In this exercise, you will:

- Learn how to make a method for GC-MS analysis
- Analyze GC chromatograms and MS spectra
- Make a calibration curve for quantification
- Calculate the concentration of analyte
- Gain experience in GC-MS usage
Humic substances represent a major fraction of the natural organic compounds in the environment. One of characteristics of HS is their heterogeneity in terms of elemental composition, molecular size distribution and chemical functionality. Bearing in mind that humic substances can derive from any organic material, including plant and animal debris, bio-wastes and meiofauna, their heterogeneity is obvious. The elemental composition of HS is 40–60 % carbon, 30–50 % oxygen, 1–3 % nitrogen and 0.1–2 % sulfur. HS contain many functional groups such as carboxylic, alcoholic, phenolic, amino, amide and sulfhydryl groups. Oxygen functional groups are the most abundant groups in HS macromolecules. Due to the acidity of these groups, such as carboxyl and hydroxyl groups, the acid–base behavior of humic acids, an operational fraction of HS, play an important role in the acid–base balance of natural waters. The study of the pH behavior of humic acid is very significant, because the complexation of metal cations and binding of different xenobiotics, such as polycyclic aromatic hydrocarbons, pesticides etc., with humic acids is pH-dependent.
Interaction between humic substances and pesticide

Many physical, chemical, and geochemical reactions of pollutants and pesticides in natural environments are strongly governed and affected by humic compounds. The persistence of pesticides and their subsequent behavior are dependent upon the nature and the amount of humic compounds, the physico-chemical properties of the pesticides, and the environmental conditions of reaction media. Humic compounds contain a variety of functional groups, such as aliphatic and aromatic carboxyls, phenolic hydroxyls, alcoholic hydroxyls, carbonyls, quinones, methoxyls, and amino groups. These functional groups are active sites in the humic molecules. Because of the large exchange capacity of humic compounds, exchange reactions play an important role in the adsorption of pesticides. The extent of adsorption increases directly with an increase in the organic matter content.
Gas chromatography – mass spectrometry

Sample that should be analyzed by GC-MS is common as liquid solution. During the transfer into the GC, the sample is volatilized by rapid exposure to a zone kept at relatively high temperature (200-300°C) and mixed with a stream of carrier gas (Ar, He, N₂, or H₂). The resulting gaseous mixture enters the separation section, a chromatographic column. Chromatographic column is a fused-silica tubular capillary coated internally with a thin polymer film. Upon their displacement through the column, analyte molecules are partitioned between the gas carrier stream (mobile phase) and the polymer coating (stationary phase), to an extent which depends mainly on their chemical structure.

At the end of the separation section, the molecules reach a detection system in which a specific physical property (thermal conductivity) or a physico-chemical process (ionization in a flame, electron capture) gives rise to an electric signal which is proportional to the amount of molecules of the same identity. A data system permits to process these data to produce a graph of the variation of this detector signal with time (chromatogram).
Gas chromatography – mass spectrometry

Thus, four principal sections are distinguishable in the chromatograph: introduction (injector), separation (chromatographic column), detection, and data handling units. Each section has its own function and its responsibility for the quality of the analysis and the results obtained. The injection system, for example, should ideally transfer the sample to the column quantitatively, without discrimination on molecular weights or volatility, and without chemical alteration (decomposition or isomerization). It is a critical step, especially for quantitative analysis. For correct GC operation, among other conditions, this gateway to the column should remain unpolluted, clean, inert, and leak-free. The main requirement for an analyte in GC is that it should be volatile enough to be present in detectable amounts in the mobile phase. Substances with low vapor pressure will not enter the chromatographic column, will accumulate at the injection system, and may eventually clog its conduits. Very polar, thermolabile, ionic and high-molecular weight compounds are not compatible with regular GC analysis. Depending on the molecular structure of the analyte and the functional groups available, it is possible in some cases to obtain a chemical derivative which has a higher vapor pressure and is therefore more amenable to GC analysis.
Material

For this lab exercise, you will need the following chemical substances:

- Pesticide (pirimicarb), 🟢
- Ethyl-acetate (HPLC grade), 🟢⚠️⚠️
- Quinione ⚠️

For this lab exercise, you will need the following laboratory equipment:

- Vials (8 mL)
- Vials (2 mL)
- Automatic pipette
- Analytical balance (±0.0001 g)
Procedure:

I. Calibration standards: Prepare a set of pririmicarb solutions containing 2, 4, 6, 8, and 10 μg/mL in ethyl acetate.

II. Internal standard solution: Prepare the solution of quinone at 1 μg/mL in ethyl acetate.

III. Make the method for GC-MS analysis (The oven temperature was programmed from 65 °C (holding time 1 min) to 220 °C (1 min) at rate of 20 °C min-1, then to 280 °C at rate of 5 °C min-1 (4 min). Volume of 1 μL was injected in the splitless mode. Helium was the carrier gas (1.0 ml min-1) and the inlet temperature was 250 °C. The operating temperature of the MSD was 280°C with the emission energy of 70 eV. The MSD was used in the single ion-monitoring (SIM) mode.)

IV. Analyse standard solutions and make a quantification method with calibration curve

V. Unknowns: Analyze the sample solution and determine unknown concentration

VI. You will need to export chromatograms in order to present them using Origin program.
### DESCRIPTION OF REMOTE ACCESS

#### 1. NETHEM COMMUNICATION SIDES

<table>
<thead>
<tr>
<th>Role</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host side</td>
<td>participant’s PC in classroom</td>
</tr>
<tr>
<td>Guest side</td>
<td>participant’s PC in laboratory</td>
</tr>
</tbody>
</table>

**NOTE:** NETHEM Communication is defined as event that involves all kinds of internet interactions (in real-time and not in real time) between participants via devices (PCs, laptops, tablets and mobile phones).

#### 2. COMMUNICATION SOFTWARE

| TeamViewer          | Meeting: No  
|                     | Remote control: Yes  
|                     | Meeting and Remote control simultaneously: No  
| Skype               | Call 1:1: Yes  
|                     | Conference Call: No  

#### 3. COMMUNICATION HARDWARE

| Host side | 1 PC for each participant, headset, microphone, camera  
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Guest side</td>
<td>1 PC for participant, headset, microphone, camera</td>
</tr>
</tbody>
</table>

#### 4. INFORMATION EXCHANGE TYPE

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational</td>
<td>(one side is dominantly receptive)</td>
</tr>
</tbody>
</table>
|                       | Place of Educator: participant; guest side  
|                       | Number of educator(s): 1  
|                       | Place of student: participant; host side  
|                       | Number of student participant(s): up to 5  
| Consultative          | (two sides are equal in giving-receiving information)                       |
|                       | Number of host side participants: /  
|                       | Number of guest side participant(s): /  

This project has been funded with support from the European Commission. This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.
Remote Access Connection Instructions

What makes these labs different and unique from other classroom experiments is that we have incorporated a section in each activity to remotely characterize your samples from your classroom.

Request a remote lab session specifying information such as: the day, the time, and the instrument you are interested in using by visiting our web site:

http://netchem.ac.rs/remote-access

You will see the list of partners with the instruments provided to chose from.
You will be contacted by a Remote Access staff member to set up a test run to ensure you are set up properly and have the required infrastructure.
Send samples or verify the in-house sample you would like us to prepare and load for characterization.
Send your samples to the Remote Access center that you chose on your request.
There are two communications soft-ware packages, that will allow us to communicate instructions and answer questions during the session.

- TeamViewer: You can obtain a free download at:
- Skype

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Remote Access Connection Instructions

You will need:

a) Computer with administrator access to install plug-ins and software
b) An internet connection
c) Speakers
d) Microphone
e) Projector connected to the same computer
f) Web browser (Firefox preferred)

During the test run you can refer to this guide to perform the following steps, but it’s very important that you only proceed with these steps during your scheduled times. You may interfere with other remote sessions and potentially damage equipment if you log in at other times.

a) Open and logon to your Zoom/Team-viewer account. You will be given the access code to enter at the time of your test and then again during the remote session.
   ▪ If you are using the Zoom software, Remote Access staff will give you the access code.
   ▪ If you are using the Team-viewer software, Remote Access staff will give you the ID & password.

b) You should soon see the Remote Access desktop and at this point you can interact with the icons on the screen as if it were your desktop.

c) Switch to full screen mode by selecting the maximize screen option in the top right corner of the screen.

d) Upon completion of the session, move your mouse to the top right corner of the screen, and click on the X to disconnect the remote session. It will ask if you want to end the remote session. Click Yes.
Author, Editor and Referee References

This remote access laboratory was created thanks to work done primarily at University of Niš.

Contributors to this material were: _______Ivana Kostić___________

Refereeing of this material was done by: _____________________

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Please contact a NETCHEM representatives at your institution or visit our website for an expanded contact list.

The work included had been led by the NETCHEM staff at your institution.