Macedonian - Chinese Scientific and Technological Cooperation

New Project Proposal for 2016-2017

Title of project proposal:	Project number
Characterization of heavy metals contents in different plant foods from polluted sites and their impact in food chain	

Macedonian organization: Faculty of Agriculture, Goce Delčev University – Štip, Republic of Macedonia

Chinese organization: College of Environment and Safety Engineering, Shenyang University of Chemical Technology

Project period: 2016-2017

Expected period of stay in China for the Macedonian Researchers:
3 persons, 7 days
From 21.03. 2016 to 27.03.2016

Expected period of stay in Macedonia for the Chinese Researchers:
4 persons, 7 days
From 10.10. 2016 to 16.10.2016

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Project description:

Introduction

Heavy metal pollution is a major problem concerning the environment quality. Heavy metals in environment are derived from natural components or geological sources as well as from human activities or anthropogenic sources. The residence time of most heavy metals in environment is very long. There are many sources of heavy metals in environment including (Reichman 2002): 1) Natural e.g. soil parent material, volcanic eruptions, marine aerosols, and forest fires; 2) Agricultural e.g. fertilizers, sewage sludges, pesticides and irrigation water; 3) Energy and fuel production e.g. emissions from power stations; 4) Mining and smelting e.g. tailing, smelting, refining and transportation; 5) Automobiles e.g. combustion of petroleum fuels; 6) Urban/industrial complexes e.g. incineration of wastes and waste disposal; and, 7) Recycling operations e.g. melting of scrap. Metal contamination issues are becoming increasingly common, the occurrence of heavy metals in soils, both natural and polluted, has been the subject of a number of studies (Muller and Anke 1994; Sanchez-Camazano et al. 1994; Dudka et al. 1996; Caussy et al. 2003; Cui et al. 2004). Whereas metal contamination is widespread, the occurrence of heavy metals in agricultural soils is a major concern. Taken up by plants, heavy metals may enter the food chain in significant amounts. Hence, people could be at risk of adverse health effects from consuming plant food grown in soils containing elevated metal concentrations. For instance, it is estimated that approximately half of human lead intake is through food, with around half originating from plants (Nasreddine and Parent-Massin 2002). Bioavailability of metals occurring in soil is the basic source of its accumulation in plant food. The uptake of metals from the soil depends on different factors such as their soluble content, soil pH, plant growth stages, types of species, etc. Plants have developed different strategies to grow on agricultural soils rich in heavy metals. In this way, the food presents the main source of human exposure to toxic metals. Plants, like all other organisms, have evolved different mechanisms to maintain physiological concentrations of essential metal ions and to minimize exposure to non-essential heavy metals. Different plant parts accumulate different levels of these potentially toxic metals. According to the Environmental Protection Agency (EPA), lead is the most common heavy metal contaminant in the environment and may be toxic to organisms even when absorbed in small amounts (Watanabe 1997). Although metals such as zinc, copper and manganese are essential trace elements for plants, they can also be dangerous at high exposure levels. At high doses of certain metal compounds, of the order of several grams, chronic toxicity or carcinogenicity as well as fatality may occur. Certain crops such as spinach, lettuce, carrot, radish, can accumulate heavy metals, e.g. Cd, Cu, Mn, Pb and Zn in their tissues (Sauerbeck 1991; Muller and Anke 1994; Hooda 1997; Bahemuka and Mubofu 1999; Cobb et al. 2000; Mattina et al. 2003; Hough et al. 2004; Zhou et al. 2005, Balabanova et al., 2015). Generally, uptake is increased in plants that are grown in areas with increased soil contamination. Among the metals, Cd and Zn are fairly mobile and readily absorbed by plant food (Mench et al. 1994). In contrast, Cu and Pb are strongly adsorbed onto soil particles reducing their availability to plants (WHO 1998). The fraction of heavy metals which can be readily mobilized in the soil environment and taken up by plant roots is considered the bioavailable fraction. The term ‘‘bioavailability’’ has been defined as the extent to which a chemical can be absorbed by a living organism and reach the systemic circulation (Kelley et al. 2002). Therefore, total metal concentrations in soil do not necessarily correspond with metal bioavailability. The bioavailability to plants of heavy metals depends on a number of physical and chemical factors in the soil. These include soil properties, e.g. pH, organic matter content, redox potential, cation exchange capacity (CEC), sulphate, carbonate, hydroxide, soil texture and clay content (Peijnenburg and Jager 2003). Apart from these factors, metal absorption by plants is influenced by the characteristics of the plants themselves (Hund-Rinke and Kordel 2003).

Since food contamination is one of the major routes for entry of metals into the humans and animals, monitoring the bioavailable pools of metals in contaminated different plant foods has generated a great interest. Pertaining to this awareness, a lot of studies should be conducted for determining the metals contents in the different plant foods and the health risk assessment should be predicted in the heavy metals polluted areas. Several investigations showed that there are significantly polluted areas with heavy metals on the territory of the R. Macedonia (Stafilov et al., 2014) and on the territory in China (Wnag et al., 2003). Therefore, is the importance of conducting further investigations for characterization of heavy metals contents in different plant foods from polluted sites and their impact in food chain.

Research Project

Bioavailability of metals occurring in soil is the basic source of its accumulation in plant food. The impact of soil pollution on the food chain presents a challenge for many investigations. Availability of metals in a potentially polluted soil and their possible transfer and bioaccumulation in different plant food samples will be examine. Also, the entrancements of the potentially toxic metals in food chain and possible health risk assessment will be examined.

Investigated area:

Appropriately, mines' surroundings and urban areas were selected as investigation areas to determine the anthropogenic impact on the agricultural land and food quality (in terms of pollution with toxic metals). However, the environmental pollution impact on the food chain has been very poorly investigated. Previous investigation showed that the most affected areas with poly-metallic pollution within the territory of the Republic of Macedonia are:

Copper mine and flotation plant "Bučim" near the town of Radoviš	Salminem et al., 2005; Balabanova et al., 2013; 2014; 2015, Stafilov et al., 2010; 2014
Lead-zinc mine "Toranica" near the town of Kriva Palanka	Salminem et al., 2005; Stafilov et al., 2014; Angelovska et al., 2014
Lead-zinc mine "Sasa" near the town of Makedonska Kamenica	Balabanova et al., 2015
Lead-zinc smelter plant near the town of Veles	Pančevski et al., 2014a; 2014b; Stafilov et al., 2008; 2014
Lead-zinc mine "Zletovo" near the town of Probištip	Stafilov et al., 2014
Ferro-nickel smelter plant "FENI" near the town of Kavadarci	Bačeva et al., 2009; 2011; 2012; Stafilov et al., 2014

Using these above mention data from the given references for the soil polluted areas, will be constructed the sampling networks and density of the network will be predicted. Dense sampling network will be constructed very close to the pollution sources as well more distanced from the "hot spot".

Different plant species will be selected for analysis, including: fruits, vegetables, crops and various herbal plants. Also, soil samples from the agricultural lands will be collected from the same location where the plants samples are planted. Two locations will be selected as control locality, where no significant pollution source appears. The Ovče pole region and the Strumica close environ will be selected as control areas, due to data given from Salminem et al. (2005). Thirteen localities will be selected as sampling location. Sample coordinates will be obtained via the global positioning system.

Site	Locality	Site characterization	latitude	longitude
1	Strumica region	Reference area	41.459544°	22.643910°
2	Ovče Pole region	Reference area	41.820084°	22.088079°
3	Village Lakavica	Reference area	41.651964°	22.236578°
4	Village Topolnica	Cu-mine environ	41.656611°	22.377807°
5	Village Bučim	Cu-mine environ	41.670875°	22.338845°
6	Village Sasa	Pb-Zn mine environ	42.076538	22.550724°
7	Village Uzem	Pb-Zn mine environ	42.221951°	22.424537°
8	Village Zletovo	Pb-Zn mine environ	41.982859°	22.234099°
9	Probištip	Pb-Zn mine environ	41.992765°	22.186003°
10	Veles	Pb-Zn smelter plant environ	41.710874°	21.764810°
11	Village Orizari	Pb-Zn smelter plant environ	41.686485°	21.735027°
12	Kavadarci environ	Fe-Ni smelter plant environ	41.437109°	22.009962°
13	Kavadarci environ	Fe-Ni smelter plant environ	41.423170°	22.017556°

Methods:

a) Plants samples preparation

Collected plant species will be transfer to the laboratory where the physical-chemical preparation will be applied. Firstly, plants will be washed with water and rinsed with distillated water, to ensure that they are thoroughly cleaned and any outside contamination has been removed. Plant samples will be totally dissolved using microwave digestion will be applied for total digestion of the plant samples according to EPA-Method 3052.

b) Soil samples pre-treatment (total and extractability forms)

Soil samples transferred to the laboratory, firstly will be physical prepared for the chemical digestion. For total digestion, an open wet digestion method with a mixture of acids will be applied . Two methods will be applied for the physical-chemical and total digestion:

- ISO 14869-1: Soil quality- Dissolution with hydrofluoric and perchloric acids.
- ISO 11464:1994, Soil quality- Pretreatment of samples for physic- chemical analysis.

Three methods were applied for the study of the plant availability of the elements: (1) Extraction with deionized H_2O that provides information on the actual availability of the elements in the soil solution; (2) Extraction with 0.1 M hydrochloric acid (HCl) displacing potentially available forms that are not easily extracted. The immobilization of the heavy metals in the contaminated soil is carried out through chelating and washing with mineral acids. In this way the efficiency in the extraction of heavy metals from the soil is determined. An effective pH level is required to enable remediation of heavy metals from contaminated soils. Because of the differences in the affinities of heavy metals with soil and soil constituents the effective pH levels are likely to vary depending on the type of the heavy metals and the strength of their bonding with the soil. (3) Extraction of the soluble species of trace elements in a mixed buffered solution (pH= 7.3) of triethanolamine (0.1 mol/L) with $CaCl_2$ (0.01 mol/L) and diethylenetriaminepentaacetic acid (0.005 mol/L), which is often recommended for extraction of toxic or biogenic metals (Bačeva et al., 2013).

c) Determination of metals contents

Inductively coupled plasma mass spectrometer (ICP-MS) and atomic emission spectrometry with inductively coupled plasma, ICP-AES will be used for all elements measurements. The following 35 elements will be recorded: Li, Be, B, Na, Mg, Al, Si, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Te, Ba, Tl, Pb, and Bi.

d) Chemicals/Reagents

For all analytical procedures, ultrapure water will be used (0.065 μS/cm), obtained from water purification system. All of the reagents will be with trace select purity, due to the traces analysis. Multi-element certificate standard will be used for the construction of the calibration curves for quantification of metals contents. Tuning solution internal standard solution will be use for correction of the drifts for external calibration curves.

e) Data processing

1) Basic statistic

The values obtained for the contents of the investigated elements will be statistically processed using basic descriptive statistics. Box–Cox transformation was used for data transformation. Bivariate statistic method was applied to check the data about the correlations between elements contents.

2) Multivariate statistic methods

Multivariate statistic methods (principal component analysis-PCA and factor analysis-FA) will be applied to reveal the associations of the chemical elements (Žibret and Šajn, 2010).

3) Biological distribution of metals from soil to plant/plant parts

Biological Concentration Factor (BCF) will be calculated as metal concentration ratio of plant roots to soil as given by Malik et al. (2010) and Yoon et al. (2006). Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root Li et al., (2007). Biological Accumulation Factor (BAF) present ratio of heavy metal in shoots to that in the soil (Li et al., 2007).

4) Averages daily intakes doses (ADD)

Additionally, the average daily intakes of both macro elements and trace elements (with special emphasis on heavy metals) will be calculated for the Macedonian and Chinese populations. During the exposure assessment stage, an average daily intake dose (ADD, μg/(kg·d)) will be use to quantify the oral exposure dosage for deleterious heavy metals. The daily intake amount of metals depends on both the heavy metal concentration and the amount of the respective food consumed. The ADD through consumption of food and soil will be calculated according to Song et al., (2015).

5) *Human Health Risk Assessment*

The hazard quotient (HQ) or hazard index (HI), a ratio of estimated exposure dose (ADD) and reference dose (RfD), characterizes the health risk of non-carcinogenic adverse effects due to exposure to toxicants (Song et al., 2015).

6) *Areal distribution*

The maps of heavy metal spatial distribution in plant foods and soil will be generated using the Kriging method with variogram interpolation. All of the maps will be extracted using Surfer soft. 8. (Golden Software, Inc. 809 14th Street, Golden, Colorado 80401-1866, USA).

Comparative analysis will be statistically proceeded in order to see the impacts of the heavy metals pollution in Macedonia vs. China.

Results and perspectives:

The obtained data will help in defining the zones with potential health risk of introducing heavy metals in the food-chain. Also, the results will help in findings tend to confirm the trend evidenced by studies carried out in other countries and which expressed concerns in particular for nutritive elements from one site and heavy metals from other site. Therefore studies aimed at the evaluation of the nutritional status of these two nutrients in well-identified segments of the Macedonian and Chinese population should be addressed. Therefore, greater attention should be paid to the potential health risks posed by the consumption of local plant food with high heavy metal concentrations and the ingestion/inhalation of contaminated soils around industrial/mining districts.

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