Quantification of oligo-elements and heavy metals in the fruits (pods and seeds) of the introduced tree Gleditsia triacanthos L. in Algeria

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ABSTRACT

Gleditsia triacanthos L. is a moderately fast growing tree. It was used like feedstock since it can provide a source of proteins and metabolic energy. The tree was introduced in Algeria since 1949 by the French colonists.

In order to valorize the natural substances of this introduced species, the coupled plasma mass spectrometry (ICP-MS) was employed for the determination of essential and nonessential elements in both seeds and pods.

The results showed that the quantification of heavy metals using the ICP-MS method showed that the fruits of G. triacanthos L. are very rich on potassium (178,68± 7,31mg.kg⁻¹ for seeds and 164,27± 7,78mg.kg⁻¹ for pods), phosphorus (75,027± 4,17 mg.kg⁻¹ for seeds and 13,06± 0,16 mg.kg⁻¹ for pods) and calcium (58,36± 15,66mg.kg⁻¹ for seeds and 60,64± 4,52 mg.kg⁻¹ for pods). The concentrations of oligo-elements and heavy metals in both seeds and pods decreased in the following order: K>P>Ca>Mg>Na>Fe>Si>Zn>Mn>Al>Cu>Cd>Pb>As>Cr.

It arises that the fruits are rich in oligo-elements and the concentrations of heavy metals is lower than the normal range which confer a great interest for many industries.

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Introduction
Honey locust (G. triacanthos L.) is a woody species (genus Gleditsia) in Leguminosae family, native to North America. This species can tolerate a wide range of climatic and soil conditions [1] and is spread in America, Middle Europe and Mediterranean countries [2]. It is drought resistant, light lover, thorny and show moderate to fast growth speed [3]. It is considered by numerous authors [4] as common invader. Although, G. triacanthos L. can be competitive for the native species and can form an ecologic danger. Its seeds are composed of the testa (27%), the embryo (29%) and the endosperm (34%) [5]. The pods are edible and can be used as vegetable or fermented to produce alcoholic or non-alcoholic beverage. Pods and foliage are valuable fodder for all classes of livestock [1]. The honey locust tree flowers are attractive sources of nectar for bees and, thus, good honey source. The trunk, pods, and bark are also used in many ethno-medicines [1].

There is a growing interest for the mineral content and nutrition of higher plants and their importance in agriculture, foods and Human health. Experiments in cell culture and in intact organisms reveal the importance of trace elements in many metabolic processes and functions throughout the life cycle. Human as well as animal studies originally showed that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine could reduce individual risk factors, including those related to cardiovascular disease [6]. These heavy metal ions are also essential micronutrients for plant metabolism but when present in excess, these, and non-essential metals such as Cd, Hg, Ag and Pb, can become extremely toxic [7].

Although the efficacy of plants for curative purposes is often accounted for in terms of its organic constituents, many studies showed that intakes of mineral elements could reduce the risk factor of individuals. It is cited that the main function of iron (Fe) is to promote oxygen transport (hemoglobin), electron transport (cytochromes), muscle metabolism and immunity, manganese (Mn) is an enzyme activator: hydrolase, kinases, carboxylases and transferases and zinc (Zn) is used in immunity, endocrinology (insulin, thyroid, etc.), and antioxidant and night vision. Copper (Cu) is used in electron transfer, antioxidant, superoxide, dismutase, etc. and cobalt (Co) is a component of vitamin B12. Chromium (Cr) is a component of glucose tolerance factor, metabolism of fat. Molybdenum (Mo) is used in the metabolism of sulphur/sulphide oxidase, uric acid metabolism (xanthine oxidase). Selenium (Se) as an antioxidant: glutathione peroxidase, thyroid metabolism (deiodinase) and liver. Fluorine in the structure of enamel and bone. Iodine a component of thyroid hormones, as they can play other important roles in alimentary systems, sources of macro and trace elements [8].

As in many other countries over the world, the investigation of heavy metals in herbal products is widely interested. The organic compositions and accompanied inorganic elements in several herbal products have been well determined using different advanced analytical techniques. Among them, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) proved to be the technique of choice to analyze and determine a large number of heavy metals in a wide range of samples.

The aim of this work was carried out in order to quantify the oligo-elements and heavy metals in Gleditsia triacanthos L. fruits (seeds and pods separately) growing under semi-arid conditions, using the ICP-MS technic.

Materials and Methods
The pods of G. triacanthos L. were collected from the university of Djillali Liabes (ITMA) Sidi Bel Abbes during November 2014. The pods were dried in the shade during 3 months. The seeds were separated manually then crushed separately. The resulting flour was preserved in glass bottles, safe from the light for further use.

The analysis of the samples was realized by metallic dosage in the obtained solution by using inductively coupled plasma mass spectrometry (Jobin-Yvon 70 ICP) ULTIMA AND JY70. A small quantity (0.5 g) of dry matter was mineralized with 2mL of sulphuric acid (H2SO4), 6mL of nitric acid (HNO3) and 6mL of oxygenated water (H2O2). This mixture was heated for 30 min. The mineral deposit was cooled and filtered by Whatmanashless filter and then supplemented to 25mL of 0.1M HNO3. All procedures of handling were carried out without contact with metals, to prevent cross-contaminations. All experiments were carried out in triplicate.

Data Analysis
The results were expressed as mean ± standard deviation (s.d.; n=3). SPSS software (version19.0) was used for the statistical analysis. One-Way Analysis of variance (ANOVA) was performed to test significant differences between seeds and pods composition. The correlations were investigated using a bivariate Pearson correlation method at P<0.05 and P<0.01. Finally the Principal Components Analysis (PCA) was carried out on the data set of oligo-elements and heavy metal.

Result and Discussion
Inductively coupled plasma mass spectrophotometry (ICP-MS) has become a popular technic in the multi element
analysis since the first commercial instrument became available in 1980s. Semi quantitative analysis by ICP-MS has proven to be a powerful tool for fast screening, in addition, it does not require the element of interest to be present in the calibration standard [9], making it especially useful for the analysis of unknown samples.

In this study, the analysis of samples was carried out by using ICP-MS technic. The concentration of oligo-elements and heavy metals was determined with different mode equipment to compare results in seeds and pods of *G. triacanthos* L.

The concentrations of oligo-elements and heavy metals in both seeds and pods decreased in the following order (Tab.1): K>P>Ca>Mg>Na>Fe>Si>Zn>Mn>Al>Cd>Pb>As>Cr. The potassium is the major compound with 178.68mg.kg⁻¹ and 164.27mg.kg⁻¹ respectively for seeds and pods. In plants, this element is an essential macronutrient composing up to 10% of the plant dry weight [10]. It plays a vital role in a wide range of biophysical and biochemical processes. It fulfills a number of important functions related to enzyme activation, as well as the neutralization of negative charges, the maintenance of cell turgor, plant growth and organ movement [11].

Table 1: Total concentration of oligo-elements and heavy metals in honey locust (*G. triacanthos* L.) compared to the normal range in plants cited by different authors.

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Seeds (mg.kg⁻¹)</th>
<th>Pods (mg.kg⁻¹)</th>
<th>Normal range in plants (mg.kg⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>0.39± 0.27</td>
<td>0.54± 0.04</td>
<td>200–≥1000</td>
<td>[12]</td>
</tr>
<tr>
<td>As</td>
<td>0.047± 0.006</td>
<td>0.036± 0.005</td>
<td>5</td>
<td>[13]</td>
</tr>
<tr>
<td>Ca</td>
<td>58.36± 15.66</td>
<td>60.64± 4.52</td>
<td>1830.2 – 2042.5</td>
<td>[14]</td>
</tr>
<tr>
<td>Cd</td>
<td>0.071± 0.000</td>
<td>0.072± 0.002</td>
<td>2</td>
<td>[15]</td>
</tr>
<tr>
<td>Cr</td>
<td>0.037± 0.011</td>
<td>0.12± 0.018</td>
<td>0.006 – 18</td>
<td>[16]</td>
</tr>
<tr>
<td>Cu</td>
<td>0.09± 0.007</td>
<td>0.0036± 0.000</td>
<td>0.4 – 45.8</td>
<td>[17]</td>
</tr>
<tr>
<td>Fe</td>
<td>1.294±0.436</td>
<td>0.79± 0.11</td>
<td>640 – 2486</td>
<td>[18]</td>
</tr>
<tr>
<td>K</td>
<td>178.68± 7.31</td>
<td>164.27± 7.78</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Mg</td>
<td>25.57± 3.78</td>
<td>6.36± 0.049</td>
<td>0.73 – 1.41</td>
<td>[19]</td>
</tr>
<tr>
<td>Mn</td>
<td>0.615± 0.036</td>
<td>0.136± 0.004</td>
<td>15 – 100</td>
<td>[20]</td>
</tr>
<tr>
<td>Na</td>
<td>12.06± 10.80</td>
<td>2.25± 0.46</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>P</td>
<td>75.02± 4.17</td>
<td>13.06± 0.16</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Pb</td>
<td>0.06± 0.02</td>
<td>0.09± 0.01</td>
<td>3</td>
<td>[21]</td>
</tr>
<tr>
<td>Si</td>
<td>1.15± 0.67</td>
<td>1.37± 0.31</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Zn</td>
<td>0.63± 0.10</td>
<td>0.12± 0.04</td>
<td>1 – 160</td>
<td>[17]</td>
</tr>
</tbody>
</table>

Phosphorus is the second most abundant elements in seeds (75.027mg.kg⁻¹); succeed by Calcium, Magnesium and Sodium. Phosphorus (P) is an essential part of the process of photosynthesis and involved in the formation of all oils, sugars, starches, etc [22].

Another essential element for plants is calcium whose role has been well documented [23]. This earth alkali metal has an important role in plant physiology, including involvement in the responses to stress, and controls numerous processes and its availability is essential in the biochemistry of plants. The result (Tab.1) shows that *G. triacanthos* seeds contain 58.36 mg.kg⁻¹ of calcium. Meanwhile, its pods contain 60.64mg.kg⁻¹.

*Gleditsia* seeds and pods are also very rich in magnesium, they present about 25.57mg.kg⁻¹, 6.36mg.kg⁻¹ respectively. Exceeding the normal range in plants cited by [19] (Tab.1). It is a part of the chlorophyll in all green plants and essential for photosynthesis. Magnesium is absorbed as the Mg²⁺ ion and is mobile in plants. It leaches from the soil like calcium and potassium. It serves as an activator for many enzymes required in plant growth processes and stabilizes the nucleic acids. The magnesium concentration of tissues considered as deficient, sufficient, or toxic depends on what growth parameter is being measured in the crops. In many food crops, classification of nutrient sufficiency is based on harvestable yields and quality of the edible plant parts [24].

In other hand, reactions between iron and manganese are commonly observed and the ratio of these two metals in both growth medium and plant tissue seems to be more

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important to plant metabolism than their concentrations [17]. The result shows in Tab. 1 that iron in *G. triacanthos* seeds was 1.294mg.kg⁻¹; however in pods it was 0.79mg.kg⁻¹. Iron (Fe) is an essential micronutrient for plant growth. It is considered as the key metal in energy transformations needed for syntheses and other life processes of the cells [25]. Although iron itself is not considered toxic, it is environmentally significant because of its interaction with metals that are toxic. Iron oxides adsorb many elements and participate in the attenuation of most trace and heavy metals.

Other elements like silicium, zinc, manganese, aluminum and copper are also important to plant metabolism. According to Lefaucheur [26], Silicium is one of the most abundant alkali metals in nature, it represent about 28% of the Earth’s crust and plays multiple functions in plant metabolism, structure, solidity and flexibility. It is present in seeds with 1.15mg.kg⁻¹ and in pods with 1.37mg.kg⁻¹. Zinc is a vital element for plant nutrition, structuration and/or enzymes catalysis (superoxide dismutase, alcohol dehydrogenase, and RNA polymerase). According to Kabata-Pendias & Pendias [17], the normal range of Zn in plants is about 1–160mg.kg⁻¹. Our results are lower than the literature data (0.63mg.kg⁻¹ for seeds and 0.12mg.kg⁻¹ for pods).

Manganese (Mn) is an important plant micronutrient and is required by plants in the second greatest quantity compared to iron. Like any other element, it can have a limiting factor on plant growth if it is deficient or toxic in plant tissue. It is used in plants as a major contributor to various biological systems including photosynthesis, respiration, and nitrogen assimilation. This element is also involved in pollen germination, pollen tube growth, root cell elongation and resistance to root pathogens [27]. As indicated in Tab.1, seeds of *G. triacanthos* contain 0.615mg.kg⁻¹ and pods contain 0.136mg.kg⁻¹.

Aluminum (Al) is the most abundant metal on the planet and the third most common element in the earth’s crust and indicated that the normal range in plants is about 200-≥1000 [12]. The result shows in Tab.1 that aluminum in *G. triacanthos* seeds was 0.39mg.kg⁻¹; however in pods it was 0.54mg.kg⁻¹. In both seeds and pods it was very lower than norms.

Copper is a structural and catalytic component of several proteins and enzymes involved in electron transfer and redox reactions [28]. Its presence is necessary for plant growth in low concentration. *Gleditsia* seeds and pods as shown in Tab. 1 contain 0.09mg.kg⁻¹, 0.0036mg.kg⁻¹ respectively.

Cadmium, lead and Chromium are considered as non-essential and toxic metals to plant growth [7]. As indicated in Tab. 1, the maximum values of Cd, Pb, and Cr were proposed by [15,16,21]. The average concentrations of these elements in honey locust seeds and pods are lower than the normal range.

In general, the results have shown that accumulation of oligo-elements and heavy metals in seeds and pods of *G. triacanthos* L. carried out by using ICP-MS instruments did not reach phytotoxic concentrations or toxic levels (see Tab. 1 for reference values). Also, the results have shown that the accumulation of oligo-elements and heavy metals in seeds was more than in pods.

![Fig. 1: comparison between oligo-elements and heavy metals concentrations in *G. triacanthos* L. seeds and pods.](image)

Due to the application of one way analysis of variance (ANOVA), it was possible to record statistically significant difference between oligo-elements and heavy metals composition of *G. triacanthos* L. seeds and pods. On the content of these two parts of the fruit; there was statistically significant influence on the concentration of P, Mg, Ca, K, Na, Zn, Cr and Mn (<0.001) and Cu (<0.05). Also, statistically significant correlations (<0.05 and <0.01) between concentrations of the metals studied in *G. triacanthos* fruits (seeds and pods separately) revealed significant positive correlations (Tab.2). Significant positive relationships (<0.01) were observed inter alia for the following assemblages: Al-Ca-Si, Cu-Mg-Mn-P-Zn, Fe-Na, Mg-Mn-P-Zn, and P-Zn.

Table 2: Single tailed Pearson Correlation of oligo-elements and heavy metals in *G. triacanthos* L. fruits (seeds and pods)

In order to confirm the obtained results by ANOVA and Spearman correlations, we have used the principal components analysis (PCA) to compare between *Gleditsia* seeds and pods mineralogical profiles. In PCA represented in rotated space (Fig.2), the first component F1 explained...
about 61.11% of the total variance, the second component F2 about 28.26% and the third component F3 about 7.25%. Total contribution from these components is 96.63% of the total variation. As can be observed on Fig.1, we have obtained 4 groups: G1 (Mg-Mn-As-K-Cu), G2 (Na-Fe), G3 (Al-Ca-Si), G4 (Pb-Cr-Cd).

The delivery of metals to seeds depends upon uptake by the mother plant, and subsequent transport to developing seeds. Due to the potential toxicity of excess levels of metals, their uptake and distribution and the intracellular concentration must be carefully regulated. Metals are normally found at the highest concentrations in the roots, and at the lowest concentrations in the reproductive tissues [29]. This is because metals are sequestered into the vacuoles of root and shoot tissue and the subsequent availability of free metals in the symplast can be low. For example, concentrations of Cd and Ni in soybean were 30 and 20 times, respectively, higher in roots than in leaves. Cadmium concentration was lowest in the seeds, whereas the concentration of Ni was the same in both leaves and seeds [29].

The environmental factors and the type of plant influence the bioaccumulation of heavy metals. Thus, the concentration of essential and non-essential heavy metals in medicinal plants beyond permissible limit is a matter of great concern to public safety all over the world. An assessment of heavy metals tolerance should be based upon a comprehensive analysis of the interaction between the accumulation of heavy metals in plants and the metal’s status in soils [25].

The maximum values for heavy metals in herbal drugs and extracts have been discussed by several authors. In 1998, Kabelitz [30] published a detailed evaluation of a database on heavy metals, which included more than 12 000 samples originating from quality control analyses by several pharmaceutical companies.

**Conclusion**

The seeds and pods of *G. triacanthos* L. are very rich in basic elements like potassium, calcium and magnesium and at the same time, the concentrations of heavy metals like chromium, lead and cadmium which are considered as toxic elements are very lower than the normal range in plants cited by different authors. However, we note that seeds are rich than pods in potassium, phosphorus, magnesium and

### Table 2: Single tailed Pearson Correlation of oligo-elements and heavy metals in *G. triacanthos* L. fruits (seeds and pods)

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>As</th>
<th>Ca</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>Pb</th>
<th>Si</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.05</td>
<td>1</td>
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<tr>
<td>Ca</td>
<td>0.93**</td>
<td>-0.428</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.267</td>
<td>-0.621</td>
<td>0.300</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Cr</td>
<td>0.375</td>
<td>-0.776</td>
<td>0.113</td>
<td>0.552</td>
<td>1</td>
<td></td>
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<tr>
<td>Cu</td>
<td>-0.450</td>
<td>0.832**</td>
<td>-0.153</td>
<td>-0.333</td>
<td>-0.935**</td>
<td>1</td>
<td></td>
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<td></td>
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<tr>
<td>Fe</td>
<td>0.356</td>
<td>0.356</td>
<td>0.623</td>
<td>-0.064</td>
<td>-0.658</td>
<td>0.671</td>
<td>1</td>
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<tr>
<td>K</td>
<td>-0.456</td>
<td>0.874**</td>
<td>-0.235</td>
<td>-0.586</td>
<td>-0.751</td>
<td>0.801</td>
<td>0.430</td>
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<tr>
<td>Mg</td>
<td>-0.217</td>
<td>0.734</td>
<td>0.090</td>
<td>-0.324</td>
<td>-0.943**</td>
<td>0.966**</td>
<td>0.829*</td>
<td>0.739</td>
<td>1</td>
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<tr>
<td>Mn</td>
<td>-0.362</td>
<td>0.790</td>
<td>-0.055</td>
<td>-0.324</td>
<td>-0.947**</td>
<td>0.994**</td>
<td>0.739</td>
<td>0.776</td>
<td>0.988**</td>
<td>1</td>
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<td>Na</td>
<td>0.445</td>
<td>0.294</td>
<td>0.680</td>
<td>-0.172</td>
<td>-0.641</td>
<td>0.587</td>
<td>0.980**</td>
<td>0.387</td>
<td>0.774</td>
<td>0.666</td>
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<tr>
<td>P</td>
<td>-0.396</td>
<td>0.809</td>
<td>-0.093</td>
<td>-0.333</td>
<td>-0.946**</td>
<td>0.998**</td>
<td>0.714</td>
<td>0.788</td>
<td>0.981**</td>
<td>0.999**</td>
<td>0.637</td>
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<tr>
<td>Pb</td>
<td>0.802</td>
<td>-0.715</td>
<td>0.536</td>
<td>0.160</td>
<td>0.671</td>
<td>-0.816*</td>
<td>-0.204</td>
<td>-0.752</td>
<td>-0.661</td>
<td>-0.764</td>
<td>-0.078</td>
<td>-0.783</td>
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<tr>
<td>Si</td>
<td>0.952**</td>
<td>-0.498</td>
<td>0.951**</td>
<td>0.437</td>
<td>0.276</td>
<td>-0.277</td>
<td>0.511</td>
<td>-0.428</td>
<td>-0.052</td>
<td>-0.190</td>
<td>0.548</td>
<td>-0.224</td>
<td>0.653</td>
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<tr>
<td>Zn</td>
<td>-0.416</td>
<td>0.838**</td>
<td>-0.111</td>
<td>-0.302</td>
<td>-0.891*</td>
<td>0.988**</td>
<td>0.685</td>
<td>0.853*</td>
<td>0.953**</td>
<td>0.980**</td>
<td>0.592</td>
<td>0.984**</td>
<td>-0.825*</td>
<td>-0.245</td>
<td>1</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

![Component Plot in Rotated Space](http://www.pacificejournals.com/aabs)
sodium. The obtained results remain so equilibrated which militate in favor of the use of honey locust fruits (seeds and the pods) in many industries like the production of animal feeds and in the field of the cosmetic.

In other hand, *G. triacanthos* L. well known as an invading and competing the autochthones species, it is very important for us to valorise its co-products especially in developing countries.

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None

**Competing Interests**

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None

**Reference**