POSTER COMMUNICATIONS

Use of Wild Edible Plants as Environmental Indicators and as Ingredients for the Creation of new Functional and Enriched Products

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The main research activities planned in the first two years of the PhD project have been completed. Firstly, two wild edible plants (WEPs) species (*M. sylvestris, F. vulgare*) and different sampling places were identified. Amounts of major bioactive compounds (tocols, carotenes, xanthophylls, riboflavin and thiamine) were determinedand two suitable analytical procedures were used for the determination of polycyclic aromatic hydrocarbons (PAHs) and heavy metals. In addition, preliminary analyses were conducted to assess the contribution of anthropogenic sources on the contamination levels of the areas chosen for sampling.

Utilizzo delle piante selvatiche commestibili come indicatori ambientali e come ingredienti per la preparazione di nuovi prodotti funzionali e arricchiti

Le principali attività di ricerca previste nei primi due anni di dottorato sono state completate. In primo luogo, sono state identificate due specie di piante selvatiche commestibili (*M. sylvestris*e *F. vulgare*) e diverse località per il campionamento. Sono state determinate le quantità presenti dei principali composti bioattivi (tocoli, caroteni, xantofille, riboflavina e tiamina) e, parallelamente, sono state utilizzate due procedure analitiche idonee per la determinazione degli idrocarburi policiclici aromatici (IPA) e dei metalli pesanti. Inoltre, sono state condotte analisi preliminari per valutare il contributo delle sorgenti antropiche sui livelli di contaminazione delle aree scelte per il campionamento.

**Key words**: Wild edible plants, bioactive compounds, vitamins, environmental pollution, PAHs, health.

# **1. Introduction**

In accordance with the PhD thesis project previously described (Ianiri G, 2022), this poster reports the results of the main research activities planned in the first two years of the PhD project concerning:

(A4) the set up of analytical procedures suitable for the analysis of polycyclic aromatic hydrocarbons and heavy metals in WEPs under study;

(A5) the sampling activities, which were carried out choosing three places with different anthropic impact, specifically Rotello, Termoli and Rome;

(A6) the realization of preliminary analyses for the quantitative determination of bioactive compounds and of PAHs and heavy metals.

# **2. Materials and Methods**

As it regards the determination of tocols, carotenes, xanthophylls, riboflavin and thiamin, methods already developed by (Panfili G, 2003, 2004)were used. The materials and methods used for the determination of polycyclic aromatic hydrocarbons and heavy metals are briefly described. The analytical protocol for the determination of PAHs was divided into three steps. In the first step, 2 grams of fresh WEPs leaves were placed in a beaker to which 30 mL of high-purity cyclohexane was added to minimize interference. Extraction was performed in an ultrasonic bath for 30 min. Finally, the extract was filtered on filter paper and collected in an Erlenmeyer flask to avoid exposure to light. In the second step, the extract was concentrated to a volume of about 5 mL through a rotary evaporator. The concentrate was then passed over a column containing anhydrous sodium sulfate. Finally, the extract was reduced to a volume of 100 μL using a gentle stream of purified nitrogen. The last step involves analysing the extract by injecting 1μL into the single quadrupole gas chromatography mass spectrometry (6890-GC/5973-MSD) system. For the determination of heavy metals, mineralization of organic matter was carried out for each sample. To 2 grams of freeze-dried sample, 10-15 mL of a mixture of nitric acid and sulphuric acid 1:3 v/v was added and then samples were placed on a hot plate for 1 hr. The whole samples were then brought to a final volume of 50 mL and analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

# **3. Results and Discussion**

Table 1 shows the content of bioactive compounds in *M. sylvestris* and *F. vulgare* sampled in Rotello and Termoli.Detected carotenoids included lutein, zeaxanthin, violaxanthin, neoxanthin, β-cryptoxanthin, anteraxanthin, α-carotene, 13-cis- β-carotene, β-carotene and 9-cis-β-carotene. Detected tocols were α-Tocopherol, β-Tocopherol, γ-Tocopherol~~.~~

***Table 1*** *Contents of total carotenoids and total tocols (mg/100g of fresh weight) with respective standard deviations (SD) (n=3). Contents of riboflavin and thiamine (mg/kg of fresh weight )with respective SD (n=3).*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **M. sylvestris****Rotello** | **M. sylvestris****Termoli** | **F. vulgare Rotello** | **F. vulgare Termoli** |
| **Total Carotenoids** | 31.70 ± 4.20 | 43.00 ± 2.20 | 27.30 ± 2.20 | 13.30 ± 0.30 |
| **Total Tocols** | 3.90 ± 0.50 | 9.50 ± 2.50 | 3.70 ± 1.80 | 2.20 ± 0.01 |
| **Riboflavin** | 0.46 ± 0.01 | 0.85 ± 0.23 | 0.90 ± 0.03 | 0.38 ± 0.02 |
| **Tiamin** | 62.47± 27.12 | 56.73 ± 0.51 | 16.01± 6.73 | 7.25 ± 0.01 |

From the data shown in Table 1, the amounts of vitamin A, as Retinol Equivalent (RE in µg/100g) and vitamin E, as Tocopherol Equivalent (TE in mg/100g) were calculated. Given the Recommended Daily Allowance (RDA) reported in Annex XIII, part A, of EU Regulation 1169/2011, the percentages of the RDA provided by the consumption of 100 grams of *M. sylvestris* and *F. vulgare* were calculated (Table 2).

***Table 2*** *Percentage of the RDA covered by the intake of 100 grams of fresh M. sylvestris and F. vulgare.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **M.sylvestris Rot.** | **M.sylvestris Ter.** | **F. vulgare Rot.** | **F. vulgare Ter.** |
| **Vitamin A** | 144 | 279 | 147 | 93 |
| **VitaminE** | 31 | 74 | 28 | 17 |
| **Riboflavin** | 3 | 6 | 7 | 3 |
| **Tiamin** | 568 | 516 | 145 | 66 |

The consumption of 100 grams of *M. sylvestris* and *F. vulgare* sampled in Rotello and Termoli largely covered the RDA of vitamin A, vitamin E, and thiamine. On the other hand, for riboflavin, the amounts of all samples did not reach the recommended daily dose. Given the high amounts of bioactive compounds of the two analysed WEPs, new food formulations will be developed to realise products intended to fulfil nutritional deficiencies and, in general, to provide health benefits for consumers.Given the spontaneous nature of WEPs, they can contain multiple organic and inorganic micro-pollutants that are extremely harmful to health (Terzi M, 2013). Before using WEPs in new food formulations, it is, therefore, necessary to evaluate the levels of the main contaminants potentially present, such as PAHs and heavy metals. This activity can also be used to establish the influence of anthropogenic sources on the levels and profiles of PAH and metal contamination in wild plants. This consequently could allow the possibility of using wild plants as environmental indicators. Table 3 shows the levels of the determined heavy metals and the sum of the polycyclic aromatic hydrocarbons of *M. sylvestris* sampled in Rotello and Rome. Among the PAHs determined is benzo[a]pyrene, the target molecule used for carcinogenic risk assessments. The Low Detection Limits (LODs) of Pb, Cd and Co were respectively 0.2, 0.005 and 0.1 µg/kg.

***Table 3*** *Heavy metals and PAHs concentrations ± SD in µg/Kg of fresh weight in M. Sylvestris leaves.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Pb** | **Cd** | **Cr** | **Co** | **Cu** | **Total PAHs** |
| **M.sylvestris Rome** | 1,94 ± 0,02 | 0,01 ± 0,00 | 0,73 ± 0,02 | 0,55 ± 0,07 | 21,34 ± 1,36 | 550 ± 1 |
| **M.sylvestris Rotello** | <LOD | <LOD | 0,25 ± 0,09 | <LOD | 1,69 ± 0,09 | 297 ± 2 |

Rotello and Rome were chosen to highlight the contribution that a large urban center, like the city of Rome, has on the state of contamination of wild plants. In fact, this is marked both for the metal component and for the PAHs. The aim is to make the population aware of the risk of collecting wild plants that grow, or are located, close to emission sources, or in territorial contexts characterized by the presence of numerous anthropic activities. Further activities related to the use of WEPs as environmental indicators will be carried out during the third year of the PhD course.

# **4. References**

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