Macedonian - Chinese Scientific and Technological Cooperation

New Project Proposal for 2020-2021

Title of project proposal: **DEVELOPMENT OF SENSITIVE AND PRECISE BIO-INDICATIVE CHEMOMETRIC MODEL FOR CHARACTERIZATION OF CRITICALLY AFFECTED AREAS WITH AIRBORNE TOXINS**	**Project number**

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

Chinese organization: College of Information Engineering, Shenyang University of Chemical Technology, Shenyang, Liaoning Province, China.

Project period: 2020-2021

Expected period of stay in China for the Macedonian Researchers:
_4__ persons, _7__ days
From 07.06. 2020 to 13.06.2020

Expected period of stay in Macedonia for the Chinese Researchers:
_4__ persons, _7__ days
From 07.03. 2021 to 13.03.2021

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Project description:

GENERAL INTRODUCTION:

Anthropogenic and non-anthropogenic activities have increased throughout the world. The consequence is air pollution. Air pollution has created a lot of problems which have affected living and non-living things. To avoid or reduce these problems, constant monitoring of the air should be ensured. The conventional techniques is costly because it requires a lot of money and time consuming. Biomonitoring has been the alternative method. Moss, lichens and plants are biomonitors available to entrap air pollutants. However, different species of mosses have been used for biomonitoring in a number of different ways which may lead to rather different results, and some kind of classification seems necessary at this point. *Epigeic* mosses (growing on the ground) are preferred in the regional surveys in Europe. Trace elements may be absorbed on the moss from the atmosphere either as soluble chemical species in wet deposition or contained in particles from dry deposition. Part of the trace element content of particulates may eventually be released by weathering and reabsorbed by the moss. Whereas uptake efficiencies for particulate-bound trace elements are generally poorly known, Ions may be subject to active uptake into cells or attached on the moss surface by physical and chemical forces. Methods are available to distinguish between intracellular and surface-bound fractions of elements. Main problem with issue moss-biomonitoring are reveal as: a) transport of soluble compounds from the soil into moss tissue, particularly during periods withexcessive soil/water contact. Although mosses do not have a root system, influence from this source cannot be disregarded, in praticular in areas with low atmospheric deposition and b) windblown mineral dust from local soil.

As far as the surface bound fraction is concerned, little is known about *the* ***binding mechanisms****,* but the fact that different metals show rather large differences in their ***retention capacitie***, indicates that both simple ***cation exchange*** on negative surface charges and ***complex formation with ligands*** on the moss surface are involved. Furthermore, the last two decades in developed countries mosses has been increasingly tested as bio-filters for airborne toxins, with emphasis for the xenobiotic's molecules and heavy melas.

Air pollution is significant environmental issue in both countries (Republic of North Macedonia and Republic of China). For that purpose, the main idea of the project is enchasing and cost effective and bio-indicative chemometric tool for precise and sensitive monitoring of air pollution. The second goal of this project is introducing bryophytes as "green filters" for short-term air-pollution fighters. Furthermore, the interchangeable moss transplants from both countries will be improving on both geographical regions (Europe-Asia).

RESEARCH PROJECT ACTIVITIES:

1. Identification and determination of dominant moss samples distributed in the Republic of North Macedonia and Republic of China.
2. Chemical characterization of the moss species distributed in both countries (tracing the species with lower background chemical composition), using chromatographic tecniques for organic compounds and mass spectroscopy with ICP and ion-chromatography for inorganic compounds.
3. Application of moss-bag technique for monitoring air-pollution across selected critically affected areas in both countries.
4. Creation of data base for pollution index using the interchange moss transplants R. North Macedonia and R. China. (selected representative moss transplants will be transfer from North Macedonia to China and opposite, furthermore the species will be tested and examine for their hazards accumulation ability.
5. Moss tissue examination – dominant surface bio-chemo-physic processes: using scanning electron microscopy.
6. Generating moss transplants base for further biotechnology with these plants.

7. Methodological standardization for exposure conditions: effect of height and exposure time.
8. Moss-bags validation vs. current state-of-the-art methods for air pollution monitoring
9. Validation will be performed in several different regions, at industrial, urban, and unpolluted environments.
10. To develop a method and perform an initial validation of its usefulness for the detection of atmospheric small-scale pollution focus using moss samples.

ANALYTICAL PERFORMANCE:

The scientific research centers from both countries/universities (www.ugd.edu.mk and www.syuct.edu.cn) will be involved in order to establish the project main goals.

Trace and rare-earth element analysis

Quadrupole-inductively coupled plasma with mass spectrometry (Q-ICP-MS) will be apllied in order od elemental analysis of moss tissue. ICP-MS is a powerful tool for elemental analysis, providing LODs for more than 70 elements at low concentrations,typically parts per billion or trillion (ppb or ppt). Conventional ICP-MS systems cannot provide the lowest LODs, because of the polyatomic interferences that can enhance the background signal or overlap the signals of the most abundant isotopes. The use of a DRC can remove unwanted interferences by creating specific chemical reactions with a supplementary gas, which improves the selectivity and the sensitivity dramatically. For the present investigation the Q-ICP-MS instrument model 7500cx, Agilent Technologies, at UNILAB, Faculty of Agriculture, Goce Delcev University-Stip, R. Macedonia: www.unilab.ugd.edu.mk .

Characterization of organic composition

High-performance liquid chromatography (HPLC) and gas chromatography with mass spectrometry (GC-MS) will be apllied for determination of the organic base of the moss tissue. Chromatographic data will improve the posibility of using moss samples for bioindication of certain xenobiotics. Spetial atention will be given to organic airborne toxins.

Scan analysis

To better understand the factors leading to metal binding in complex organic matrix and trace metal fate, electron microscopic characterization of samples will be conducted. Scanning electron microscopy can revealed the presence of trace metal specific incorporation in organic matrix in close association with biological structures with morphologies consistent. For the present investigation, scanning electron microscope model Tescan Vega3-LMU, will be used in order of characterization of the morphology, structure, and metal binding mechanisms. Furthermore, another approach will be introduced. Preliminary investigation have been implemented for improving this technique for monitoring of surface dust physical **transport.**

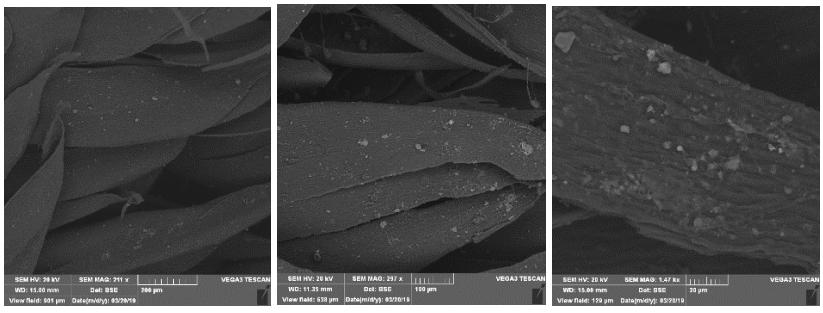


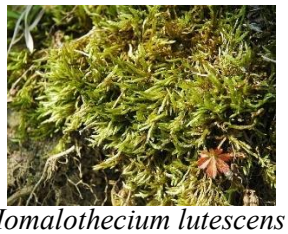
Image: Balabanova B, Lazarova M, 2019 (Preliminary investigation for generating main issues of the project proposal).

MOSS TRANSPLANTS BASE – collecting moss samples

Moss samples will be collected according to the adopted European protocol (http://icpvegetation.ceh.ac.uk/) also given in Fernandez et al., 2017 [1]. After the moss samples were

collected, dry cleaning has been involved in order to reduce as it possible the contamination from soil surface dust particles and other plant species. Both moss species previously were improved for interchange use in air pollution studies [2] The investigation will be started with validation on two moss samples two species (*Homalothecium lutescens* and *Homalothecium sericeum*) from the genus *Homalothecium*. These both samples have been previously used in several biomonitoring programs.

Homalothecium sericeum (Hedw.)
Image: Balabanova B, 2019



Homalothecium lutescens (Hedw.)
Image: Balabanova B, 2019

Transplants base will be generated in the laboratories of the both Universities and for each moss transplant species will be tested in vitro and in vivo. The research groups from both Universities will test the moss samples in vivo at selected polluted areas. Further more comparative analysis will be done between the both countries. Non-polluted area will also be included in order to calibrate the data which will be obtained for the critically polluted sites.
Despite this start-up species from the genus *Homalothecium*., various moss species will be examined separately in China and in Republic of North Macedonia, including specious from the genus *Sphagnum, Hypnum and Pleurosium.*

DETERMINATION OF DUST PARTICLE SURFACE TRAPING in moss tissue

Control moss samples will be tested for dust accumulation and scan analysis will help in better understanding of the physical processes of the phenomena of "moss green tables", which is a new trend in green environment brought in to the air polluted zones. Novel and much more effective model will be tested in order to improve the moss carpet use as bioindication filter in air polluted zones. Moss transplants will be transfer to nylon mosquito net with porosity of 2 mm. Spatial The "moss tables" will be monitor in vivo and in vitro (laboratory camber where the dust will be introducing in sequenced intervals and within controlled conditions). In vivo, two location in each country will be selected for testing the "moss tables" (1st location will be the control locality where air pollution is very low and monitored through the year; and 2nd location is extremely polluted area, already improved with significant air pollution in certain part of the year).

MOSS BAG TECHNIQUE

Moss bags will be prepared as follows: nylon mosquito net, with a mesh of approximately 2 mm, will be cut cut in pieces of a 12 × 12 cm, where 5 g of moss prepared material (model organisms) will be inserted and closed with a nylon thread. At each exposure site, the bags of each moss (in two replicates) will be exposed approximately 2 m above the ground for 4-6 weeks, in both years. The location will be carefully selected in order to obtain several exposure identification (industrial zone, heavy traffic zones, mining areas).

DATA PROCCESING

Moss accumulation index

To assess the accumulation of each toxin by moss species, relative accumulation factors (RAF) will be calculated using Equation (1):

$$RAF = (C_{exposed} - C_{initial})/C_{initial} \quad (1)$$

where $C_{exposed}$ is the content of each element after exposure and $C_{initial}$ is the content before exposure. Contaminations factors (CF), used to assess the degree of anthropogenic influence, will be calculated using Equation (2):

$$CF = C_{moss}/C_{background} \quad (2)$$

Artificial Neural Networks (ANN)

ANN are defined as structures comprising densely interconnected adaptive simple processing elements, called artificial neurons (or nodes), which can perform massively parallel computations for data processing and knowledge representation .

Classification and Regression Trees (CARTs)

CARTs is a powerful and flexible classification tool. It handles both ordered and categorical predictor variables. The final classification rule has a simple form, which is easy to interpret and to use for future classification. CART takes into account the fact that different relationships may hold between variables in different parts of the data. It does automatic stepwise variable selection and calculates the importance rank of the variables. CART calculates misclassification error estimated by both re-substitution and cross-validation .

Linear Discriminant Analysis (LDA)

LDA is probably the most frequently used supervised pattern-recognition method and the most studied one. LDA is based on the determination of linear discriminant functions, which maximize the ratio of between-class variance and minimize the ratio of within-class variance. In LDA, classes are supposed to follow a multivariate normal distribution and be linearly separated. LDA can be considered, as PCA, a feature-reduction method in the sense that both, LDA and PCA, determine a smaller dimension hyperplane on which the points will be projected from the higher dimension .

Cluster Analysis (CA)

CA or clustering is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar (in some sense or another) to each other than to those in other clusters. In this way, we categorize the samples in order to succeed with discrimination.

Principal Component Analysis (PCA)

PCA is a technique that, by the reduction of the data dimensionality, allows their visualization, while retaining as much as possible of the information present in the original data. So, PCA transforms the original measured variables into new uncorrelated variables called principal components (PCs). Each PC is a linear combination of the original measured variables. This technique affords a group of orthogonal axes that represent the directions of greatest variance in the data. The first PC (PC1) accounts for the maximum of the total variance, the second (PC2) is uncorrelated with the first and accounts for the maximum of the residual variance, and so on, until the total variance is accounted for .

Analysis of Variance (ANOVA)

ANOVA is a collection of statistical models, and their associated procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes t-test to more than two groups. Performing multiple two-sample t-tests would result in an increased chance of committing a type I error. For this reason, ANOVA is useful in comparing two, three, or more means and has been used to compare elemental profiles of foods of different origin.

COORDINATION MEETINGS AND TRANSFER OF KNOWLEDGE

One time events (Two coordinative meetings) will be organized in order to gather the reasearhers teams from the both countries.

- ***1st Coordinative project meeting***, which will be organized at "Goce Delcev" University, Shtip, North Macedonia (June 2020). Both research teams from North Macedonia and China will meet to organize the planed activities, manage the human resources and laboratory activities. The main goal of the

meeting will be: creating valuable, suitable and accurate chemometric models for predicting and measuring the contamination level in air and creating a regional and global scale of experience for determining the anomalous parts of the Environment. Furthermore, the general conclusions will be presented to the students as plenary lectures to the students from the University, at the **Students workshop day*.**

- ***2nd Coordinative project meeting***, which will be organized at Shenyang University for Chemical Technolgies in Shenyang in China (March 2021). Both research teams from North Macedonia and China will meet to summarize the data base to compare the obtained research data, and to organize the promotion of the obtained research results. The general outcome from the coordinative meeting will be proposing **new ideas for national and regional strategies for Environmental protection.**

TRANSFER OF KNOWLEDGE - ***Scientific Student conference and workshop days*** for enclosing the transfer of the knowledge to the youth. The summery conclusions (as brochure, book of abstracts and scientific monography) shall be promoted after enclosing the one-time events.

TIMEFRAME OF THE PROPOSED PROJECT ACTIVITIES
The proposed project activities will be organized in six trimesters in the time-frame of two years.

Project activity	Jan.-March 2020	April-June 2020	Sep.-Dec. 2020	Jan.-March 2021	April-June 2021	Sep.-Dec. 2021
Field-work: preparation sampling strategy, moss samples collection (2nd half of March)						
Determination of moss samples, laboratory analysis, preparation of moss bag, preparation of moss tables and *in vitro* analysis						
1st Coordinative project meeting, dissemination of data results and project activities						
1st Student workshop days (2 days event)						
Installation of moss bag, and *moss tables in vivo* analysis						
Generating annual report of the project activities						
Data processing, preparing scientific papers for publishing						
2nd Coordinative project meeting dissemination of data results and project activities						
2st Student workshop days (2 days event)						
Presenting research data summary at scientific symposiums and conferences						
Generating final report						

References

[1] Fernández JA, Boquete MT, Carballeira A, Aboal JR (2015) Sci. Total. Environ. 517:132-150
[2] Balabanova B, Stafilov T, Sajn R, Baceva K (2017) J. Environ. Sci. Health, Part A. 52(3): 290-301

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