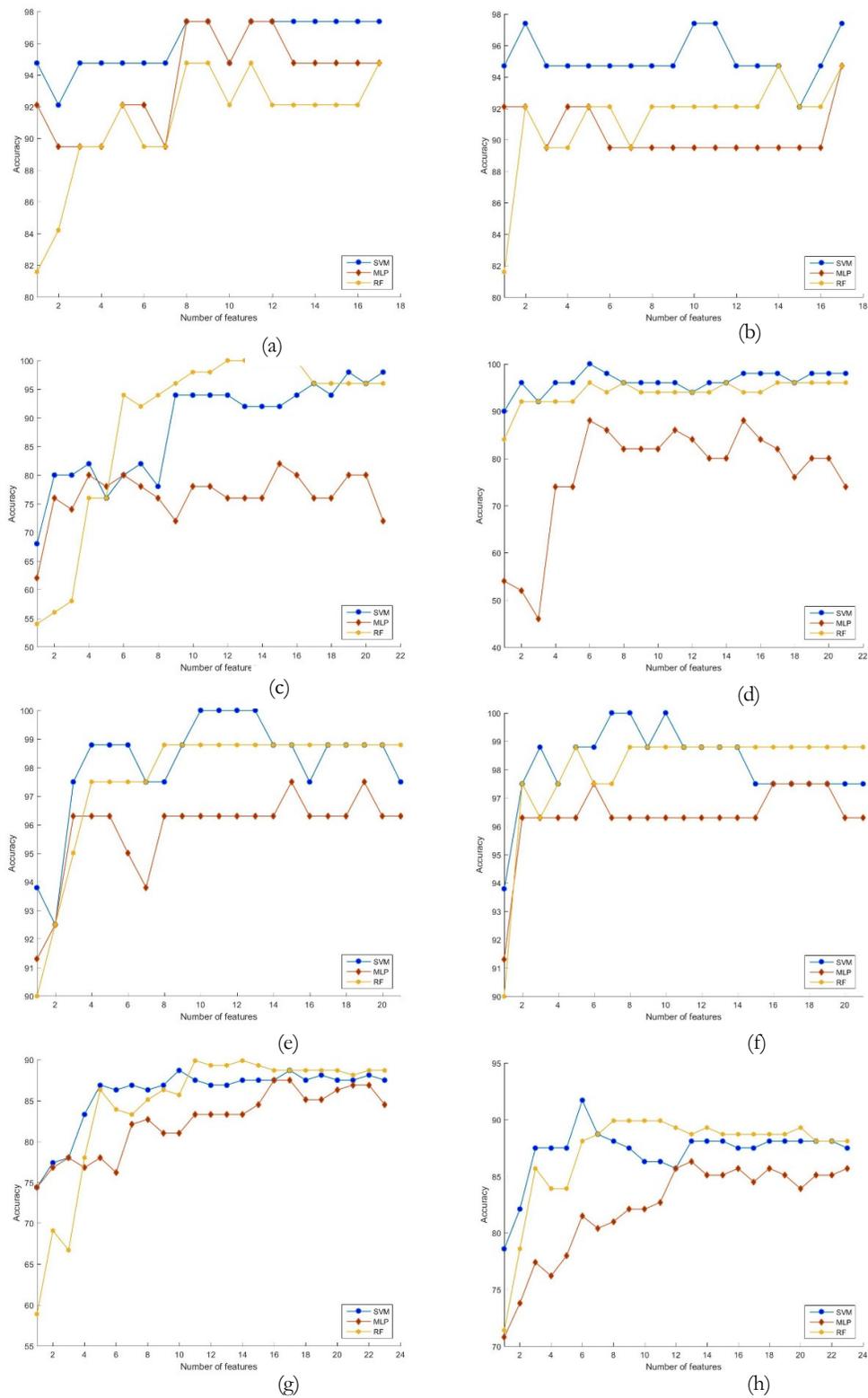
Lettuce	Organic	Element	Al	B	Ba	Ca	Cd	Co	Cr	Cu
		Mín-max	12.9 – 4940	26.3 – 96.4	4.7 – 98.4	3799 – 16830	0 – 3	0 – 1.6	0 – 4.7	5.7 – 15
		Mean $\pm$ SD	776.7 $\pm$ 971.8	55.4 $\pm$ 14.4	19.8 $\pm$ 17.3	9440 $\pm$ 2704.2	0.3 $\pm$ 0.6	0.14 $\pm$ 0.4	0.8 $\pm$ 1	9.2 $\pm$ 1.8
		Element	Fe	Hg	K	Mg	Mn	Mo	Na	Ni
		Mín-max	40.2 – 1917	0 – 3.8	13629 – 84253	4.2 – 12779	22.3 – 394.1	0 – 3.2	668.1 – 13774	0 – 2
		Mean $\pm$ SD	455 $\pm$ 410.1	0.2 $\pm$ 0.7	38206 $\pm$ 12500	7290 $\pm$ 2977	82.3 $\pm$ 72.1	0.6 $\pm$ 0.7	5585 $\pm$ 2510	0.4 $\pm$ 0.5
		Element	P	S	Se	Si	Ti	V	Zn	---
		Mín-max	2374 – 10233	2560 – 7444	4 – 28.7	264.1 – 3625	0 – 11.2	0 – 4.9	25.5 – 90.2	--
		Mean $\pm$ SD	6529 $\pm$ 1558	4934 $\pm$ 1305	17.5 $\pm$ 5.9	722.8 $\pm$ 495.8	2.2 $\pm$ 3	1.1 $\pm$ 1.5	45.7 $\pm$ 14.7	--
	Conventional	Element	Al	B	Ba	Ca	Cd	Co	Cr	Cu
		Mín-max	15 – 10237	13 – 90	4.4 – 257.4	134 – 13373	0 – 3.1	0 – 0.5	0 – 7.1	2 – 24.4
		Mean $\pm$ SD	764 $\pm$ 1545.2	36 $\pm$ 18.3	30.5 $\pm$ 45.3	8370 $\pm$ 2389.6	0.17 $\pm$ 0.5	0.005 $\pm$ 0.1	0.6 $\pm$ 1.3	8.7 $\pm$ 4.1
		Element	Fe	Hg	K	Mg	Mn	Mo	Na	Ni
		Mín-max	41.2 – 4607	0 – 4.3	71.4 – 68937	21.3 – 11972	16.4 – 327	0 – 2.4	26.7 – 9431.3	0 – 1.8
Mean $\pm$ SD		477.9 $\pm$ 755.1	0.6 $\pm$ 1.2	34401 $\pm$ 13757	6907 $\pm$ 2296.3	96.2 $\pm$ 81.6	0.6 $\pm$ 0.7	4249 $\pm$ 2350.7	0.3 $\pm$ 0.4	
Element		P	S	Se	Si	Ti	V	Zn	---	
Mín-max		6.9 – 10054	123 – 7621	3.6 – 24	12.2 – 2292	0 – 14.9	0 – 10	2.1 – 140.8	--	
Mean $\pm$ SD		6887 $\pm$ 1701.6	3840 $\pm$ 1177	14.1 $\pm$ 5.5	469.9 $\pm$ 500.1	1.9 $\pm$ 3.6	0.7 $\pm$ 1.9	57 $\pm$ 28.6	--	

**Table 3.** Feature order based on feature selector

F-score (Fs)	Tomato	Si	B	Cu	S	Na	Mg	Ca	Se	K	Ba	Mo	Ni	Fe	Zn	P	Al	Mn	--	--	--	--	--	--
	Pepper	Cu	Ni	Mo	B	Se	K	Mn	Na	Zn	Si	S	Co	Cr	Cd	P	Ba	Ca	Hg	Al	Mg	Fe	--	--
	Onion	Mn	Ni	Ba	Ca	Zn	Se	Cu	B	Fe	Na	K	Si	Co	Cr	Pb	Mg	Al	Mo	P	S	Hg	--	--
	Lettuce	B	S	Se	Na	Si	Zn	Co	Ca	Hg	K	Ba	Ni	V	P	Cr	Mn	Cu	Cd	Mg	Ti	Fe	Mo	Al
Chi-squared (Chi)	Tomato	Si	Ca	B	Mg	P	Na	Cu	S	Mn	Zn	Ba	K	Al	Fe	Mo	Ni	Se	--	--	--	--	--	--
	Pepper	Si	Zn	Ca	Ni	Na	Cu	Al	B	Ba	Cd	Co	Cr	Fe	Hg	K	Mg	Mn	Mo	P	S	Se	--	--
	Onion	Mn	Ca	Fe	Ni	Na	Zn	Cu	Ba	B	Se	Al	Co	Cr	Hg	K	Mg	Mo	P	Pb	S	Si	--	--
	Lettuce	Si	B	S	Na	Cu	Ba	Cr	Se	Fe	Zn	Co	Al	Ca	Cd	Hg	K	Mg	Mn	Mo	Ni	P	Ti	V

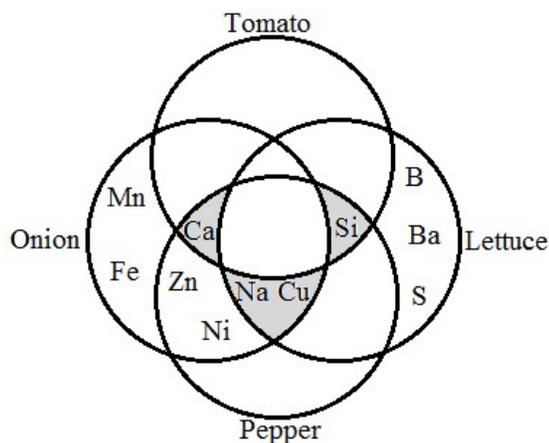
**Table 4.** Classifier result combined with F-score (Fs) and chi-squared (Chi). The best accuracy are shown in bold.

Subset	Tomato						Pepper						Onion						Lettuce					
	SVM		MLP		RF		SVM		MLP		RF		SVM		MLP		RF		SVM		MLP		RF	
	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi	Fs	Chi
#01	95	95	92	92	82	82	68	90	62	54	54	84	94	94	91	91	90	90	74	79	74	71	59	71
#02	92	<b>97</b>	89	92	84	92	80	96	76	52	56	92	92	97	92	96	92	97	77	82	77	74	69	79
#03	95	95	89	89	89	89	80	92	74	46	58	92	97	99	96	96	95	96	78	87	78	77	67	86
#04	95	95	89	92	89	89	82	96	80	74	76	92	99	97	96	96	97	97	83	87	77	76	78	84
#05	95	95	92	92	92	92	76	96	78	74	76	92	99	99	96	96	97	99	87	87	78	78	86	84
#06	95	95	92	89	89	92	80	<b>100</b>	80	88	94	96	99	99	95	97	97	97	86	<b>92</b>	76	81	84	88
#07	95	95	89	89	89	89	82	98	78	86	92	94	97	<b>100</b>	94	96	97	97	87	89	82	80	83	89
#08	97	95	97	89	95	92	78	96	76	82	94	96	97	100	96	96	99	99	86	88	83	81	85	90
#09	97	95	97	89	95	92	94	96	72	82	96	94	99	99	96	96	99	99	87	87	81	82	86	90
#10	95	97	95	89	92	92	94	96	78	82	98	94	100	100	96	96	99	99	88	86	81	82	86	90
#11	97	97	97	89	95	92	94	96	78	86	98	94	100	99	96	96	99	99	87	86	83	83	90	90
#12	97	95	97	89	92	92	94	94	76	84	100	94	100	99	96	96	99	99	87	86	83	86	89	89
#13	97	95	95	89	92	92	92	96	76	80	100	94	100	99	96	96	99	99	87	88	83	86	89	89
#14	97	95	95	89	92	95	92	96	76	80	100	96	99	99	96	96	99	99	87	88	83	85	90	89
#15	97	92	95	89	92	95	92	98	82	88	100	94	99	97	97	96	99	99	87	88	84	85	89	89
#16	97	95	95	89	92	95	94	98	80	84	100	94	97	97	96	97	99	99	87	87	87	86	89	89
#17	97	97	95	95	95	95	96	98	76	82	96	96	99	97	96	97	99	99	89	87	87	84	89	89
#18	--	--	--	--	--	--	94	96	76	76	96	96	99	97	96	97	99	99	87	88	85	86	89	89
#19	--	--	--	--	--	--	98	98	80	80	96	96	99	97	97	97	99	99	88	88	85	85	89	89
#20	--	--	--	--	--	--	96	98	80	80	96	96	99	97	96	96	99	99	87	88	86	84	89	89
#21	--	--	--	--	--	--	98	98	72	74	96	96	97	97	96	96	99	99	87	88	87	85	88	88
#22	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	88	88	87	85	89	88
#23	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	87	87	84	86	89	88



**Figure 2.** Classifier combined with features selection: (a), (c), (e), (g) data tomato, pepper, onion and lettuce with F-score, respectively. (b), (d), (f), (h) data tomato, pepper, onion and lettuce with Chi-squared, respectively.

Diagram (Figure 3) shows the behavior of combined classifiers according to Table 4. B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Se, Si, and Zn were the elements that contributed the most to each data set for the classification of samples grown in organic and conventional systems using SVM as the classifier (Figure 3; Table 4). Thus, the influence of Si, Ca, Na and Cu on the classification of cultures is clear.



**Figure 3.** Diagram of the elements responsible for greater accuracy

SVM combined with chi-squared outperformed MLP and RF in all data sets, reaching an accuracy level of 97% in tomato samples using just two features (Si e Ca) (Table 5). The bell pepper samples reached an accuracy level of 100% using six features (Si, Zn, Ca, Ni, Na, and Cu), and the onion samples also reached 100% accuracy using seven features (Mn, Ca, Fe, Ni, Na, Zn and Cu), and for lettuce samples, the highest accuracy level (92%) was reached using six features. Samples sets responsible for these results are presented in (Figure 3). More than 90% of organic samples were correctly classified (specificity) (95%, 100%, 100% and 91%) in the case of tomato, pepper, onion, and lettuce samples, respectively). This shows that the data mining technique, wherein classification algorithm and feature selection are combined, is very effective for classifying samples that were grown in organic and conventional systems.

**Table 5.** Performance measures detailed for the best results of models computed

Data	Tomato	Pepper	Onion	Lettuce
Feature subset	#2 (Chi-squared)	#6 (Chi-squared)	#7 (Chi-squared)	#6(Chi-squared)
Classifier	SVM	SVM	SVM	SVM
Accuracy (%)	97.4	100.0	100.0	91.7
Sensitivity (%)	100.0	100.0	100.0	92.5
Specificity (%)	94.7	100.0	100.0	90.9

#### 5.4. Discussion

It was possible to sort the five elements present in higher concentration as an exercise which compares the two systems (Table 2). For tomato, the sequence was  $K > P > S > Mg > Ca$  in the organic system, and

K>P>Mg>S>Ca in the conventionally grown tomatoes. The average concentrations of K, P, and S were higher in organic tomatoes, while Mg and Ca concentrations were higher in the conventional ones. Herencia, García-Galavís, Dorado, & Maqueda (2011) also observed higher P content in organic tomatoes compared with conventionally grown tomatoes in Spain. In some condition, the application rate of organic composts is calculated based on nitrogen content, Herencia, García-Galavís, Dorado, & Maqueda (2011) believed that this can lead to higher P availability. For example, in their experiment, the organic compost utilized contained almost three times more P than the mineral fertilizer (127 kg ha<sup>-1</sup> and 44 kg ha<sup>-1</sup> of P respectively) providing the same quantity of N (200 kg ha<sup>-1</sup> of N).

In the bell pepper samples collected from both organic and conventional farms, the sequence was K>P>Mg>S>Ca>Na, and the content of all these elements was higher in the organic bell pepper. This result disagrees with (López, Fenoll, Hellín, & Flores, 2013), who grew bell pepper under both organic and conventional systems in greenhouses and found the highest content of macronutrients in conventionally produced vegetables. The composts used as fertilizers in organic systems release nutrients in a slow, but constant, rate over the vegetable growing cycle (López, Fenoll, Hellín, & Flores, 2013). Thus, if the average time of the growing cycle of bell peppers under organic systems was higher than that of the bell peppers collected from conventional farms, this difference could have promoted more nutrient absorption.

Organic and conventionally grown onions had the following sequence: K>S>P>Ca>Mg, and only Mg had a higher level in organic onions. For the lettuce samples, the sequence was K>Ca>Mg>Na>S, with the highest levels found in the organic samples. (Kelly & Bateman, 2010) observed no differences ( $\alpha=0,05$ ) in Ca content between lettuce organically and conventionally produced, and credited this result to the association between plants and arbuscular mycorrhizal fungi (AMF). AMF are more present in organic systems, which could promote more availability and absorption of elements. However, unfavorable practices (like the use of certain biocides), as well as management practices prior to conversion (such as high P input, for example) may result in AMF with poor efficiency (Gosling, Hodge, Goodlass, & Bending, 2006). This could be only one of several soil-plant-microorganism interactions that result in different elemental constitutions and make it difficult to establish a standard for systems under different farm conditions

Hunter et al. (2011) carried out a survey of 66 papers which used differences in mineral, vitamins and pro-vitamin contents for comparison of vegetables, fruits, legumes and grains organically and conventionally produced. (Hunter et al., 2011) observed that the absolute values of nutrients are often higher in organic products and that differences between vegetables is more evident than between fruits. However, Hoefkens et al. (2010) evaluated organic and conventional samples of carrot (*Daucus carota*), tomato, lettuce, spinach (*Spinacia oleracea*) and potatoes (*Solanum tuberosum*), and did not find any differences ( $\alpha=0.01$ ). Plants have different absorption, translocation and accumulation systems (involving processes of transport, chelation, and sequestration), and the shooting of most of the elements to the leaves (as in leaf vegetables such as lettuce) is frequently higher than in fruits (Clemens, 2001).

Krejčová, Návesník, Jičinská and Černohorský (2016) determined the levels of 18 elements (among major, micro and potentially toxic elements) in carrots grown under organic, conventional and garden systems, but only found differences ( $\alpha=0.05$ ) for eight elements when they reduced the number of variables. The concentration of elements in the edible parts of crops are influenced by countless and complex factors, such as plant genotype, soil properties and composts utilized during the production cycle, as well as environmental conditions and interactions between elements (Herencia et al., 2011). This complexity of factors evidences that a mean comparison on its own is not a tool that is sufficiently robust and reliable to classify products as organically or conventionally produced.

Assays based on food fingerprinting will soon become a powerful tool in authenticity processes which focus on the complex food matrix (Riedl, Esslinger, & Faulh-Hassek, 2015). However, this kind of approach works with a massive data set, with many variables. Thus, powerful mathematical and statistical tools are required to efficiently analyze these data. In our study, the classification algorithm which showed the best results (accuracy, sensitivity, and specificity) was the Support Vector Machines (SVM). SVM is an appropriate tool that could be chosen for classification when discrimination between samples is not linear (Hernandez-Sanchez et al., 2012), Hernandez-Sanchez et al. (2012) discriminated organically produced mangoes (*Magnifica indica* L.) from conventional mangoes produced in the Canary Islands using the metal content in the samples and SVM as a classification method.

Two and seven elements were necessary for a satisfactory discrimination of organic and conventionally grown tomato and onion samples respectively. For bell pepper and lettuce groups, the best classification was obtained with six elements (Figure 3). The difference in the numbers of elements necessary for classification could reflect not just the absorption and translocation paths, but also the differences and similarities between organic and conventional management of each vegetable. For example, tomato production systems could have more restrictive management, while the production of lettuce is more variable, even within each system itself. (Diniz, Pistonesi, Alvarez, Band, & de Araújo, 2015) used elemental composition and successive projections algorithm to attest to the origins of black and green tea (*Camellia sinensis*) in Argentina and Sri Lanka. Reaching satisfactory classifications using but a few elements is extremely positive, once the reduced number of elements required provides an easier, faster and less expensive analytical method.

The control and supervision of organic products are based on the production stage, but there is no direct inspection of the final product sold to the consumers. Furthermore, the current procedures for inspection and certification need to be improved based on new analytical and data mining methods (Laursen, Schjørring, Kelly, & Husted, 2014). The method tested in our study showed to be a promising alternative, once the edible parts of vegetables (which can be easily sampled in markets) had been used, and accessible extraction techniques had been established. The technique suggested herein does not replace the current inspection system, but could be an important supportive tool in the investigative process.

Considering the scenario of vegetable production in Brazil (where the commercialization of organic products is not always strictly linked to certifying stamps), our study provides a viable alternative to enforcement agencies, because we were able to classify these four important vegetables commonly consumed by humans by combining the quantification of elemental composition with machine learning techniques.

## 5.5. Conclusion

The techniques used in this study were efficient (accuracy levels in the upper 90%) in classifying tomato, pepper, onion, and lettuce grown in both organic and conventional systems. The Support Vector Machine outperformed Multilayer Perceptron and Random Forest for all four sample groups (tomato, pepper, onion, and lettuce). The reduced number of elements required to classify the different types as conventional or organic crops is an important positive point because it evidences the low operational cost of the process of authentication of these vegetables.

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## References

- Araujo, C. L., Nogueira, A. R. A., & Nobrega, J. A. (2002). Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials, *57*(April), 2121–2132.
- Aschemann-witzel, J., Maroscheck, N., & Hamm, U. (2013). Are organic consumers preferring or avoiding foods with nutrition and health claims? *Food Quality and Preference*, *30*(1), 68–76.
- Barbosa, R. M., Batista, B. L., Barião, C. V., Varrique, R. M., Coelho, V. A., Campiglia, A. D., & Barbosa, F. (2015). A simple and practical control of the authenticity of organic sugarcane samples based on the use of machine-learning algorithms and trace elements determination by inductively coupled plasma mass spectrometry. *Food Chemistry*, *184*, 154–159.
- Bergsma, W. (2013). A bias-correction for Cramér's V and Tschuprow's T. *Journal of the Korean Statistical Society*, *42*(3), 323–328.
- Bigot, C., Meile, J., Kapitan, A., & Montet, D. (2015). Discriminating organic and conventional foods by analysis of their microbial ecology: An application on fruits. *Food Control*, *48*, 123–129.
- Brasil. (2007). Decreto no 6.323, de 27 de dezembro de 2007. Regulamenta a Lei nº 10.831, de 23 de dezembro de 2003, que dispõe sobre a agricultura orgânica, e dá outras providências., 2.
- Breiman, L. (2001). Random forests. *Machine Learning*, *45*(1), 5–32.
- Chandrashekar, G., & Sahin, F. (2014). A survey on feature selection methods. *Computers and Electrical Engineering*, *40*(1), 16–28.
- Charlebois, S., Schwab, A., Henn, R., & Huck, C. W. (2016). Food fraud: An exploratory study for measuring consumer perception towards mislabeled food products and its influence on self-authentication intentions. *Trends in Food Science & Technology*, *50*, 211–218.
- Chawla, N. V., Bowyer, K. W., Hall, L. O., & Kegelmeyer, W. P. (2002). SMOTE: Synthetic minority over-sampling technique. *Journal of Artificial Intelligence Research*, *16*, 321–357.
- Chen, Y.-W., & Lin, C.-J. (2006). Combining SVMs with various feature selection strategies. In *Feature extraction* (pp. 315–324). Springer.
- Chih-Wei Hsu, Chih-Chung Chang, and C.-J. L. (2008). A Practical Guide to Support Vector Classification. *BJU International*, *101*(1), 1396–400.
- Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*.
- Cortes, C., & Vapnik, V. (1995). Support-vector networks. *Machine Learning*, *20*(3), 273–297.
- Cristianini, N., & Shawe-Taylor, J. (2000). An Introduction to Support Vector Machines and other kernel based learning methods. *Ai Magazine*.
- Danezis, G. P., Tsagkaris, A. S., Brusic, V., & Georgiou, C. A. (2016). Food authentication: state of the art and prospects. *Current Opinion in Food Science*, *10*, 22–31.

- Diniz, P. H. G. D., Pistonesi, M. F., Alvarez, M. B., Band, B. S. F., & de Araújo, M. C. U. (2015). Simplified tea classification based on a reduced chemical composition profile via successive projections algorithm linear discriminant analysis (SPA-LDA). *Journal of Food Composition and Analysis*, *39*, 103–110.
- Gosling, P., Hodge, A., Goodlass, G., & Bending, G. D. (2006). Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment*.
- Gundersen, V., Bechmann, I. E., Behrens, A., & Sturup, S. (2000). Comparative Investigation of Concentrations of Major and Trace Elements in Organic and Conventional Danish Agricultural Crops . 1 . Onions ( *Allium cepa* Hysam ) and Peas ( *Pisum sativum* Ping Pong ), (1997), 6094–6102.
- Guyon, I., & Elisseeff, A. (2003). An introduction to variable and feature selection. *Journal of Machine Learning Research*, *3*(Mar), 1157–1182.
- Haykin, S. (2008). *Neural Networks and Learning Machines*. Pearson Prentice Hall New Jersey USA 936 pLinks (Vol. 3).
- Herencia, J. F., García-Galavís, P. A., Dorado, J. A. R., & Maqueda, C. (2011). Comparison of nutritional quality of the crops grown in an organic and conventional fertilized soil. *Scientia Horticulturae*, *129*(4), 882–888.
- Hernandez-Sanchez, C., Luis, G., Moreno, I., Camean, A., Gonzalez, A. G., Gonzalez-Weller, D., ... Hardisson, A. (2012). Differentiation of mangoes (*Mangifera indica* L.) conventional and organically cultivated according to their mineral content by using support vector machines. *Talanta*, *97*, 325–330.
- Hoefkens, C., Sioen, I., Baert, K., De Meulenaer, B., De Henauf, S., Vandekinderen, I., ... Camp, J. Van. (2010). Consuming organic versus conventional vegetables: The effect on nutrient and contaminant intakes. *Food and Chemical Toxicology*, *48*(11), 3058–3066.
- Hornik, K., Stinchcombe, M., & White, H. (1989). Multilayer Feedforward Networks are Universal Approximators, *2*, 359–366.
- Hunter, D., Foster, M., McArthur, J. O., Ojha, R., Petocz, P., & Samman, S. (2011). Evaluation of the micronutrient composition of plant foods produced by organic and conventional agricultural methods. *Critical Reviews in Food Science and Nutrition*, *51*(February 2014), 571–582.
- IBGE, I. B. de G. e E. (2006). Censo Agropecuário: Brasil, Grandes Regiões e Unidades da Federação. *Coordenação de Marketing/ Centro de Documentação E Disseminação de Informações - CDDI*, 1–775. Retrieved from [http://www.ibge.gov.br/home/estatistica/economia/agropecuaria/censoagro/brasil\\_2006/Brasil\\_censoagro2006.pdf](http://www.ibge.gov.br/home/estatistica/economia/agropecuaria/censoagro/brasil_2006/Brasil_censoagro2006.pdf)
- Kelly, S. D., & Bateman, A. S. (2010). Comparison of mineral concentrations in commercially grown organic and conventional crops--Tomatoes (*Lycopersicon esculentum*) and lettuces (*Lactuca sativa*). *Food Chemistry*, *119*(2), 738–745.
- Kochkina, E. (2015). *Analysis and evaluation of classification models for disease detection using human gut metagenomic data*. Chalmers University of Technology.
- Kohavi, R. (1995). A study of cross-validation and bootstrap for accuracy estimation and model selection. *International Joint*, *14*(0), 0–6.
- Kohavi, R., & Provost, F. (1998). Glossary of Terms. *Machine Learning*, *30*(2–3), 271–274.
- Kotsiantis, S. B., Zaharakis, I. D., & Pintelas, P. E. (2006). Machine learning : a review of classification and combining techniques, (2006), 159–190.
- Krejčová, A., Návesník, J., Jičínská, J., & Černohorský, T. (2016). An elemental analysis of conventionally, organically and self-grown carrots. *Food Chemistry*, *192*, 242–249.
- Kuhn, M. (2016). Package “caret”: Classification and Regression Training.

- Laursen, K. H., Mihailova, A., Kelly, S. D., Epov, V. N., Bérail, S., Schjoerring, J. K., ... Husted, S. (2013). Is it really organic? – Multi-isotopic analysis as a tool to discriminate between organic and conventional plants. *Food Chemistry*, *141*(3), 2812–2820.
- Laursen, K. H., Schjoerring, J. K., Olesen, J. E., Askegaard, M., Halekoh, U., & Husted, S. (2011). Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *Journal of Agricultural and Food Chemistry*, *59*(9), 4385–4396.
- Laursen, K. H., Schjoerring, J. K., Kelly, S. D., & Husted, S. (2014). Authentication of organically grown plants--advantages and limitations of atomic spectroscopy for multi-element and stable isotope analysis. *TrAC Trends in Analytical Chemistry*, *59*, 73–82.
- López, A., Fenoll, J., Hellín, P., & Flores, P. (2013). Physical characteristics and mineral composition of two pepper cultivars under organic, conventional and soilless cultivation. *Scientia Horticulturae*, *150*, 259–266.
- Maione, C., Batista, B. L., Campiglia, A. D., Barbosa, F., & Barbosa, R. M. (2016). Classification of geographic origin of rice by data mining and inductively coupled plasma mass spectrometry. *Computers and Electronics in Agriculture*, *121*, 101–107.
- Mathworks, I. (2013). MathWorks Announces Release 2013b of the MATLAB and Simulink Product Families. *MathWorks*, 1–3.
- Mirai, S. G. (2016). Package “XLConnect”: Excel Connector for R. Retrieved from <https://cran.r-project.org/web/packages/XLConnect/XLConnect.pdf>
- Notton, G., Paoli, C., & Diaf, S. (2013). Estimation of tilted solar irradiation using Artificial Neural Networks. *Energy Procedia*, *42*, 33–42.
- Pang-Ning, T., Steinbach, M., & Kumar, V. (2006). *Introduction to data mining. Library of Congress*.
- Pearson, K. (1992). On the Criterion that a Given System of Deviations from the Probable in the Case of a Correlated System of Variables is Such that it Can be Reasonably Supposed to have Arisen from Random Sampling. New York: In Breakthroughs in Statistics.
- R Development Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *R Foundation for Statistical Computing, Vienna, Austria*. Retrieved from <http://www.r-project.org/>
- Riedl, J., Esslinger, S., & Fahl-Hassek, C. (2015). Review of validation and reporting of non-targeted fingerprinting approaches for food authentication. *Analytica Chimica Acta*, *885*, 17–32.
- Romanski, P., Kotthoff, L., & Kotthoff, M. L. (2016). Package FSelector: Selecting Attributes, 18. Retrieved from <https://cran.r-project.org/web/packages/FSelector/FSelector.pdf>
- Rumelhart, D. E., Hinton, G. E., & Williams, R. J. (1986). Learning representations by back-propagation of errors, *323*, 533–536.
- Smola, A. J., Bartlett, P., Scholkopf, B., & Schuurmans, D. (1999). Advances in Large Margin Classifiers. *Advances*.
- Smola, A. J., & Schölkopf, B. (1998). *Learning with kernels*. Citeseer.
- Tang, J., Alelyani, S., & Liu, H. (2014). Feature Selection for Classification: A Review. *Data Classification: Algorithms and Applications*, 37–64.
- Zumel, N., & Mount, J. (2014). *Practical Data Science with R*.

## 6. FINAL REMARKS

Although some of the samples were collected in areas with long history of soil use, we could attest that, regardless of the production system, the PTEs contents in soils were (in most of the samples) below the threshold limits imposed by the Brazilian legislation, not representing an acute harm for the people directly exposed. Among the vegetables collected, lettuce leaves had the higher capacity to accumulate PTEs, onions had an intermediate capacity, while the fruit vegetables (tomato and bell pepper) absorbed relatively less PTEs. Lettuce was the most relevant horticultural crop sampled, and because of that, we used the soil-transfer factor (calculated to testify the capacity of the lettuce to uptake PTEs from the soil) to assess the human risk, using the equation established for Brazilian conditions. Calculating the risk concentration of the PTEs allowed us to compare the risk of each PTEs, in both systems (organic and conventional), and four scenarios (rural and urban, for adults and infants). Based on the risk concentration values, it was clear that the organic system represents a lower potential harm situation for humans than the conventional production of lettuce in the state of Pernambuco. The human risk assessment is a useful index to complement the traditional environmental studies based on the PTEs concentrations in plants and soils. The uptake of PTEs by plants is the result of a complex system where physiologic characteristics of each plant interact and react to the soil proprieties. In a short way, the phytoavailability of a PTE depends on its interaction with the soil matrix. The kinetic experiments signaled to the importance of the soil proprieties. The presence and application of organic matter had an importance greater than the aging effect on the Cu desorption. The formation of strong Cu-DOC complexes seems to be crucial for the Cu phytoavailability and mobility in the soil. These results are particularly interesting for garden soils (or other systems where the load of organic amendments is intense), because the application of organic composts changes the dynamic of the PTEs and could increase their availability. In this way, a closer monitoring of PTEs levels in soils under constant amendment is important to secure the environmental viability of the agricultural system. The data mining technique called machine learning models was used to classify our vegetable samples based on the production system and the elemental composition, and such presented a high efficiency using just a few variables (mainly macro elements). These finds have a big potential for practical uses for enforcement agencies, once we attested the capacity of directly differentiate organic and conventional commonly consumed vegetables using just their edible parts, what can be easily sampled in markets and supermarkets.