T.C.
MUĞLA SITKI KOÇMAN UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF GEOLOGICAL ENGINEERING

BEHAVIOR OF COPPER (CU) AND ZINC (ZN) IN SOILS AMENDED WITH AGRICULTURAL EFFLUENTS, ISOTOPIC APPROACH

MASTER OF SCIENCE

HATİCE TURAN

NOVEMBER 2021
MUĞLA

H. TURAN DEPARTMENT OF GEOLOGICAL ENGINEERING MASTER THESIS MUĞLA 2020

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THESIS CONFIRMATION

The thesis, prepared by **HATİCE TURAN,** titled as **BEHAVIOR OF COPPER (CU) AND ZINC (ZN) IN SOILS AMENDED WITH AGRICULTURAL EFFLUENTS, ISOTOPIC APPROACH** has been accepted unanimously/majority by the jury listed below that fulfils the necessary conditions for master's degree of Department of Geological Engineering at 04/11/2021.

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I declare that all the results, information, and documents that I obtained and presented during my thesis were obtained by me personally and within the scope of this thesis; it complies with academic and scientific ethical rules. According to the academic and scientific ethical rules, I also declare that all original information and results which were not obtained during this thesis were cited.

Hatice TURAN

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ÖZET

TARIMSAL ETKİLERLE DEĞİŞTİRİLEN TOPRAKLARDA BAKIR (CU) VE ÇİNKO (ZN) DAVRANIŞI, İZOTOPİK YAKLAŞIM

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Toprak, dünyadaki yenilenemeyen en önemli doğal kaynaklardan biridir. İnsanlar ve canlı organizmalar için gıda üretimi ve habitat desteği sağlar. Ağır metallerin neden olduğu toprak kirliliği bu kaynağın sürdürülebilirliğini olumsuz etkileyebilir. Dünyada tarımsal topraklarda domuz bulamacı (PS) uygulaması, toprakların fiziko-kimyasal özelliklerinin iyileştirilmesine yardımcı olan ve tarımsal verimi artıran, sıklıkla kullanılan bir stratejidir. Bununla birlikte, domuz gübresi yüksek seviyelerde Bakır (Cu) ve Çinko (Zn) konsantrasyonları içerir ve sonuç olarak büyük domuz bulamacı uygulamaları, PS ile değiştirilmiş topraklarda Cu ve Zn konsantrasyonlarında artışa neden olur. Bu çalışmanın amacı, izotopik yaklaşımı kullanarak PS ile değiştirilmiş topraklarda antropojenik ve doğal Cu katkıları arasında ayrım yapmaktır. Doğal ve PS ile değiştirilmiş toprak örnekleri, Brezilya'nın Santa Maria eyaletinden örneklenmiş ve Cu izotopik bileşimleri için analiz edildi. En yüksek Cu ve Zn konsantrasyonları, değiştirilmiş toprağın yüzey horizonlarında gözlenmiştir. Değiştirilmiş topraklarda analiz edilen δ^{65}Cu'ın izotopik izleri, doğal topraklarda gözlemlenenlerden farklıdır, ancak kullanılan domuz gübresinin izotopik imzalarına yakındır. Karışım hesaplamaları, domuz bulamacından gelen Cu oranının, değiştirilen toprakların yüzey ufuklarında biriken toplam Cu'ın %30'una kadar ulaşabileceğini göstermiştir.

Anahtar Kelimeler: Domuz Bulamacı, Ağır Metal, Cu, Zn, Konsantrasyon, İzotopik Analiz.

ABSTRACT

BEHAVIOR OF COPPER (CU) AND ZINC (ZN) IN SOILS AMENDED WITH AGRICULTURAL EFFLUENTS, ISOTOPIC APPROACH

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Soil is one of the most important non-renewable natural resources on Earth. It provides support for food production and habitat for humans and living organisms. Soil pollution caused by heavy metals can negatively affect the sustainability of this resource. In the world, pig slurry (PS) application on agricultural soils is a frequently used strategy which helps improving the physico-chemical properties of soils and increases the agricultural yield. However, pig manure contains high levels of Copper (Cu) and Zinc (Zn) concentrations and consequently the massive pig slurry applications cause an increase of the Cu and Zn concentrations in PS-amended soils. The aim of this work was to discriminate between anthropogenic and natural Cu contributions in PS-amended soils, using the isotopic approach. Soil samples of natural and PS-amended soils were sampled in Santa Maria, Brazil and analyzed for their Cu isotopic compositions. The highest Cu and Zn concentrations were observed in the surface horizons of amended soil. The isotopic signatures of δ^{65}Cu analyzed in amended soils are different from those observed in natural soils, but close to the isotopic signatures of the pig manure used. Mixing calculations showed that the proportion of Cu coming from the pig slurry can reach up to 30 % of the total Cu accumulated in the surface horizons of the amended soils.

Keywords: Pig Slurry, Heavy Metal, Cu, Zn, Concentration, Isotopic Analysis.

To my lovely family

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LIST OF ABBREVIATIONS

δ	Delta(d) Notation for Isotopic Signature as per Mil (‰)
f	Fractional Isotopic Abundance
m	Molar quantities of interest element
[Cu]	Cu Concentration, $\mu g\ g^{-1}$
$\sum$	Summation Symbol
Cu	Copper
Zn	Zinc
N	Nitrogen
P	Phosphorous
S	Stock of each element, $\mu g\ g^{-1}$
D	Depth, cm
PS	Pig Slurry
OM	Organic Matter
BD	Bulk Density, g cm^3
CEC	Organic Exchange Capacity
GPC	Gel Permeation Chromatography
IEC	Ion-Exchange Chromatography
PFA	Polytetrafluoroethylene
XAS	X-ray Absorption Spectroscopy
XRD	X-ray Powder Diffraction
SRC	Single Reaction Chamber
UFSM	Universidade Federal de Santa Maria
NOD-P-1	Manganese Nodule Reference Material
SEM-EDS	Scanning Electron Microscopy with X-ray Microanalysis
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MC ICP-MS	Multi-Collector Inductively Coupled Plasma Mass Spectrometry

1. INTRODUCTION

1.1 Purpose and Scope

Soil is an important complex environmental media of the Earth which has high level heterogeneity due to solid, liquid, and gaseous components interaction (Zornoza et al. 2015). It is a support for plants and a habitat for animals and microorganisms, and it has various chemical and physical properties (Morgan 2016; Ye et al. 2012). Therefore, soil quality is extremely important for the environmental system and its sustainability (Ye et al. 2012). In recent years, increased heavy metal input such as arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), zinc (Zn), etc. in the topsoil produced negative impacts on vegetation and living organisms, and consequently on the human health (Morgan 2016). Behavior of heavy metal in the soil changes depending on soil physico-chemical and mineralogical properties. Several anthropogenic activities, such as agricultural activity, industrial and mining activity, etc. negatively affect the soil quality. For instance, heavy metal concentration in the soil is started to increase with several agricultural activities (Morgan 2016).

In the world, a global pig stocks increased 2.5 times from 1960 to 2010 (FAO 2020) and while large distribution of pig population is found in Asia, half the world’s pigs are produced in China (Robinson et al. 2011). According to the research, the total number of pigs is 57.35 million in intensive systems in Eastern Europe and Central Asia in 2005 (Jondreville, Revy, and Dourmad 2003). While pig production number rate of increase is 2.5 % between 2018 and 2019, they expect that this rate may increase to 4.5 % between 2019 and 2020 in Brazil (Silva and Flake 2020). The pig production is increasing year by year due to increasing food demand and activities in agriculture, but at the same time contributes to a regular increase in the quantities of produced waste, i.e., pig slurry (PS).

Copper (Cu) and Zinc (Zn) are present in pig body as minor components and play important role in many metabolic functions. Especially Cu is necessary for the protection of tissues from oxidative stress (Daudén, Quílez, and Vera 2004; Hernández et al. 2006). Pig nutrients are often enriched in Cu and Zn to ensure a sufficient supply of these elements for the animals. However, these metals are not metabolized completely by pigs and most of the Cu and Zn are found in the slurry (Belon et al. 2012). Pig slurry application on agricultural soils is a widely used practice because it helps to improve the physico-chemical properties and fertility of PS-amended soils and consequently increases the agricultural yield and financial benefits (De Conti et al. 2016; Keller and Domergue 1996). Thus, Cu and Zn concentrations in soil increase due to intensive pig manure application. These metals can be transported in soluble or particulate form into water streams (De Conti et al. 2016; Dos Santos et al. 2013). They might have dangerous consequences for living organisms and human life.

The preliminary research was about the impact of the long-term pig slurry applications on soils in 1972 (Batey, Berryman, and Line 1972). This study had shown that Cu concentration in soil increased with pig slurry applications and pointed out the need of further investigations about firstly Cu then also Zn. Moreover, studies on pig slurry began to intensify (McGrath, Fleming, et al. 1982; Poole et al. 1983, 1990). It was observed that the slurry was applied to the grass at different amounts and the amount of copper varied differently than control based on the amount of pig slurry (McGrath, Fleming, et al. 1982). According to the soil types, increasing copper amount differ in soil which pig slurry at a rate of 20 m^3ha^{-1} was applied 3 times by year (Poole et al. 1983). It has been shown that microbial activity increases in soils that have massive pig slurry application (Cote and Ndayegamiye 1989). It has been interpreted that copper in the pig slurry can act as a ligand and that zinc can act as a metal due to interaction differences between metals and matters (Castilho, Brunt, and Bruins 1993). Copper and Zn contents which are not susceptible to mobilization were observed to increase to over 70% (Formentini et al. 2015). Cu and Zn concentrations in soils after massive pig slurry applications with different quantity (50, 100 and 200 m^3 ha^{-1} $year^{-1}$ of PS) increased by 20.27 %, 23.49 %, 43.81 % and 47.21 %, 62.65 %, 87.35 % in 0-5 cm , respectively (Formentini et al. 2015). Copper interacts with organic matter more quickly than Zn therefore Cu accumulates in the upper soil, while Zn accumulates in deeper layers in the soil (Formentini et al. 2015, 2016).

Behavior of metals differ in aqueous, solid, and gaseous forms depending on oxidation state, electron configuration, ionic radius, and the presence of various ligands (Reeder, Schoonen, and Lanzirotti 2006). Speciation controls the flux of metal species in systems with solution and solid interactions (Forstner 1993). Metal speciation plays a key role for determining toxicity, mobility, distribution, and bioavailability in various media (Reeder et al. 2006; Tessier, Campbell, and Bisson 1979). X-ray absorption spectroscopy (XAS) is a powerful element-specific technique for in-situ characterization of the speciation (Reeder et al. 2006).

Copper and Zn exist in pig nutrient as $CuSO_4$, CuO (oxidation stage I) and ZnS, and in pig manure as Cu_2S (oxidation state II) and ZnS respectively (Formentini et al. 2016; Legros et al. 2013). Zn concentration in control soil and in PS-amended soil (200 m^3 ha^{-1} $year^{-1}$ of PS) reached 100.4 mg kg^{-1} and 188.1 mg kg^{-1}, respectively in the soils of the experimental site in Campos Novos, Santa Catarina State, Brazil (Formentini et al. 2015). Furthermore, investigation of speciation of Zn in these soils showed that Zn is found as 30 % Zn-Fe (oxyhydr) oxide and 70 % Zn-Kaolinite in control soil but, 27 % Zn-Fe (oxyhydr) oxide, 54 % Zn-Kaolinite and 19 % Zn-OM in amended soil. The sulfate form of Zn present in the PS, i.e., ZnS was not recorded in the PS-amended soil which indicating a radical change of Zn speciation (Formentini et al. 2016). Indeed, it is not possible to discriminate between a natural and anthropogenic metal in the soil that has received massive pig slurry applications using the speciation analyses.

Stable isotopes of metals have been also extensively used in the literature successfully for identifying the origin and tracing the anthropogenic contamination in soil (Chen et al. 2013; Mattielli et al. 2009; Sivry et al. 2008).

First initiatives for on the stable isotope chemistry of Cu started in 1958 (Albarède 2004). Therefore, Cu and Zn isotopic composition applications started using thermal ionization mass spectroscopy in 1972 (Albarède 2004). Today, isotopic approach has been using for many elements in the soil (Probst, Hernandez, and Probst 2003). It was determined using the isotopic approach 50 % of the total Cu concentration present in abandoned vineyard soils comes from anthropogenic sources whereas it changes in cultivated soils up to 75% (El Azzi et al. 2013). Additionally, some stable isotopes of metals were performed successfully for the determination of the origin of the industrial metal contamination (Gelly et al. 2019). Unfortunately, there is limited data about isotopic signatures of contaminants from animal waste (Fekiacova et al., 2015).

This study has been completed at CEREGE (Centre for Research and Teaching in Environmental Geoscience, Aix en Provence, France), laboratory methods were carried out in the study conducted under the supervision of Assoc. Prof. Zuzana FEKIACOVA and Assoc. Prof. Emmanuel Doelsch.

The main objective of this study is to determine the concentrations and isotopic compositions of Cu and Zn in soils that have received pig slurry applications from 2000 - 2006. Besides, discrimination between the anthropogenic and natural sources of Cu and Zn in soils that have received massive pig slurry applications is attempted in this study.

General literature review regarding the pig slurry applications on agricultural soils is summarized in Table 1.1.

Table 1.1. Summary of the general literature survey for this study

	Reference	Purpose	Result	Importance with this study
1	Batey et al. 1972	This study aimed to change the Cu level when applied to the grass depending on the amount of pig manure.	-It was determined that the Cu content of grass was increased when pig slurry application was applied to the soil.	This study is the first paper in soil related to pig slurry applications.
2	Gracey et al. 1976	This study aimed to determine the effect of applying high level of copper-rich pig slurry to grassland on the health of grazing sheep.	-The Cu storage was increased in the livers and kidneys of sheep related to the greater availability of copper on the copper sulphate area.	Studies have started to focus on the field of animal health.
3	McGrath, Fleming, et al. 1982; McGrath, McCormack, et al. 1982; Poole et al. 1983, 1990	Studies to determine the effects of applying copper-rich pig slurry to grassland had started with Pot experiments such as rainfall, species, and soil-copper incorporation experiments. The study had continued to determine the spread of different amounts of pig slurry in different types of soils. Then, the effect on grazing sheep was investigated in the soil which has received massive pig slurry application. Therefore, sheep feeding experiments were done.	-It was observed that the copper effect changed according to the plant species in the soils in which the pig slurry application was applied. -Spreading has been linked to many reasons: sward density, rate of application, time interval between spreading and sampling and rainfall pattern. According to the intensity of rainfall, the copper content of harvested herbage was changed. -When the exposure of sheep to grazing grassland treated with copper-rich pig slurry, their fecal copper was increased. -It was observed differences between slurry copper and sulfate copper due to chemical speciation with sheep feeding experiments.	Spreading pig slurry studies have started to intensify.
4	Cote and Ndayegamiye 1989	This study purposed to determine the effects of a long-term application of pig slurry and solid cattle manure on soil microbial activity and to identify chemical changes.	-Organic carbon, CEC (Cation Exchange Capacity), microbial activity, and potentially mineralizable nitrogen were increased with solid cattle manure and pig slurry. -Soil microbial activity is improved with long-term pig slurry applications.	It is prominent that differences in fertilization practices affect the soil.
5	Castilho et al. 1993	This study's purpose is to determine dissolved organic matter, cadmium, copper, and zinc in pig slurry and soil solution. Nominal molecular mass fractions were performed using size exclusion chromatography.	-While cadmium and zinc complexes showed rapid dissociation in pig slurry liquid molecular size fractions, copper complexes did not dissociate in pig slurry liquid using GPC (Gel Permeation Chromatography) fractions. -Copper from pig slurry is showed different properties than cadmium and zinc.	This study is presented the first study for the determination of copper and zinc in pig slurry using chromatography.
6	L'Herroux et al. 1997	Behaviors of metal in the soil that has received pig slurry applications had determined using sequential extraction method.	-Metals which came from pig slurry accumulated in the upper layer. -Metal enrichments had found in different soil fractions with speciation analyses. -Copper and Zn mainly were observed in the organic fraction and hydroxides, respectively.	This study has represented the background of the behavior of metals in the soil with pig slurry applications.

Table 1.1. (Continuation)

7	Jondreville et al. 2003	Copper and Zn are important minor body components for pig body. Therefore, pig feeds are usually supported with Cu and Zn. This paper was aimed to determine the environmental effects of Cu and Zn with manure applications.	-It is considered that the most effective method to minimize environmental pollution is lowering Cu and Zn dietary supply in pig diets. -It has been proposed to intensify the research for clarification.	Copper and Zn metals are important metal for pig protection tissues. These metals can cause environmental pollution.
8	Legros et al. 2010	Copper is accumulated in topsoil which has pig slurry applications. The aim of this study was to understand Cu speciation using multi technique approach (MicroX-ray Fluorescence Spectroscopy, X-ray Absorption Near-edge Structure Spectroscopy).	-Cu speciation exists in raw pig slurry samples as Cu(I)$_2$S (96%) and Cu (II)-acetate (4%). -In pig slurry, the majority oxidation state of Cu is oxidation state I. But previous studies show that Cu exists in pig feed as $CuSO_4$ or CuO (oxidation state II). This complication had explained by the very low solubility of Cu_2S at Cu accumulation at the soil received intensive pig slurry spreading.	Cu speciation mostly exists in different oxidation states in pig slurry (Cu(I)$_2$S) and pig feed ($CuSO_4$ and CuO).
9	Lourenzi et al. 2011, 2012	The aim of this study was to determine nutrient distribution, to explain for nitrogen (N) and phosphorus (P) contamination and evaluate the alterations of the chemical properties related to soil acidity in the soil profile under no-tillage after pig slurry applications.	-In soil depth, it is not determined any increase in mineral nitrogen. But it was recovered increasing levels of available phosphorus (P) and potassium (K). This is proof of transfer to deeper layers. -Pig slurry application has a negative environmental effect. -Soil acidity was decreased with pig slurry applications at 8 cm depth. -Accumulation of Ca^{2+} and Mg^{2+} were observed in the surface soil layers. -The levels of soil organic matter were increased with successive applications of pig slurry in deeper layers.	Soil samples were taken from the same study area with Lourenzi et al. 2012.
10	Legros et al. 2013	The aim of this study was to determine the fate and behavior of Cu and Zn following pig slurry spreading in water soil plant system.	-It was determined that the Cu and Zn mass received from pig slurry were not transferred plant systems. -The Cu mass (Cu_2S) received from pig slurry was recovered in the 0 – 20 cm depth. -Zn mass received from pig slurry was stored in 20 – 60 cm depth.	This study helps to the interpretation of behavior of Cu and Zn.
11	Formentini et al. 2015	The purpose of this study was to determine the effects of long-term pig slurry application at different rates, on Cu and Zn accumulation. Selective sequential extraction was applied in amended and non-amended soils.	-Copper and Zn contents were observed up to 70% in the residual fraction. -It was observed that amount of pig slurry is a different effect on accumulation of the Cu and Zn concentrations in the soil layers.	This study is one of the most background studies related to Cu and Zn accumulation and fractionation in the soil with pig slurry applications.
12	Fekiacova et al. 2015	The aim of this paper was to determine whether it is possible to characterize Cu and Zn contamination in soils using their isotopic signatures and whether it is possible to identify determination of conditions for tracing Cu and Zn pollution using isotopic approach.	-Contaminants have a large isotopic signature interval. It was not detected any formulas on the possibility of tracing soil Cu and Zn pollution with their isotopic signatures. -Copper and Zn isotopes are an effective tool for tracing the pollution except in calcareous soils for Zn.	Literature has limited data about isotopic analysis on agriculture.

Table 1.1. **(Continuation)**

13	El Azzi et al. 2013; Gelly et al. 2019; Sivry et al. 2008	General purpose of these studies to determine discrimination pollution between anthropogenic and natural using a combination of geochemical and isotopic methods.	-Isotopic signatures were used successfully for discrimination sources of pollution.	An isotopic signature is an effective tool for distinguishing of pollution between anthropogenic and natural.
14	Silva et al. 2016	The aim of this paper was to evaluate the effect of pig slurry applications on the biological composition and activity of the soil in pasture areas.	-According to the time related to exposed to pig slurry application to the soil, the edaphic fauna was affected, and the composition and diversity of this fauna were altered.	This study helps to understand the biological composition and activity of the soil in pasture areas.
15	Da Rosa Couto et al. 2015	The aim of this paper was to determine the accumulation and distribution of Cu and Zn in the 0 – 0.4 m soil layer with pig slurry application from 3 years to 30 years.	-In the soil surface layer, soluble, exchangeable, mineral, organic, and total Cu and Zn fractions had changed with more than 17 years of pig slurry application. -Copper and Zn concentrations decreased through the soil profile.	This study represents background studies related to Cu and Zn accumulation in the soil with pig slurry applications.
16	De Conti et al. 2016	The aim of this study was to determine the soluble, available, and total Cu and Zn concentrations and the distribution of their chemical species in the solution in a Hapludalf soil received pig slurry applications and plant cultivation.	-The concentrations of Cu and Zn are increased in surface soil with received pig slurry applications and the concentration of Cu is increased in the soil solution at depth. -The plants provide soil conditions with chemical species complexed with dissolved organic carbon and Cu and Zn in free forms exit in a small amount.	Soil samples were taken from the same location with this study.
17	Formentini et al. 2016	The aim of this study to assess Zn speciation using combination of three approaches (X-ray powder diffraction (XRD), scanning electron microscopy with X-ray microanalysis (SEM-EDS), and XAS) in the pig slurry matrix and the soil that had received pig slurry amendments.	-Zinc exists in the pig slurry matrix with the presence of nano sized ZnS particles. However, ZnS was not detected in pig slurry amended soil after pig slurry applications.	Speciation analysis is not enough tool for the discrimination source of Zn in pig slurry amended soil.
18	Tiecher et al. 2017	The aim of this study to evaluate the impact of successive pig slurry applications on phosphorus (P) forms in the soil, and on some biological and biochemical parameters related to the soil phosphorus (P) cycle.	-Phosphorus applied in pig slurry is stored in the soil in inorganic form as 83 – 98 %. -Organic carbon limited the increase of organic phosphorus in the soil. -The chemical, biological, and biochemical components of the soil P cycle are affected by pig slurry application to the soil.	This study helps to evaluate biological and biochemical parameters in the soil with pig slurry applications.
19	Moraes Tavares et al. 2019	The objective of this study was to determine some chemical and physical attributes of soil after pig slurry application in soil under a no-tillage system.	-Pig slurry applications with the dose of 50 m^3 ha^{-1} tended to promote mineralization of organic matter and improved the soil structuring degree with an increase in the size of aggregates. -Pig manure affected on the soil properties, and this effect was greater in the surface layers (0 – 10 and 10 – 20 cm). -Pig manure is a complementary practice to mineral fertilization on agriculture.	The objective of the study leads to understand the general literature of the subject.

2. MATERIAL AND METHOD

2.1 Study Area

The study area is located in Santa Maria, the southern state of Brazil in the central of Rio Grande do Sul (Figure 2.1). All experiments were conducted at Federal University (Universidade Federal de Santa Maria, UFSM) in Santa Maria which is in 29°43’12” S – 53° 43’ 4W coordinate (De Conti et al. 2016; Lourenzi et al. 2012).

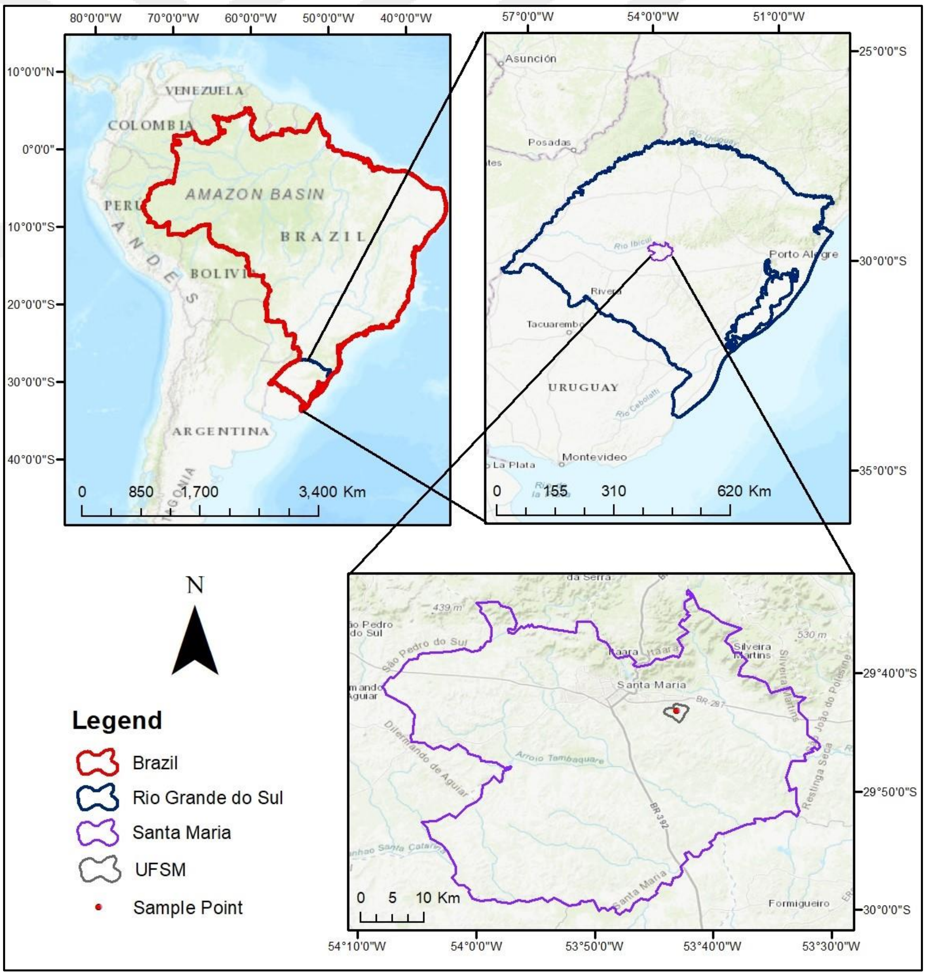

Figure 2.1. Location map of the study area

Soil columns is based on 6 years long-term experiment. 170 g kg^{-1} clay, 300 g kg^{-1} silt, 530 g kg^{-1} sand (sandy loam texture) and 16 g kg^{-1} organic matter in 0 - 0.1m were used in this study (De Conti et al. 2016).

2.2 Sampling and Pre-treatment of the Samples

The rate of pig slurry was performed 80 m^{3}ha^{-1}year^{-1} and pig slurry applications are applied two times for each year. The samples were collected at the end of 6 years before the last application in slices, using a shovel. Then soil slices were stratified into the desired layers (Figure 2.2).

Figure 2.2. Sampling process a- Sampling with the help of a shovel b, c, and d- Layering process e- After sampling[1]

In this study, UFSM 11 - 20 and UFSM 101 - 120, as from bottom soil to topsoil were examined under 2 groups of samples. These samples represent no PS (Pig Slurry) application (control soil) and 11 PS application (PS-amended soil), respectively (Table

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[1] These photos were taken by Fábio Mallmann (unpublished photo).

2.1). Additionally, samples are from the same location have already been studied by de Conti et al., 2016. In their study, the soils are ranged as 0 - 5, 5 - 10, - 20, 20 - 30, 30 - 40, 40 - 60 cm depths and focusing point of their study is to appreciate the soluble, available, and total Cu and Zn concentrations and the distribution of chemical species in the solution and to determine differences in soil which has pig slurry applications and plant cultivation.

Table 2.1. Selected soil horizons and soil profiles (Profile 1 and 2 represent no PS application and maximum PS application, respectively)

Layer (cm)	Profile 1	Profile 2
0 - 5	UFSM-20	UFSM-110
5 - 10	UFSM-19	UFSM-109
10 - 15	UFSM-18	UFSM-108
15 - 20	UFSM-17	UFSM-107
20 - 25	UFSM-16	UFSM-106
25 - 30	UFSM-15	UFSM-105
30 - 35	UFSM-14	UFSM-104
35 - 40	UFSM-13	UFSM-103
40 - 50	UFSM-12	UFSM-102
50 - 60	UFSM-11	UFSM-101

Standards reference materials GSS-3 and Manganese Nodule NOD-P-1 are certified values on total Cu and Zn concentrations. These both reference materials were treated with the samples in order to monitor and check the accuracy of the analyses. Concentrations in NOD-P-1 and GSS-3 are 11500 (±49) μg g^{-1}, 11.4 (±1.1) μg g^{-1} and 1600 (±6) μg g^{-1}, 31 (±3) μg g^{-1} for Cu and Zn, respectively (AQSIQ 2003; Wilson 2007).

Selected samples were grinded to obtain homogeneous fine fraction using agate mortar and pestle (Figure 2.3). Soil samples were prepared in order to start pre-treatment of the sampling process (Figure 2.3a). Collected soil samples tubes were shook to obtain homogeneous medium (Figure 2.3b). Approximately 5 g of soil samples was placed in the mortar (Figure 2.3c). Soil samples were grinded with agate mortar and pestle (Figure 2.3d). Grinded soils were transferred to experimental tubes (Figure 2.3f).

Agate mortar and pestle were cleaned with ultra-pure water (milli-Q$^{\copyright}$) and quartz powder (Figure 2.3g, Figure 2.3h and, Figure 2.3i). Then, these experimental equipment were cleaned with ethanol in order to avoid contamination (Figure 2.3j).

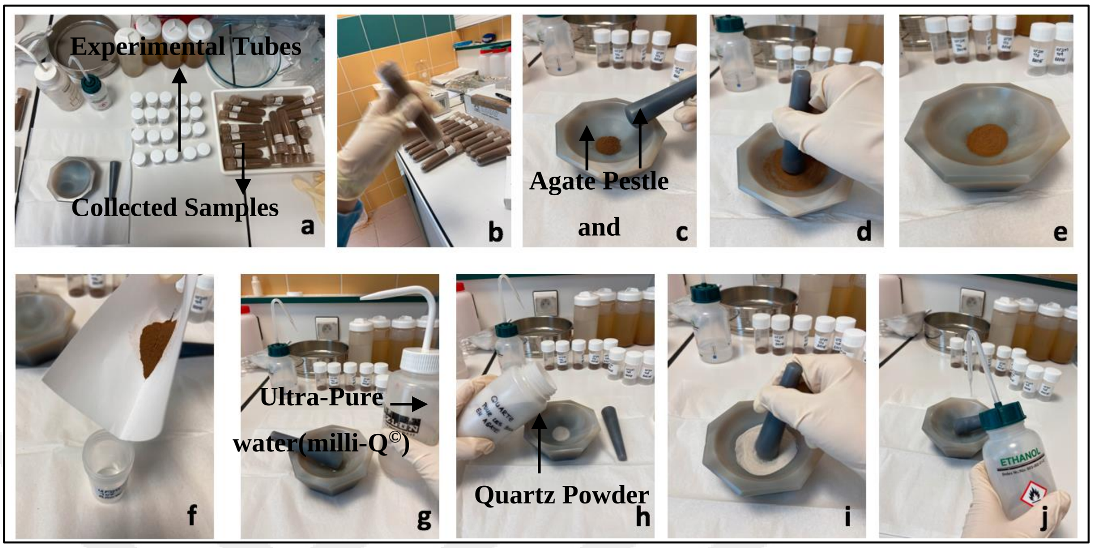

Figure 2.3. Pre-treatment of the sampling process a- Collected samples b- Shaking process for homogenizing distribution of the samples c- Transfer process of the samples to the agate mortar d- Grinding by agate pestle and agate mortar e- After homogenizing f- Transfer process of the samples g- Cleaning processes with ultra-pure water (milli-Q$^{\copyright}$) for used agate mortar and pestle h- Cleaning process of agate mortar and pestle with quartz powder i- Grinding of quartz powder with agate mortar and pestle during 2 minutes j- Cleaning process of the agate mortar and pestle with ethanol

2.3 Calcination Process

Organic matter was removed from each sample using a calcination method that is called “heating to high temperatures in air or oxygen” by (IUPAC 2014). This step has several objectives such as decrease in the quantity of the material to be digested; destruction of the organic matter contained in the samples by thermal decomposition. It is important to avoid the negative effect of organic matter on the experimental accuracy by heating the soil at 450°C. Organic matter can prevent solubilization of some elements which have a high affinity for the organic matter. Furthermore, presence of organic matter in the analysis solution might produce interferences during

ICP-MS (Inductively coupled plasma mass spectrometry) and MC-ICP-MS (Multi-collector Inductively coupled plasma mass spectrometry) analyses.

Samples were weighted (~0.2 g) in the porcelain cups using analytical balance, Sartorius La 230 S and transferred to the Nabertherm (30 - 3000°C) oven for calcination process (Figure 2.4). General working principle of Nabertherm (30 - 3000°C) oven takes advantage of time - temperature correlation. These processes were showed in Table 2.2.

Table 2.2. Time - Temperature correlation for laboratory oven

	Time	**Temperature (C°)**
T_0 - T_1	1 hour (up to)	250
T_1 - T_2	1 hour (stable)	250
T_2 - T_3	1 hour (up to)	450
T_3 - T_4	4 hours (stable)	450

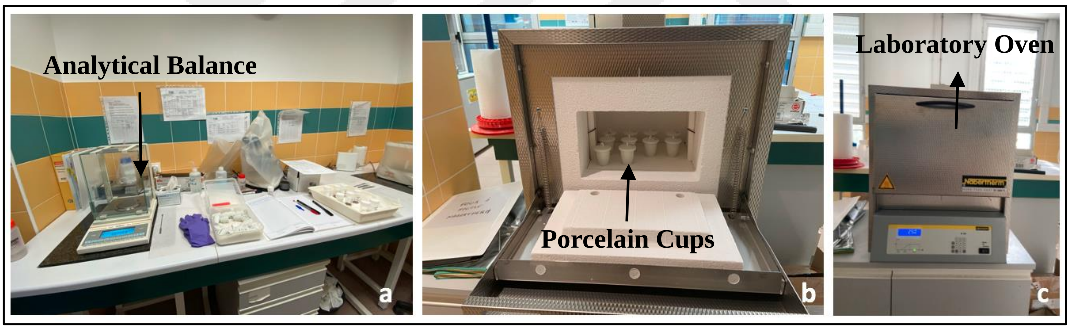

Figure 2.4. Calcination Process a- Weighting and transfer process of samples to porcelain cups for calcination b- Placing the samples in the oven c- Adjustment processes for time and temperature correlation

2.4 Dissolution Process

After calcination, all samples were prepared for dissolution. Dissolution is a transformation process from the solid matrix to the solution. Digestion was performed using Milestone UltraWave Single Reaction Chamber (SRC) Microwave Digestion System (Figure 2.5). Firstly, all samples were transferred microwave tubes previously neutralized to eliminate the unwanted electrostatic charge, using Sartorius YIB01-0DR ionizing fan and weighted using Analytical balance XP205. Average weight loss was up to 4.47% due to calcination (Appendix A). Then, the following digestion protocol was used: the samples were digested with 2 mL of concentrated HNO_3 and 1.5 mL of concentrated HCl for 1 hour, then 0.5 mL of concentrated HF was added. After, all samples were put into the UltraWave Single Reaction Chamber (SRC) Microwave Digestion System. According to the procedure, digestion process was started with pressure adjustment at 35 bar.

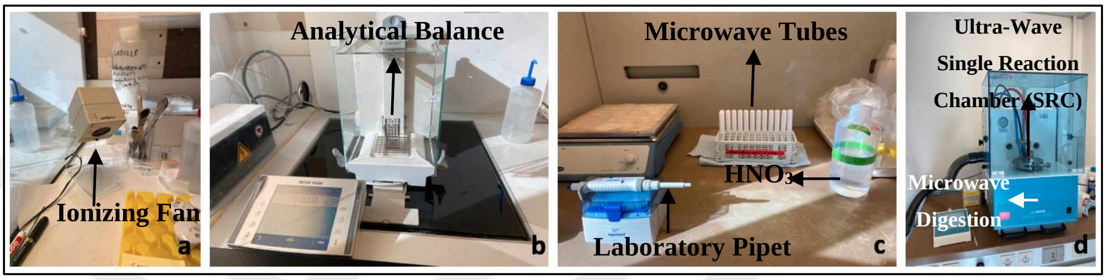

Figure 2.5. Dissolution (mineralization) process a- Ionizing fan for neutralize the electric charge b- Sample transfer process with analytic balance scaling sample weight c- Prepared samples for the Microwave digestion. d- Ultra Wave Single Reaction Chamber (SRC) Microwave Digestion System

After microwave procedure, all samples were transferred into pre-cleaned polytetrafluoroethylene (PFA) sample vials with screw cap for further treatment. The digestion solution was evaporated, then concentrated HNO_3 (15 drops) and 10 minutes in ultrasonic bath were applied to each sample in order to break the aggregates and destroy the residual white particles. Then the HNO_3 was evaporated 2 more times. After the HNO_3 evaporation, 3 mL of concentrated HCl was added and samples were heated at 110 °C for 48 hours then evaporated to dryness.

For chromatographic separation, the samples were dissolved in 1.5 mL of 7N HCl+0,001% H_2O_2 and heated during 1 night on a heating plate at 70°C, to ensure complete dissolution (Figure 2.6).

Figure 2.6. a- When samples were evaporated on the hot plate at 110°C b- Ultrasonic bath c- Adding 1.5 mL of 7N HCl +0,001% H_2O_2 HCl to samples before chromatographic separation

2.5 Chromatographic Separation

Ion-exchange chromatography (IEC) relies on electrostatic interactions between ions of the analyte and the resin and is used to isolate the element of interest from the matrix (Tanimizu, Asada, and Hirata 2002). The anion exchange resin AG MP-1 was used in chloride form for separation of Cu and Zn from the soil matrix. Working principle of chromatographic separation is shown in Figure 2.7, representatively.

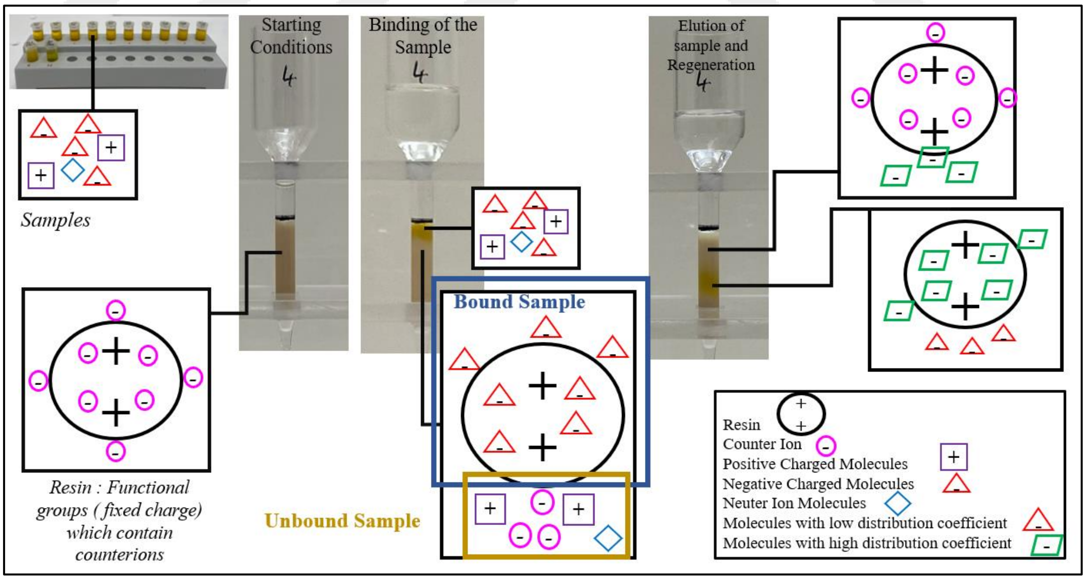

Figure 2.7. Basic working principle of chromatographic separation (adapted from Ullah, 2012)

The quartz column is packed with 1.6 mL of the resin, which is composed of an insoluble support, to which are attached the functional groups with the counterions. In the chloride form of AG MP-1, the counterion on the resin is Cl^-. Each sample consists of different kinds of molecules charged negatively, positively, or neutral. When a sample passes through the column, the molecules with neutral charge and the same charge as the functional groups (cations) are not retained on the resin and are eluted (unbound sample). The molecules that have the opposite charge compared to the functional groups (anion) are in a competition with the counterions of the resin. In a chloride solution, the dominant forms of Cu and Zn are $CuCl_3^-$, $CuCl_4^{-2}$ and $ZnCl_3^-$, $ZnCl_4^{-2}$ (Marechal and Albarède 2002) (Figure 2.8). These sample anions are exchanged onto the resin and replace the counterions (bound sample). To recover the bound sample ions, they can be eluted by introducing an ion with a higher affinity for the resin or a high concentration of an ion with equivalent or lower affinity.

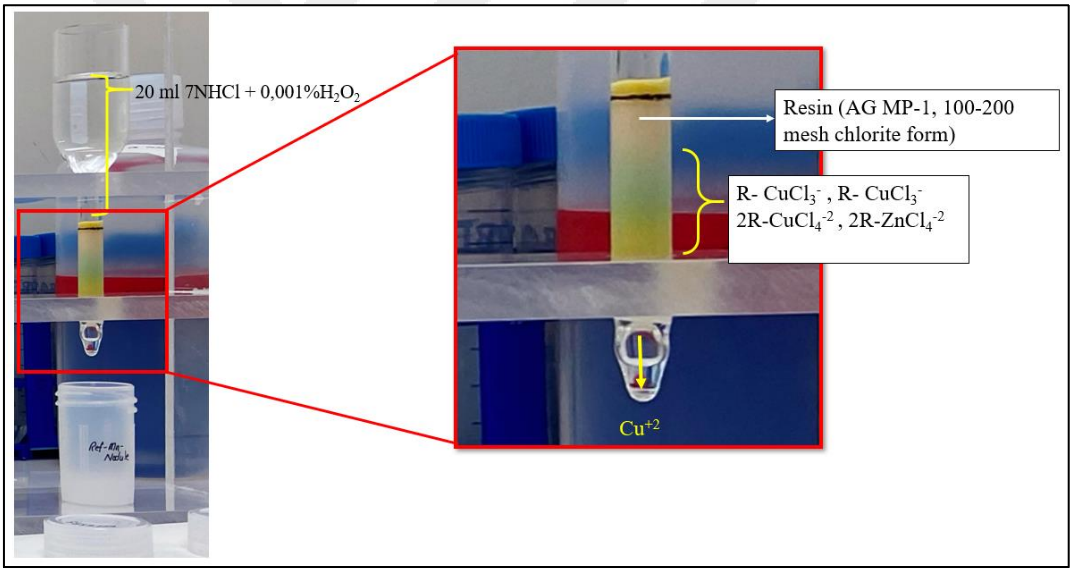

Figure 2.8. Manganese Nodule (NOD-P-1) sample from chromatographic separation

The separation protocol was developed for Cu and Zn by (Maréchal et al., 1999) and shown in Table 2.3. All resin was washed 3 times with 0.5N HNO_3 of 7 mL and H_2O (milli-Q©) of 3 mL. After oxidation of sample, centrifuge (10 min,14000 rpm) was applied for samples before load samples to columns since it accelerates the separation of solid phase and liquid phase for Cu and Zn separation (Figure 2.9). Aliquot of 0.05 µL samples injected into small experimental tubes for determination chemical recovery before chemistry.

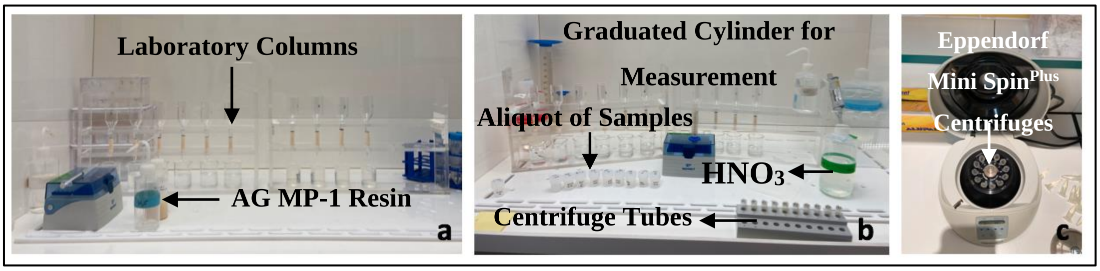

Figure 2.9. Chromatographic separation process a- Preparation of the chromatographic separation columns b- Preparation of the samples for chromatographic separation c- Centrifugation (10 min, 14000 rpm) before loading the samples into the columns

Briefly, the samples were dissolved in 1.5 mL of 7N HCl + 0.001% H_2O_2 and were loaded on the columns after conditioning. The matrix elements were eliminated with 9.5 mL 7N HCl + 0.001% H_2O_2. Copper was eluted with 20 mL of 7N HCl + 0.001% H_2O_2 and Zn was with 10 mL of 7N HCl + 0.001% HNO_3. Collected Cu and Zn fractions were dried on the hot plate at 110 °C for 12 hours.

Table 2.3. Chemical procedure for Cu and Zn Separation (after Maréchal et al., 1999)

Decompaction	H_2O	± 2 mL
Cleaning Process	0.5N HNO_3	7 mL
	H_2O	3 mL
	0.5N HNO_3	7 mL
	H_2O	3 mL
	0.5N HNO_3	7 mL
	H_2O	3 mL
Oxidation of Sample	1% H_2O_2	5 µL
Condition Column	7NHCl + 0,001% H_2O_2	6 mL
Loading the Sample	7NHCl + 0,001% H_2O_2	1.5 mL
Removing Matrix Elements	7NHCl + 0,001% H_2O_2	9.5 mL
Elute Cu	7NHCl + 0,001% H_2O_2	20 mL
Elute Fe	2NHCl + 0,001% H_2O_2	10 mL
Convert to Nitrates	0.5N HNO_3	0.75 mL
Elute Zn	0.5N HNO_3	10 mL

Copper and Zn were eluted using chromatographic separation in the laboratory (Figure 2.10).

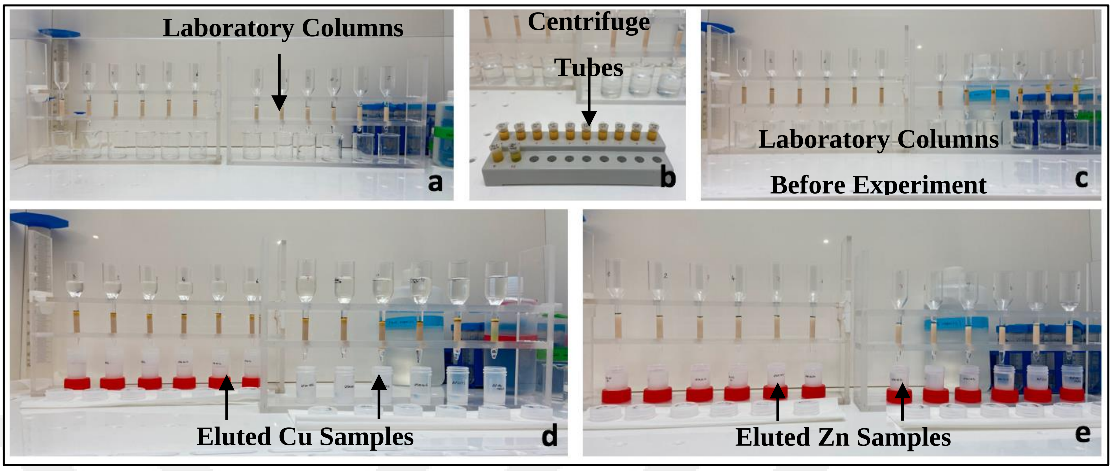

Figure 2.10. Separation processes with chromatographic separation columns a- Chromatographic separation columns before experiment b- Samples after centrifuge application c- Load samples on the columns d- Separation of Cu e- Separation of Zn

2.6 Purification

Same procedure was repeated with the Cu elution in order to obtain pure fractions and avoid interferences generated by matrix elements (Table 2.3). Eluted Cu was purified using experimental laboratory columns (Figure 2.11). All sample preparation and chromatographic separation were performed in clean lab at CEREGE (Centre for Research and Teaching in Environmental Geoscience), Aix en Provence, France.

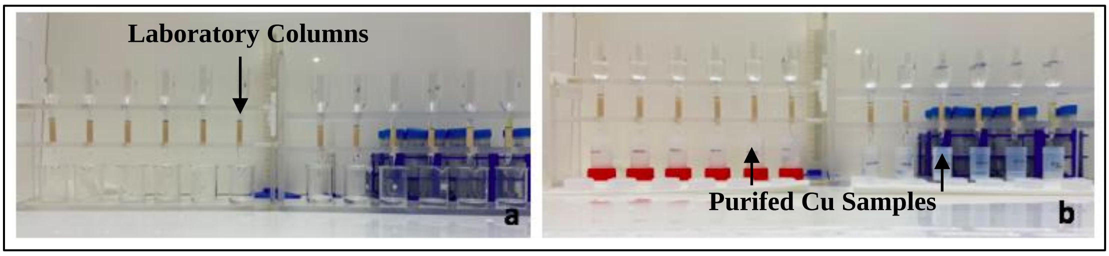

Figure 2.11. Purification Process a- Before Cu purification b- During purification of Cu

2.7 ICP-MS and MC-ICP-MS

Inductively coupled plasma mass spectrometry system (ICP-MS) is a most sensitive technique using plasma methods for the determination of trace and major elements (Aceto 2016). The general principle of a mass spectrometer consists in ionizing the molecules of the sample, separating target ions and, detecting ions of the selected mass. General working principles of mass spectroscopy is shown in Figure 2.12.

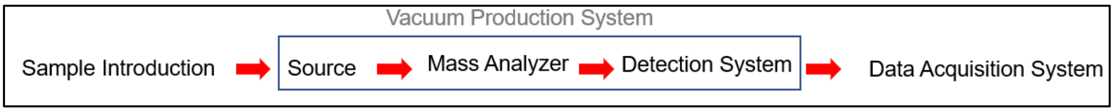

Figure 2.12. General working principles of mass spectrometry

The purpose of the vacuum is to avoid collision of target ions with the air molecules. The source represents the system for ionization of the samples. The sample aerosol is vaporized, atomized, and ionized by plasma in the source. Produced ions with sufficient energy are focused on a beam and extracted into the mass spectrometer. The target ions are separated according to m/z ratio mass analyzer system and their quantity is detected in the detection system. The purpose of the detection system is to detect, and count ions isolated and focused by the mass analyzer and translate the number of ions coming to the detector into the electric signal.

Total concentrations of Cu and Zn were determined for all samples by Quadrupole Perkin Elmer, Nexlon 300X at CEREGE (Figure 2.13). Perkin Elmer supports 5 - 20 ppb and 5 - 15 mL as concentration and volume. Six-point calibration (Standard Cu/Zn 0 (blank), 5 ppb, 10 ppb, 25 ppb, 50 ppb, 100 ppb) was used to control the spectrometer response.

Figure 2.13. Quadrupole Perkin Elmer Nexlon 300 (CEREGE 2020)

Isotopic compositions of Cu were analyzed using Thermo Neptune Plus Multi-Collector mass spectrometer (MC ICP-MS) (Figure 2.14). While Cu has 2 isotopes that masses are 63 and 65 with abundances of 69.17% and 30.83%, Zn has 5 isotopes that masses are 64, 66, 67, 68 and 70 with abundances 46.63 %, 27.90 %, 4.10 %, 18.75 % and 0.62 %, respectively (Albarède 2004). The precision of the measurement of the isotopic ratios depends on the purity of sample solution obtained by chromatographic separation. Absence of other than target elements in the sample solution (elution) helps limiting the risk of interference during the analyses. MC-ICP-MS offers high resolution analyses thanks to double focusing system and multi-collector options.

Figure 2.14. Thermo Scientific Neptune Series MC-ICP-MS (Planet Isotopes 2020)

There are 2 main differences between used ICP-MS and MC-ICP-MS. These are mass analyzer system (Quadrupole Mass Analyzer and Double Focusing Analyzer) and detection system (Figure 2.15).

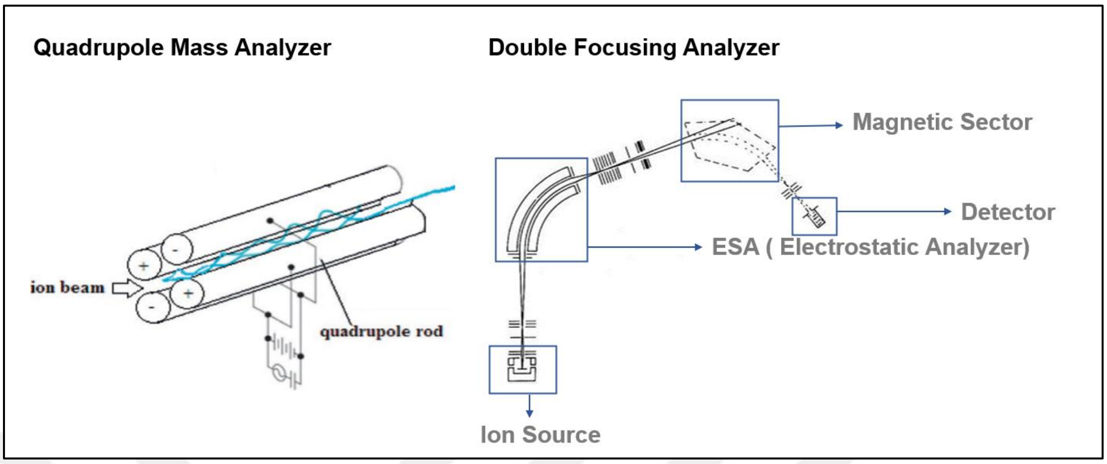

Figure 2.15. Differences of Mass Analyzer Systems between used ICP-MS and MC-ICP-MS techniques (Modified by Bateman, 2014; Beauchemin, 2017)

Perkin Elmer Nexlon 300X ICP MS has quadrupole mass analyzer system. Many elements may be analyzed in one sequence however their ions are collected element by element in a single collector.

Neptune^{Plus} has double focusing analyzer system composed of ESA (Electrostatic Analyzer) and Magnetic Sector. One element is analyzed at the same time however its isotopes are collected simultaneously in multi collectors.

Results are presented in conventional delta notation as per mil (2.1). AE633 is the Cu isotopic standard solution.

$$\boldsymbol{\delta^{65}Cu}(‰) = \left[\frac{\left(\frac{65_{Cu}}{63_{Cu}}\right) \boldsymbol{sample}}{\left(\frac{65_{Cu}}{63_{Cu}}\right) \boldsymbol{AE633}} - \mathbf{1} \right] * \mathbf{1000}$$

(2.1)

3. RESULTS

3.1 Total Cu and Zn Concentrations and Isotopic Compositions

Total Cu and Zn concentration and Cu isotopic composition in control soil and PS-amended soil are shown in Table 3.1 and Table 3.2. Recovery of the chromatographic produce was verified for each sample and yielded on average 92 % (Appendix B). Comparison of Cu concentrations obtained for GSS-3 and Mn-Nod reference materials with the certified values showed recovery of 85.1 % and 86.6 %, respectively. These lower than expected values can be explained by statistic electricity problem, which was particularly important for these two samples during transfer process of soil samples.

Table 3.1. Total Cu and Zn concentration in the control soil and PS-amended soil

***Control Soil**				***PS-amended Soil**		
Layer(cm)	**Sample**	**Cu (μg g^{-1})**	**Zn (μg g^{-1})**	**Sample**	**Cu (μg g^{-1})**	**Zn (μg g^{-1})**
0 - 5	*UFSM-20*	9,61	21,69	*UFSM-110*	76,34	94,65
5 - 10	*UFSM-19*	10,65	23,99	*UFSM-109*	29,36	47,02
10 - 15	*UFSM-18*	10,78	23,75	*UFSM-108*	19,20	37,60
15 - 20	*UFSM-17*	10,02	22,40	*UFSM-107*	12,72	28,38
20 - 25	*UFSM-16*	9,61	21,49	*UFSM-106*	12,07	26,20
25 - 30	*UFSM-15*	10,01	22,49	*UFSM-105*	10,16	22,55
30 - 35	*UFSM-14*	9,92	22,83	*UFSM-104*	10,20	24,58
35 - 40	*UFSM-13*	10,24	22,71	*UFSM-103*	11,00	24,94
40 - 50	*UFSM-12*	9,76	21,99	*UFSM-102*	10,23	22,62
50 - 60	*UFSM-11*	10,44	22,69	*UFSM-101*	10,23	21,39

*Control Soil: No pig slurry application, PS-amended Soil: Maximum pig slurry application

Table 3.2. Cu isotopic composition in the control soil and PS-amended soil

***Control Soil**				***PS-amended Soil**		
Layer (cm)	**Sample**	**d65Cu, ‰**	**± 2SD**	**Sample**	**d65Cu, ‰**	**± 2SD**
0 - 5	*UFSM-20*	-0.39	0.09	*UFSM-110*	0.05	0.11
5 - 10	*UFSM-19*	-0.43	0.16	*UFSM-109*	-0.04	0.06
10 - 15	*UFSM-18*	-0.42	0.10	*UFSM-108*	-0.12	0.07
15 - 20	*UFSM-17*	-0.26	0.03	*UFSM-107*	-0.19	0.06
20 - 25	*UFSM-16*	-0.37	0.03	*UFSM-106*	-0.27	0.11
25 - 30	*UFSM-15*	-0.32	0.12	*UFSM-105*	-0.30	0.09
30 - 35	*UFSM-14*	-0.31	0.16	*UFSM-104*	-0.29	0.08
35 - 40	*UFSM-13*	-0.36	0.15	*UFSM-103*	-0.27	0.06
40 - 50	*UFSM-12*	-0.38	0.12	*UFSM-102*	-0.31	0.11
50 - 60	*UFSM-11*	-0.63	0.12	*UFSM-101*	-0.44	0.07

* Control Soil: No pig slurry application, PS-amended Soil: Maximum pig slurry application.

3.1.1 Total Cu Distribution in the Soil Profiles

Copper concentration in the control (uncontaminated) soil is homogeneous and remains close to 10 µg g^{-1} though the whole profile. Total Cu concentration graph is shown in Figure 3.1. In the PS-amended soil (contaminated) the highest-level total Cu concentrations are observed in the uppermost layers which are 0 – 5 cm and 5 – 10 cm depths. In the upper layer, while total Cu concentration is measured as 9.61 µg g^{-1} in the control soil, this value is increased to 76.34 µg g^{-1} in PS-amended soil. The lowest total Cu concentration is observed at 25 – 30 cm depths. Total Cu concentrations after 11 years of pig slurry application do not display any change regularly.

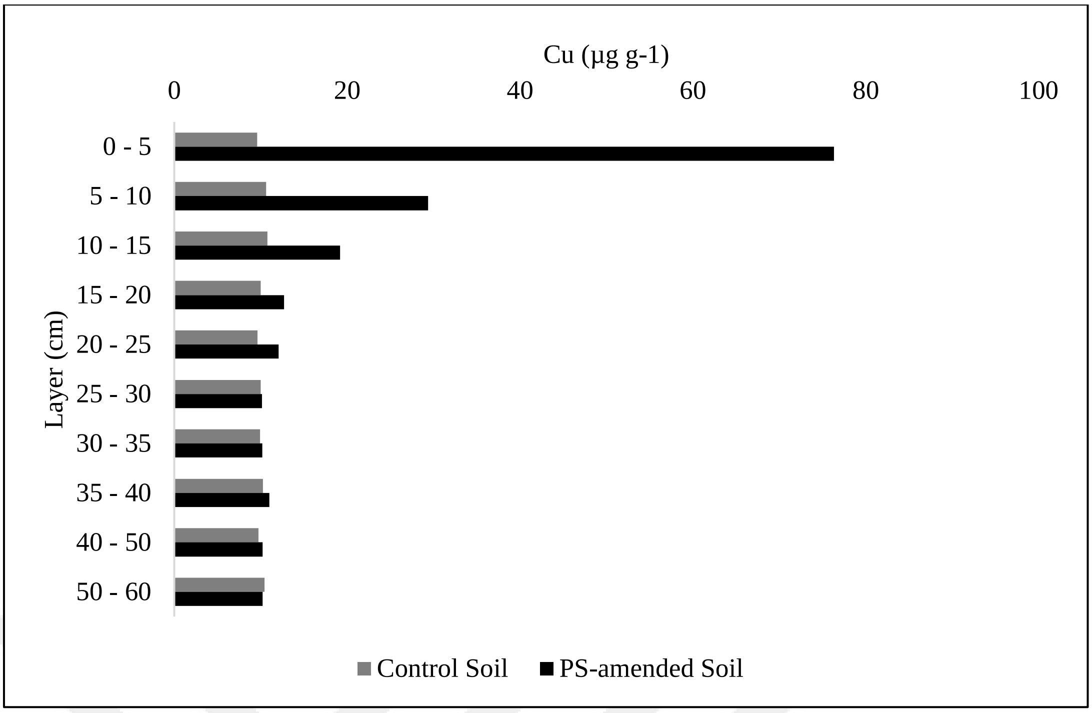

Figure 3.1. Total Cu concentrations in control soil and PS-amended soil (Control Soil: No pig slurry application, PS-amended Soil: Maximum pig slurry application)

3.1.2 Total Zn Distribution in the Soil Profile

The highest total Zn concentrations are observed in the uppermost intervals of the PS-amended soils, which are 0 – 5 cm and 5 – 10 cm depths. Total Zn concentration graph is shown in Figure 3.2. The total Zn concentration increased from 21.69 µg g^{-1} to 94.65 µg g^{-1} for the first layer. The total Zn concentrations are compared within control soil and PS-amended soil, that shows increase generally except in 50 – 60 cm depths.

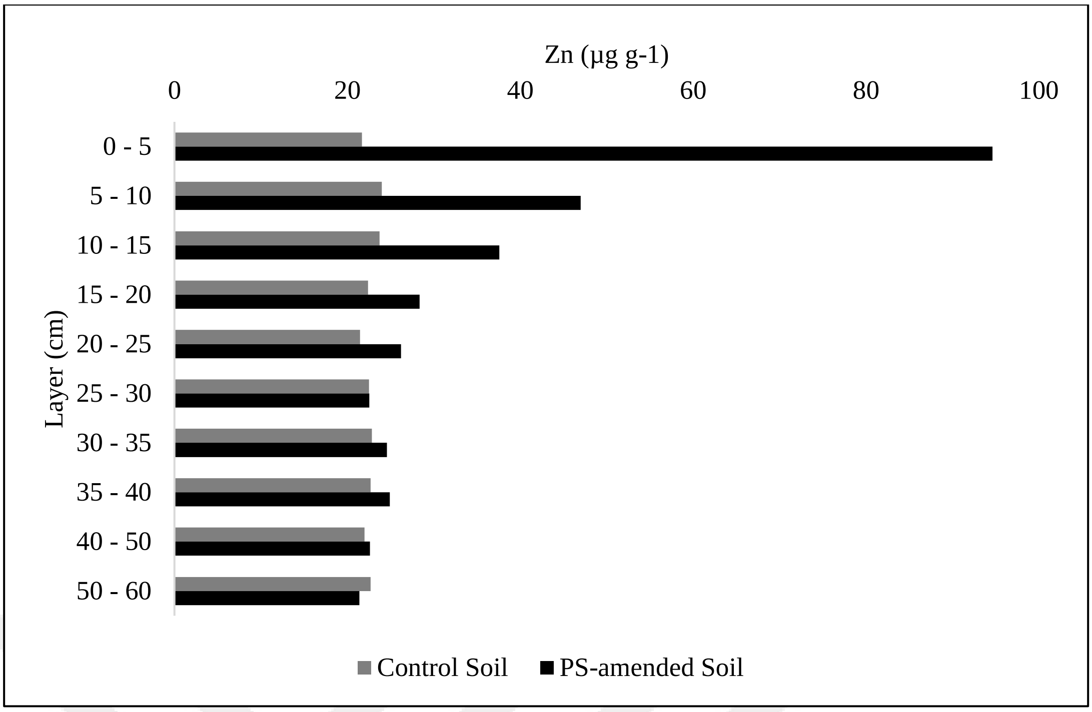

Figure 3.2. Total Zn concentrations in control soil and PS-amended soil (Control Soil: No pig slurry application, PS-amended Soil: Maximum pig slurry application)

3.1.3 Cu Isotopic Signatures

Cu isotopic signatures are shown in Figure 3.3. While δ^{65}Cu values range are differed between -0.26 ± 0.03 ‰ and -0.63 ± 0.12 ‰ in control soil, they are observed between 0.05 ± 0.11 ‰ and -0.44 ± 0.07 ‰ in PS-amended soil. Averages values of δ^{65}Cu are -0.39 ‰ and -0.22 ‰ in control soil and PS-amended soil, respectively. Isotopic composition of control soils is enriched in light isotopes compared to the PS-amended soils and the difference is particularly visible in upper soil horizons. The pig slurry sample from Santa Maria analyzed in 2019 has δ^{65}Cu value of 0.29 ± 0.08 ‰. Its Cu and Zn concentrations were 80.5 µg g^{-1} and 483.4 µg g^{-1}, respectively.[2] In past studies, only two δ^{65}Cu values for pig slurry were published and show rather heterogeneous isotopic signature of 0.12 ‰ and 0.52 ‰ (Fekiacova et al., 2015).

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[2] These data were obtained by Zuzana Fekiacova (unpublished data).

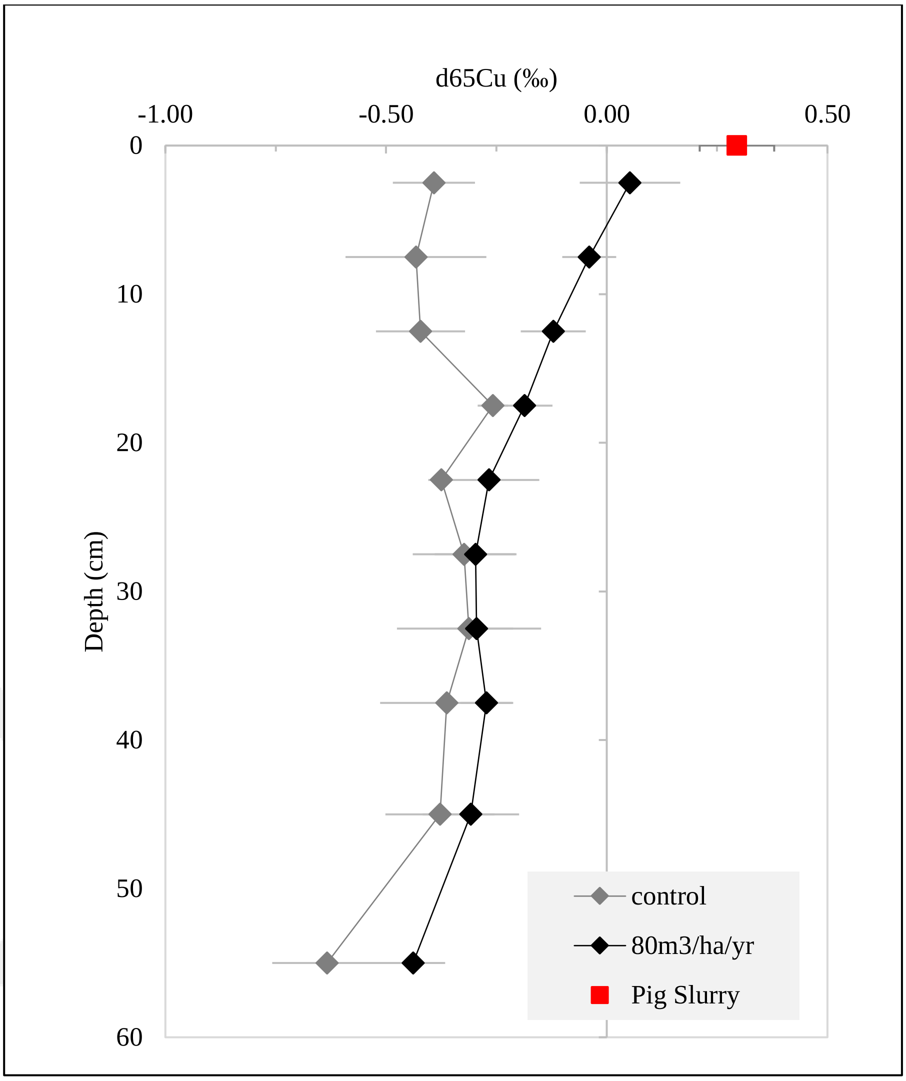

Figure 3.3. According to soil depth, Cu isotopic composition in control soil and PS-amended soil (Control Soil: No pig slurry application, PS-amended Soil: Maximum pig slurry application)

3.2 Total Cu and Zn Accumulation in the soil

Total Cu and Zn accumulated have estimated in the PS-amended soil. Copper and Zn concentration in the 0 – 5 cm are 9.61 µg g^{-1}, 21.69 µg g^{-1} and 76.34 µg g^{-1}, 94.65 µg g^{-1} in control soil and PS-amended soil, respectively. Soil bulk density within 0 – 5 cm for Luvisol soil is 1.47 g cm^{-3} (Silva et al. 2011). The stock of each elements is calculated using the in 3.1.

$$S = Soil(\mu g\ g^{-1}) * \frac{1}{1000}(mg\ g^{-1}) * BD(g\ cm^{-3}) * D(cm) * 100\left(\frac{10^{-6}}{10^{-8}}(kg\ ha^{-1})\right) \tag{3.1}$$

S: Stock of each element (kg ha^{-1})

BD: Bulk Density, (g cm^{3})

D: Depth, (cm)

In pig slurry, stock of Cu and Zn were calculated as 68.4 kg ha^{-1} and 85.6 kg ha^{-1} corresponding to application rates of 80 m^{3} ha^{-1} (De Conti et al. 2016). Using these values stock of Cu and Zn in the soil were calculated.

3.2.1 Stock of Cu in the Soil

Stock of Cu is calculated in 0 – 5 cm layer of the control soil as 7.06 kg ha^{-1}. Thus, the theoretical value of the total stock of Cu in the PS-amended soil after 11 PS applications was calculated to be 75.46 kg ha^{-1}. The real stock of Cu calculated on the basis of the analyzed Cu concentration in the PS- amended soil was found to be 56.11 kg ha^{-1}. According to these calculations, 19.36 kg ha^{-1} of Cu were leached deeper layer (Figure 3.4).

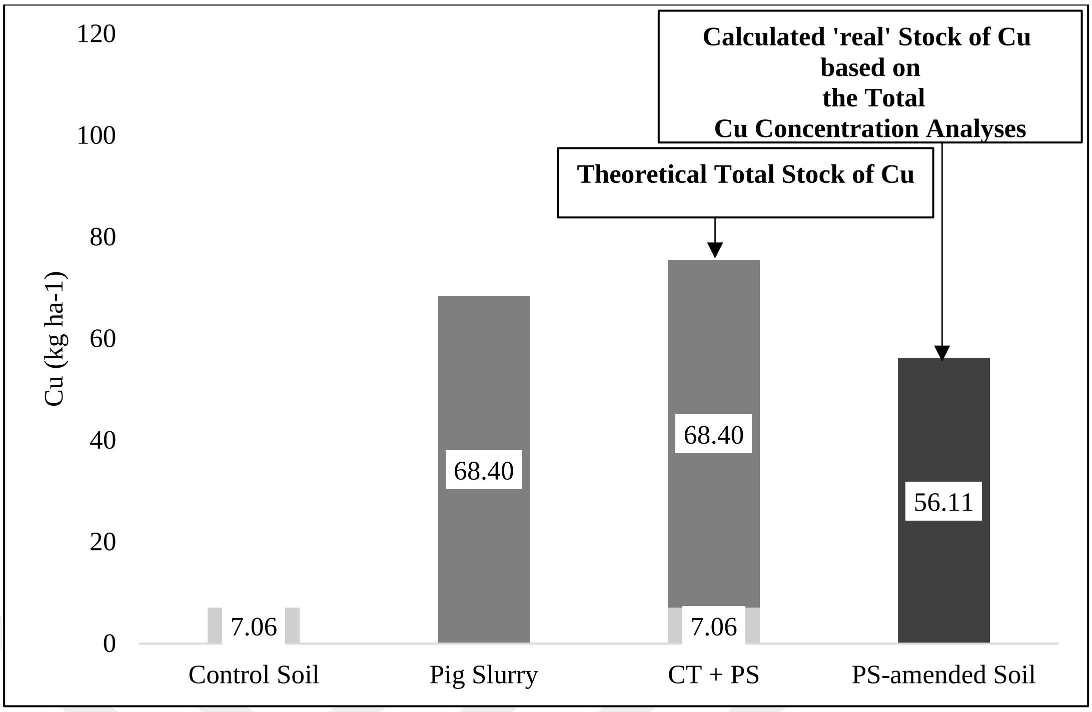

Figure 3.4. Stock of Cu in control soil, pig slurry and PS-amended soil

3.2.2 Stock of Zn in the Soil

Stock of Zn is calculated in 0 – 5 cm layer of the control soil as 15.94 kg ha^{-1}. Therefore, the theoretical stock of Zn was calculated as 101.54 kg ha^{-1}. The real stock of Zn calculated based on the analyzed Cu concentration in the PS- amended soil was determined to be 69.57 kg ha^{-1}. Furthermore, missing amount of stock of Zn is calculated 31.97 kg ha $^{-1}$ (Figure 3.5). According to these calculations, 31.49 % theoretical stock of Zn is accumulated in deeper layers.

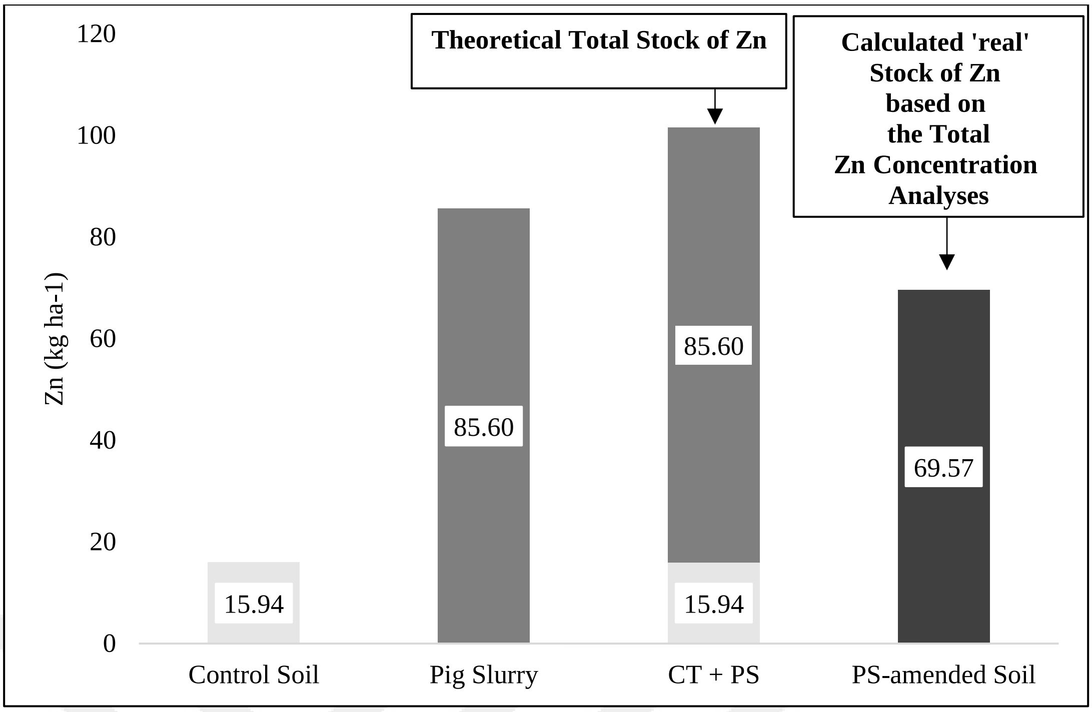

Figure 3.5. Stock of Zn in control soil, pig slurry and PS-amended soil

3.2.3 Calculation of the Anthropogenic Contribution to soil Cu Budget

Plot of δ^{65}Cu (‰) values and 1/[Cu] is shown in Figure 3.6. Error bars represent the two standard derivations (2sd) associated to δ^{65}Cu values. In a δ^{65}Cu (‰) - 1/[Cu] diagram, PS-amended Soils (black square symbol) spread along a straight-line indicating mixing between two Cu sources (natural and anthropogenic). Natural Cu pool represents by low δ^{65}Cu (‰) and high 1/[Cu], anthropogenic Cu pool characterizes by high δ^{65}Cu (‰) and low 1/[Cu] compositions. In agreement with this observation, the samples from the control soil (grey-circle symbols) have Cu concentrations and Cu isotopic compositions characteristic of the “natural Cu-end” of the mixing line. Furthermore, the previously analyzed PS sample (red symbol) lies at the proximity of the “anthropogenic Cu-end” of the mixing line. A slight shift was observed between the δ^{65}Cu value of the PS and those of the contaminated surface horizon samples. This discrepancy most likely indicates that Cu from the PS underwent transformations after its deposition on the soil, which has modified its original isotopic composition.

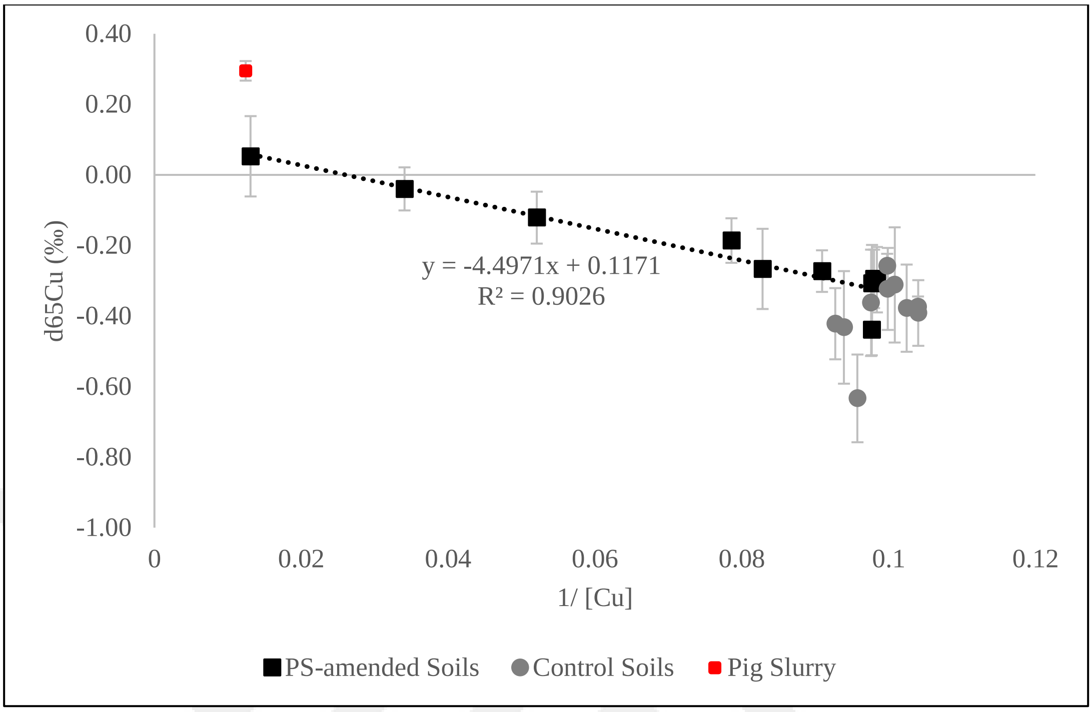

Figure 3.6. Plot of δ^{65}Cu (‰) values and 1/[Cu] in the PS-amended soil layers

Metal stable isotopes have proven to be a useful tool for metal source tracing in soils (e.g., Wiederhold, 2015). Contribution of different reservoirs can be detected using the metal isotope signatures for determination source tracing (Wiederhold 2015). The anthropogenic and natural Cu contributions are calculated in the PS-amended soil using simple mixing equation (3.2). Obtained results are shown in Table 3.3.

$$|fCu_{NATURAL}| = \frac{(\delta^{65}Cu_{PS-AMENDED} * [Cu]_{PS-AMENDED}) - (\delta^{65}Cu_{PS} * [Cu]_{PS})}{(\delta^{65}Cu_{CONTROL} * [Cu]_{CONTROL}) - (\delta^{65}Cu_{PS} * [Cu]_{PS})} * 100 \tag{3.2}$$

$fCu_{NATURAL}$: the fraction of the natural Cu in the PS-amended soil.

$\delta^{65}Cu_{PS\text{-}AMENDED}$: Measured δ^{65}Cu in the PS-amended soil, (‰)

$[Cu]_{PS\text{-}AMENDED}$: Measured Cu concentration in the PS-amended soil, (µg g^{-1})

$\delta^{65}Cu_{CONTROL}$: Measured δ^{65}Cu in the control soil, (‰)

$[Cu]_{CONTROL}$: Measured Cu concentration in the control soil, (µg g^{-1})

$\delta^{65}Cu_{PS}$: Measured δ^{65}Cu in pig slurry, (‰)

$[Cu]_{PS}$: Measured Cu concentration in pig slurry, (µg g^{-1})

Table 3.3. Percentage of anthropogenic and natural Cu in each samples soil layer calculated on the basis δ^{65}Cu (‰) values and Cu concentrations

Sample	Layer (cm)	Anthropogenic Cu (%)	Natural Cu (%)
UFSM-110	0 - 5	28.30	71.70
UFSM-109	5 - 10	12.13	87.87
UFSM-108	10 - 15	7.85	92.15
UFSM-107	15 - 20	0.82	99.18
UFSM-106	20 - 25	1.40	98.60
UFSM-105	25 - 30	0.81	99.19
UFSM-104	30 - 35	0.33	99.67
UFSM-103	35 - 40	2.60	97.40
UFSM-102	40 - 50	1.96	98.04
UFSM-101	50 - 60	7.00	93.00

Additionally, the percentages of anthropogenic – natural Cu fractions are calculated in the PS-amended soil using the Microsoft Excel Solver Add-In Tools. Solver is an add-on package that solves the solution of equations with multiple unknowns using linear modeling methods. Parameters used for the calculation were obtained. According to isotopic mass-balance, heavy isotopes in product must be equal to sum of heavy isotopes in precursors (Hayes 2004), which is mathematically expressed in 3.3.

$$\sum m_i f_i = m_1 f_1 + m_2 f_2 + \cdots + m_i f_i \tag{3.3}$$

The *m* and *f* terms represent molar quantities of the element of interest and fractional isotopic abundances, respectively.

Therefore, fraction of each reservoir can be determined as δ function in 3.4.

$$\delta_{CULTIVATED} = \delta_{ANTHROPOGENIC} * f_{ANTHROPOGENIC} + \delta_{NATURAL} * f_{NATURAL}$$

(3.4)

And 3.5 must be provided.

$$f_{ANTHROPOGENIC} + f_{NATURAL} = 1$$

(3.5)

PS-amended δ functions are calculated as empirical using by 3.6.

$$\delta_{PS-AMENDED} = \frac{[Cu]_{CONTROL} * \delta^{65}Cu_{CONTROL} * f_{CONTROL} + [Cu]_{PS} * \delta^{65}Cu_{PS} * (1 - f_{CONTROL})}{[Cu]_{PS-AMENDED}}$$

(3.6)

Equation units and explanations are given in below:

$[Cu]_{CONTROL}$: Measured Cu concentration in the control soil (sample UFSM – 11 are accepted to present the natural Cu), (µg g^{-1})

$\delta^{65}Cu_{CONTROL}$: Measured δ^{65}Cu in the control soil (UFSM – 11), (‰)

$f_{CONTROL}$: Fraction of the natural Cu sources, (-)

$[Cu]_{PS}$: Measured Cu concentration in the Pig Slurry, (µg g^{-1})

$\delta^{65}Cu_{PS}$: Measured δ^{65}Cu in the Pig Slurry, (‰)

$(1 – f_{CONTROL})$: Fraction of the anthropogenic sources = from the PS, (-)

$[Cu]_{PS-AMENDED}$: Measured Cu concentration in the PS-amended soil, (µg g^{-1})

Square of a difference was determined to minimize the difference and to optimize results between measured and calculated isotopic composition using by 3.7.

$$diff \sum = (Isotopic\ Data - Empirical\ Data)^2$$

(3.7)

Then, calculated differences are inputted into the Set Objective section in the Excel Solver Parameters Windows choosing minimum option to balance equality between differences. After variable and constraints data are inputted into the parameter section.

The % of anthropogenic and natural Cu fractions in the PS-amended soil represent variable values. The sum of these values must be equal to one (3.5). A cell is selected, and the sum of these values is written. This cell equality to one is provided on the add-on and inputted in the subject to constraints. Therefore, the Solver add-in is used for this study (Appendix C). Results are shown in Figure 3.7.

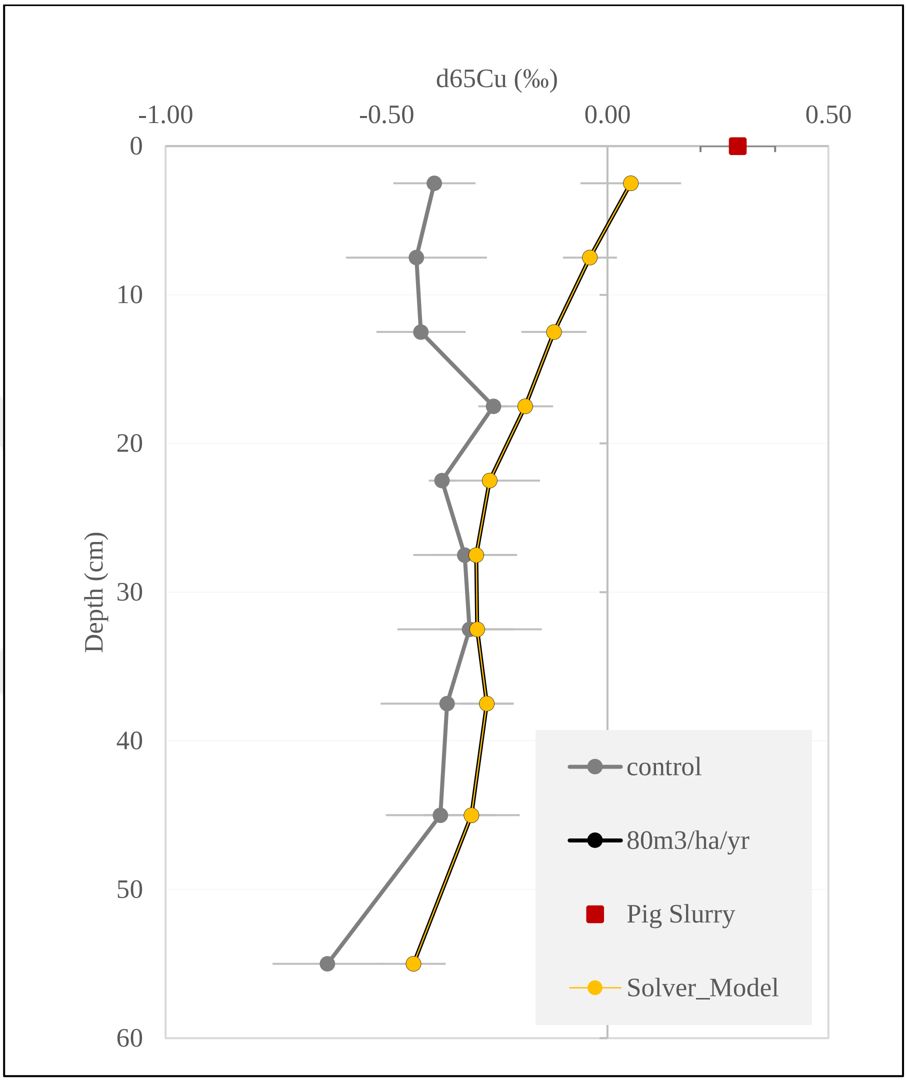

Figure 3.7. Obtained Model using by Solver Add-in

Total Cu concentration are calculated combining obtained fractioned data using by 3.8.

$$[Cu] = [Cu]_{NATURAL} * f_{NATURAL} + [Cu]_{PS} * (1 - f_{NATURAL})$$

(3.8)

Obtained total Cu concentrations in the control soil and PS-amended soil are shown in Figure 3.8 and Figure 3.9.

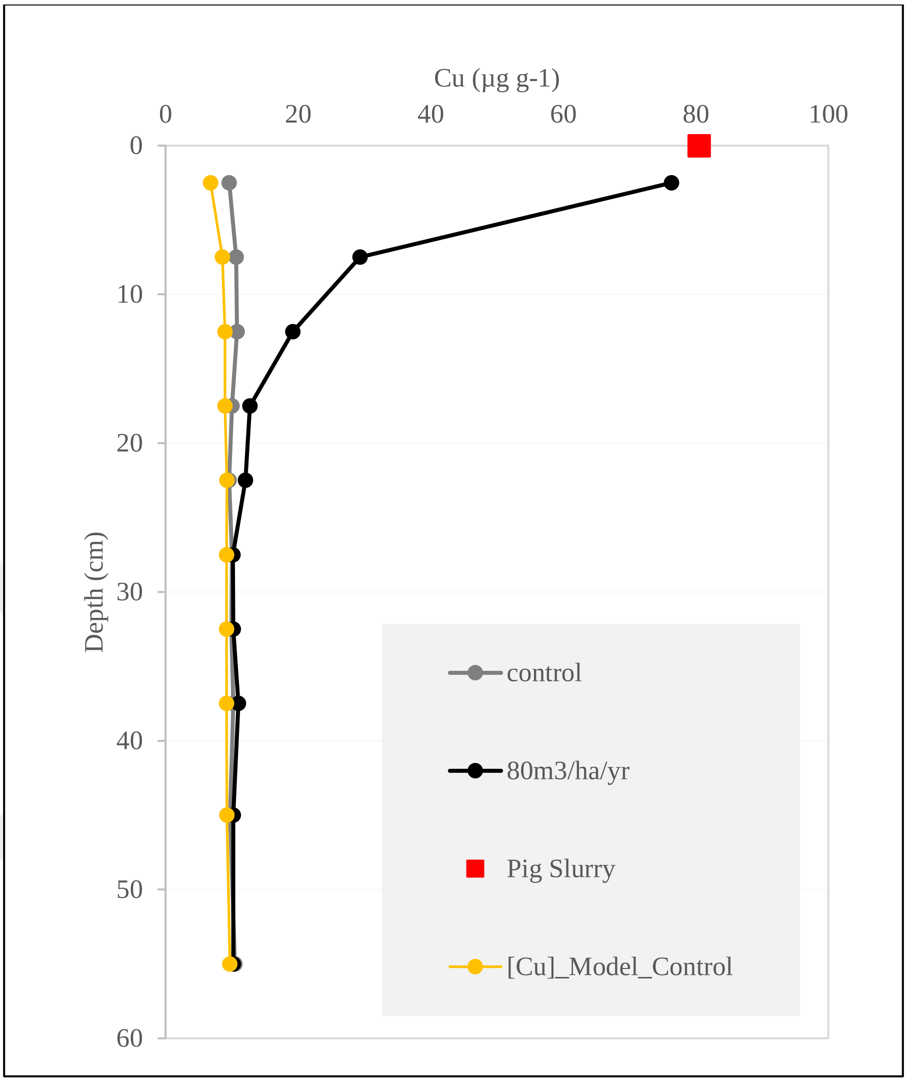

Figure 3.8. Obtained total Cu concentration in control soil using by output of the model

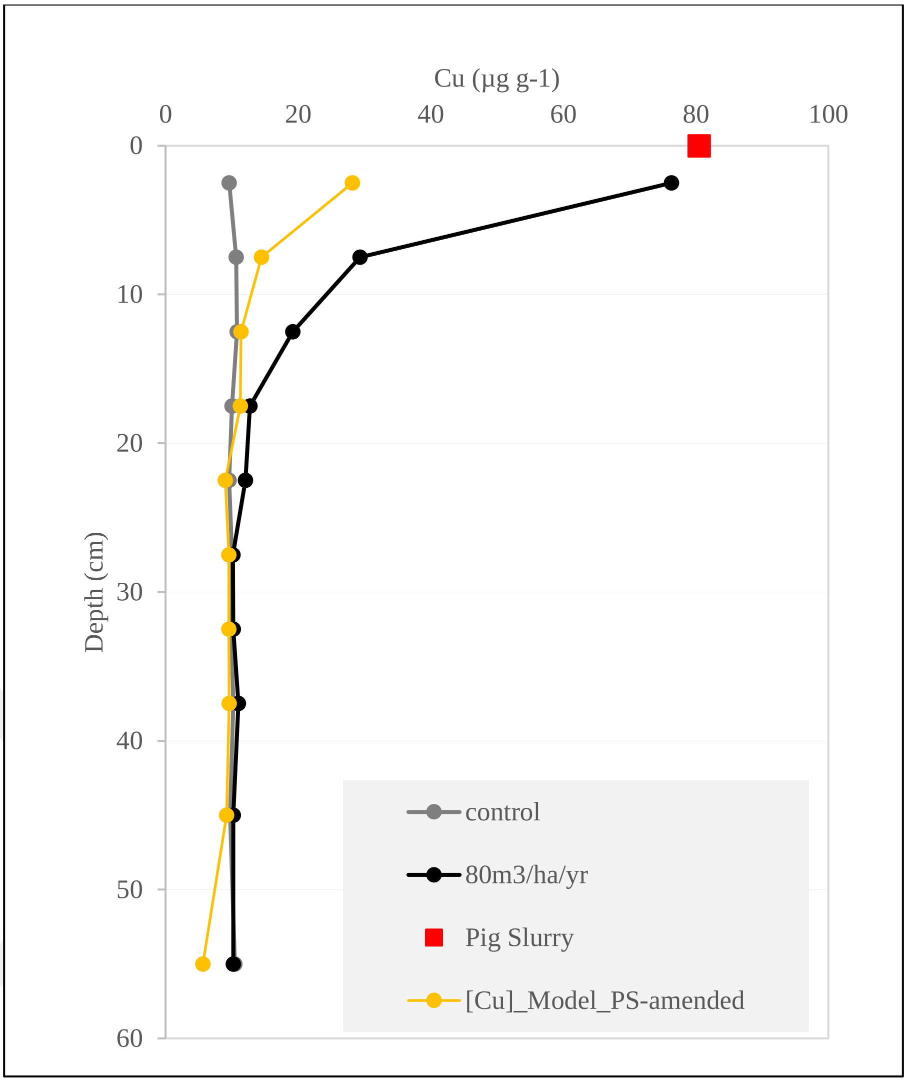

Figure 3.9. Obtained total Cu concentration in PS-amended soil using by output of the model

Calculated total Cu concentrations from model are shown in Table 3.4.

Table 3.4. Calculated total Cu concentration using anthropogenic and natural fractions obtained by Solver Add-in

Sample	Layer (cm)	[Cu] (µg g^{-1})	Sample	Layer (cm)	[Cu] (µg g^{-1})
UFSM-20	0-5	6.78	*UFSM-110*	0-5	28.20
UFSM-19	5-10	8.57	*UFSM-109*	5-10	14.45
UFSM-18	10-15	8.97	*UFSM-108*	10-15	11.37
UFSM-17	15-20	8.98	*UFSM-107*	15-20	11.26
UFSM-16	20-25	9.27	*UFSM-106*	20-25	9.01
UFSM-15	25-30	9.20	*UFSM-105*	25-30	9.54
UFSM-14	30-35	9.20	*UFSM-104*	30-35	9.57
UFSM-13	35-40	9.20	*UFSM-103*	35-40	9.59
UFSM-12	40-50	9.25	*UFSM-102*	40-50	9.19
UFSM-11	50-60	9.71	*UFSM-101*	50-60	5.64

Established total Cu concentration results with Solver Add-in are differed between 6.78 µg g^{-1} and 9.71 µg g^{-1} in the control soil. In PS-amended soil, modelled total Cu concentration values are changed between 5.64 µg g^{-1} and 28.20 µg g^{-1}. Calculated fractions were calculated as percentage (Table 3.5).

Table 3.5. Calculated anthropogenic and natural contribution for Cu using by Excel Solver Add-in

Sample	Layer (cm)	Anthropogenic Cu (%)	Natural Cu (%)
UFSM-110	0 - 5	35.04	64.96
UFSM-109	5 - 10	17.96	82.04
UFSM-108	10 - 15	14.13	85.87
UFSM-107	15 - 20	13.99	86.01
UFSM-106	20 - 25	11.20	88.80
UFSM-105	25 - 30	11.85	88.15
UFSM-104	30 - 35	11.89	88.11
UFSM-103	35 - 40	11.91	88.09
UFSM-102	40 - 50	11.42	88.58
UFSM-101	50 - 60	7.00	93.00

According to results of developed model, the highest anthropogenic contribution was observed in 0 – 5 cm depth. Then, the lowest anthropogenic contribution was determined in 50 – 60 cm depth.

4. CONCLUSION

Metals are present in soils as a result of the effect of natural and anthropogenic processes. In this study, it was aimed to determine total Cu and Zn concentrations in control soil and PS-amended soil and distinguish contributions of natural and anthropogenic sources of Cu in PS-amended soil using the isotopic approach. The following conclusion were conducted:

- Total Cu and Zn concentrations were higher in the PS-amended soil than in the control soil. The main difference was observed in the upper layers.
- Stock calculations results confirm accumulations of Cu and Zn in the surface horizons of the PS-amended soils. Furthermore, these calculations suggest that, Cu is accumulated in upper layers, while Zn is also transported to deeper layers. This observation can be explained by preferential Cu interaction with organic matter, in contrast to Zn, which shows affinity for Al and Fe-oxides rather than organic matter. Estimated stock of Zn accumulated in deeper layers is up to 1.65 times higher than total stock of Cu.
- Observed isotopic signatures (δ^{65}Cu values) were different in control soil and PS-amended soil. Similarity of the δ^{65}Cu values in the surface layers of the PS-amended soil with the Cu isotopic signature of the pig slurry provided evidence of the presence of the Cu originating from the PS in these layers. Difference of isotopic signatures decrease with depth. The anthropogenic and natural Cu contributions were determined in the PS-amended soil using the isotopic signatures and mixing calculations. Significant proportion (up to 30%) of the anthropogenic Cu was observed in the 3 upper layers. The contribution of the anthropogenic Cu decreased in deeper layers, at 30 - 35 cm depth.
- The evolution of contribution of the anthropogenic Cu was modelled in the PS-amended soils using Microsoft Excel Solver Add-In Tool. Developed model shows that up to 35.04 % of the Cu from PS remains immobilized in the upper layers. This proportion decreases rapidly with depth.
- It was observed a discrepancy between δ^{65}Cu values in the surface layers of the PS-amended soils and the δ^{65}Cu values of the pig slurry. This difference

reflects the modification of Cu isotopic signature during Cu immobilization in the upper soil layers. Further investigations are necessary to identify the process responsible for this immobilization (adsorption, complexation, etc.).

- The data showed that stable Cu isotopes are a powerful tool for studying the fate of Cu in soils. This study will be completed with the Zn isotopic analysis on the studied natural and PS-amended soils with the aim to supply data for further interpretation of the metal behavior in the PS-amended soils and for geochemical modelling.

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6. APPENDICES

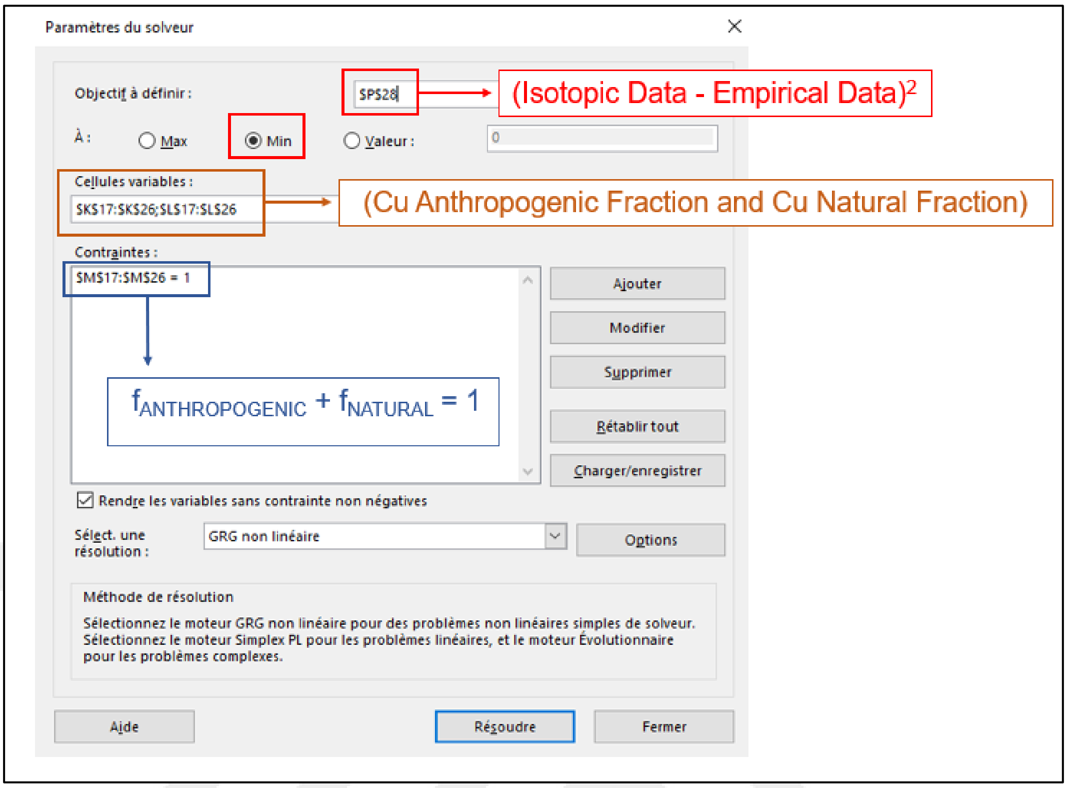
Appendix A. Calculations of Lost Weight During the Transition to the Dissolution Process.

Prepared Samples Number	**Lost Weight (%)**
UFSM-11-BROYE	3.44
UFSM-12-BROYE	4.26
UFSM-13-BROYE	4.34
UFSM-14-BROYE	4.33
UFSM-15-BROYE	4.13
UFSM-16-BROYE	3.82
UFSM-17-BROYE	3.82
UFSM-18-BROYE	4.46
UFSM-19-BROYE	3.39
UFSM-20-BROYE	4.91
UFSM-101-BROYE	3.16
UFSM-102-BROYE	2.87
UFSM-103-BROYE	2.54
UFSM-104-BROYE	4.50
UFSM-105-BROYE	4.07
UFSM-106-BROYE	4.01
UFSM-107-BROYE	4.38
UFSM-108-BROYE	0.39
UFSM-109-BROYE	5.08
UFSM-110-BROYE	6.14
REFERENCE - GSS-3	1.51
REFERENCE - Mn Nodule	18.83

Appendix B. Chemical Recovery for Cu Elute Samples.

Sample Number	Cu Elute Yield (%)
UFSM-11-Cu	91.30
UFSM-12-Cu	96.59
UFSM-13-Cu	95.18
UFSM-14-Cu	95.50
UFSM-15-Cu	91.71
UFSM-16-Cu	91.59
UFSM-17-Cu	95.05
UFSM-18-Cu	89.86
UFSM-19-Cu	90.37
UFSM-20-Cu	90.00
UFSM-101-Cu	91.20
UFSM-102-Cu	94.03
UFSM-103-Cu	90.69
UFSM-104-Cu	97.05
UFSM-105-Cu	92.01
UFSM-106-Cu	83.64
UFSM-107-Cu	96.03
UFSM-108-Cu	94.59
UFSM-109-Cu	94.33
UFSM-110-Cu	86.62
REF - GSS-3-Cu	99.39
REF - Mn Nod-Cu	91.20

Appendix C. Inputs for Excel Solver Add-In Tool.



Paramètres du solveur

Objectif à définir : $P$28

(Isotopic Data - Empirical Data)^2

À : ○ Max ● Min ○ Valeur : 0

Cellules variables : $K$17:$K$26;$L$17:$L$26

(Cu Anthropogenic Fraction and Cu Natural Fraction)

Contraintes : $M$17:$M$26 = 1

$f_{\text{ANTHROPOGENIC}} + f_{\text{NATURAL}} = 1$

☑ Rendre les variables sans contrainte non négatives

Sélect. une résolution : GRG non linéaire

Méthode de résolution

Sélectionnez le moteur GRG non linéaire pour des problèmes non linéaires simples de solveur. Sélectionnez le moteur Simplex PL pour les problèmes linéaires, et le moteur Évolutionnaire pour les problèmes complexes.

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CERTIFICATES

-Muğla Sıtkı Koçman University, International Groundwater Resilience to Climate Change and High-Pressure Conference (GRECPIMA-2019), Certificate of Attendance.

-Hacettepe University, Faculty of Engineering, “15. Student Project Exhibition”, Geological Engineering Department Hydrogeology Program Second Degree.

-Anadolu University, Open Education Faculty, Honour Certificate (2015-2016).

COMPUTER SKILLS

Microsoft Office (Word, Excel, Powerpoint), Adobe Illustrator, ArcGIS, Surfer, RorkWorks, CorelDraw, ModelMuse, AquiferWin32, GW Chart, Groundwater Vista, Hydrus, JChess, Feflow, R Software.

LANGUAGES

Turkish (Native)

English (C1)

French (B1)

Spanish (A1)

BOURSES

2019-2020: Joint Scholarship of Muğla Sıtkı Koçman Foundation & Ambassade de France en Turquie.

SOCIAL ACTIVITIES

2016-2018: Hacettepe University, Hacettepe Women’s Rugby Team.

2015-2016: Hacettepe University, Dance Sports Community.