Heavy Metal Pollution Affects the Antioxidant Potential of *Rosa canina* L. Species

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The content of vitamin C, carotenes, polyphenols, total carbohydrates and total soluble proteins was determined, alongside with the activity of the oxidative stress enzymes (superoxide-dismutase, catalase, peroxidase, isocitrat-dehydrogenase, ketoglutarat-dehydrogenase, succinat-dehydrogenase and malat-dehydrogenase) in Rosa canina L. samples collected from the heavy metals and arsenic polluted area around of Tarnița closed mine, as well as from Sucevita area (as unpolluted site). The results showed an increase in the rose hip richness of nutrients and biological-active compounds, as well as the antioxidant activity of the samples from Tarnita area. We have explained our results taking into consideration the response of rosa plants to the oxidative stress produced by the accumulation of reactive oxygen species generated by exposure to pollutant factors from the contaminated area.

Keywords: heavy metals, environmental pollution, Tarnita mine site, Rosa canina, antioxidant potential

In recent years, the profile industry has referred to the analysis of an increasing number of plants targeting the discovery of new pharmacologically active substances that serve as a major perspective for future therapy [1, 2]. Romania has a huge potential of growing and harvesting medical plants, but just a small number of them being used for therapeutic purposes [3]. Rose hip is a valuable spontaneous species, given by the high concentration of vitamins, minerals, sugar, phenolic compounds, carotenoids, tocopherols, bioflavonoids, tannins, organic acids, amino acids, volatile oils etc [2, 4, 5]. *Rosa canina* fruits have medicinal properties [6, 7], being recommended in the prevention and curative treatment of some inflammatory affections of gastric mucosa as well as against the gastric ulcer [8]. Besides, the components of such fruits have been recommended in atherosclerosis and in the chronic diseases of any inflammatory nature [9, 10], as well as the kidney and the gallbladder diseases [11]. At the same time, the rose hips have antifungal and antibacterial properties [12, 13], being also antioxidants and anti-eczematous agents. Moreover, they decrease the blood glucose level and fat corps, and there is also a hypocaloric diet based on the rose hip probiotic, having lipid-lowering, antiobese and antiulcerogenic effects [14, 15]. Some authors claim that some extract could be used in the treatment of skin diseases [15] or even against cancer [16].

Therefore, the objectiv of this work is to investigate the content of vitamin C, carotenes, polyphenols, total carbohydrates and total soluble proteins, as well as the activity of the oxidative stress enzymes (superoxide-dismutase, catalase, peroxidase, isocitrat-dehydrogenase, ketoglutarat-dehydrogenase, succinat-dehydrogenase and malat-dehydrogenase) in *Rosa canina* L. samples collected from the heavy metals and arsenic polluted area around of Tarnita closed mine. We have decided to search for a relationship between the antioxidant activity of *Rosa canina* and the presence of heavy metals in the environment, to use it as a marker of environmental pollution.

Experimental part
Materials and method
Sampling

Rose hip fruits and leaves were sampled in September – October 2015 from two areas of the Suceava County namely, Tarnita-Ostra (polluted area with heavy metals and arsenic) and Sucevita (control area). In order to cover a vast area around the mine site perimeter, the samples were picked from four different points. Similarly, the biological tissues of the reference area were sampled. The measurements were performed in triplicate, whereas the obtained data were reported as the mean of the three values, $X \pm$ the standard deviation, s_d. The fresh picked fruits were sent to the laboratory were a number of 50 rose hips were randomly selected. Thereafter, their seeds were remove and the pulp mixed. Then, the concentration of ascorbic acid, carotenes, polyphenols, sugar and proteins was determined in the resulted homogeneous mixture. The superoxide-dismutase (SOD), catalase (CAT), peroxidase (POX) and dehydrogenase Krebs cycle (DH) activity were evaluated in the foliar tissue.

Sites description
The Tarnita-Ostra area is situated in the southern region of Suceava County. On January 1st, 2007, all the activity at the copper and barite processing factory from Tarnita was stopped. In 2008, safety and consolidation works started at Tarnicioara Lake, one of the most important environmental risk areas, occupying a surface area of 28.5 ha. In 2012, the authorities initiated closure and greening works at the Tarnita-Ostra mine, which have not yet been finalized. Sucevița is located in the north-eastern part of Suceava County and represents an important tourist region due to Sucevita Monastry, located at a distance of 55 km far from Suceava city and 18 km from Radauti town.

Determination of some biochemical indicators
Ascorbic acid content, as mg of ascorbic acid per 100 g fresh weight (fw), was estimated with a 2,6-dichloro-

phenolindophenol solution [17]. The concentration of carotenes after their extraction from the biologic material with acetone and, then, with petroleum ether was determined by reading the extinction at the 460 nm wavelength against an azo-benzene solution used as standard. The results were expressed as mg carotenes/ 100 g fw. Total polyphenols content (espressed as mg gallic acid (GAE)/100 g fw) was determined by the Singleton method [18].

The total sugars in rose hips, expressed as grams of reducing sugars/100 g fw were determined using the 3,5-dinitrosalycilic acid method [19]. The absorbance was measured at 500 nm using a UV-1700 PharmaSpec Shimadzu spectrophotometer. The amount of total soluble proteins was estimated by the Bradford method [20] and expressed as g/100 g fw.

The SOD (E.C. 1.15.1.1) activity was evaluated using the Winterbourn method, CAT (E.C. 1.11.1.6) by Sinha method, POX (E.C. 1.11.1.7) by Gudkova and Degtiari method [21], and DH with triphenyl-tetrazolium [22]. The enzyme activity was expressed as units *per* mg of protein. The unit of SOD activity is defined as the amount of enzyme required to determine the 50% inhibition in the NBT reduction. A catalase unit represents the amount of the enzyme catalase which dissolves one micromole of H_2O_2 in one minute at 20°C and $pH = 7.0$. A peroxidase unit corresponds to an enzyme amount which catalyzes the decomposition of one micromole of H_2O_2 in one minute under optimal conditions.

Statistics

From each study area, multiple determinations were performed, the systematized data representing the mean of these replicas ± the standard deviation. In order to test the statistical significance of the experimental results we performed the t-Student test, the differences between the control group and the polluted area being considered significant at values of p less than 0.05 (***$p < 0.001$ - very significant; ** $0.001 < p < 0.005$ - significant; * $0.01 < p < 0.05$ - less significant; $0.05 < p < 0.5$ - not significant).

Results and discussions
Vitamin C

Our results show a rich content of vitamin C in the pulp of the fruit of the rose, the maximum values being recorded in the harvested group from Sucevita (918.7±40.4 mg/ 100 g fw), while the samples from neighboring areas, around the Tarnița barite and pirrite closed mine show a net inferior content of this vitamin (735.1±45.8 mg/100 g fw). Applying the statistical Student test to the concentration values of ascorbic acid in *Rosa canina* fruits of different areas of Suceava County showed the existence of a significant difference between the two lots, the value of the indicator expressing the probability level (p) between 0.01 and 0.05 (fig. 1).

Indeed, the accumulation of vitamin C may be affected by climatic conditions during growth and maturation, as the literature shows, with a significant decrease in the concentration of this vitamin during the baking process [23], but also as a result of air and soil pollution with various heavy metals [24-30], as well as residues from mining techniques [31, 32].

Carotene concentration

The content of carotenes follows the same trend (fig. 2), with maximum mean values in the Sucevița group (38.7 ± 1.8 mg/100 g fw) and less in the group from Tarnita mine site (29.25±3.9 mg/100 g fw). The results support data from literature that highlights the influence of
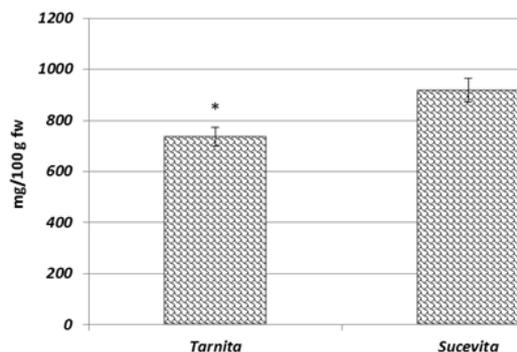

Fig. 1. Ascorbic acid content of *Rosa canina* L. fruits

environmental pollution [33, 34] with different concentrations of pesticides [35] and heavy metals [24-30, 36] on the content of photosynthetic pigments.
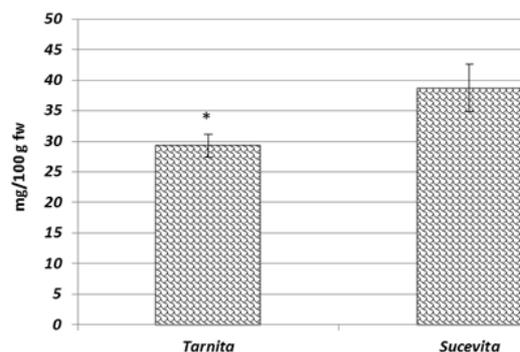

Fig. 2. Carotene content of *Rosa canina* L. fruits

Polyphenols

Regarding the content of polyphenols in *Rosa canina* fruits from the two areas of Suceava County, it can be observed that Sucevița stands out by a higher value, the concentration of polyphenols reaching the threshold of 657.5±30.8 mg GAE/100 g fw, while the mean value recorded at Tarnita was 589.3±14.5 mg GAE/100 g fw. Our results are in line with those in the scientific literature that demonstrate that the polyphenolic fraction depends both on climatic conditions and species and variety [37]. The statistical analysis of the experimental data shows the existence of significant differences ($0.01 < p < 0.05$) between the two lots in terms of the concentration of polyphenols (fig. 3).
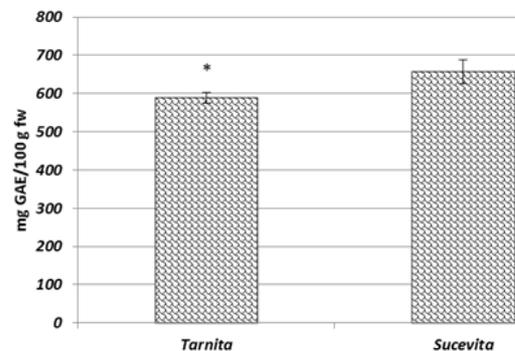

Fig. 3. Polyphenols content of *Rosa canina* L. fruits

Reducing sugars and proteins

In the samples taken from the Sucevita area, the total carbohydrate concentration reached the average threshold of 15.5±1.5 g/100 g fw and that of total soluble proteins of 1.99±0.23 g/100 g fw, while the samples taken near the Tarnita mine site exploitation showed average values of 15.0±0.6 g/100 g fw and 1.7±0.12 g/100 g fw, respectively (figs. 4-5).
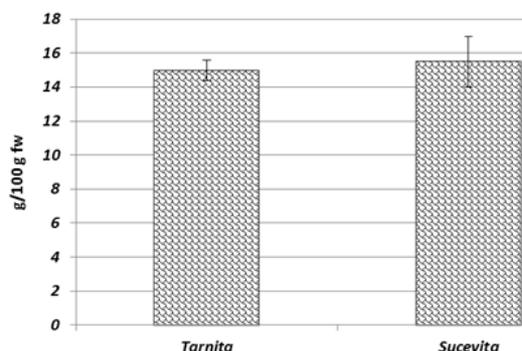
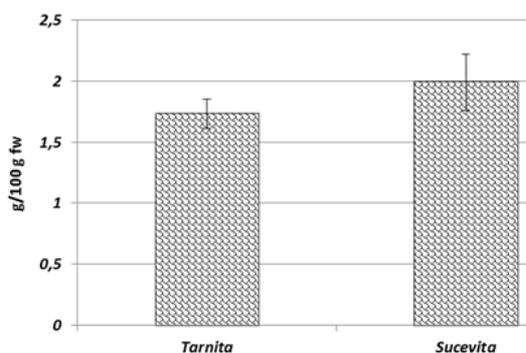Fig. 4. Sugars content of *Rosa canina* L. fruits

Fig. 5. Soluble protein content of *Rosa canina* L. fruits

SOD activity

This enzyme, which is the first barrier in the defense of living organisms against reactive oxygen species (ROS), recorded a value of 49.8±4.9 units mg^{-1} protein in the samples collected from Tarnita-Ostra, almost double compared to the control group, where an average of 26.5 ± 2.9 units mg^{-1} protein was reached.

The leaf material obtained from Tarnita showed very high values for SOD activity compared to the control, the mean activity of the batch reaching the value of 39.9±2.9 units mg^{-1} protein towards 22.9±2.2 units mg^{-1} protein in Sucevita lot (fig. 6).
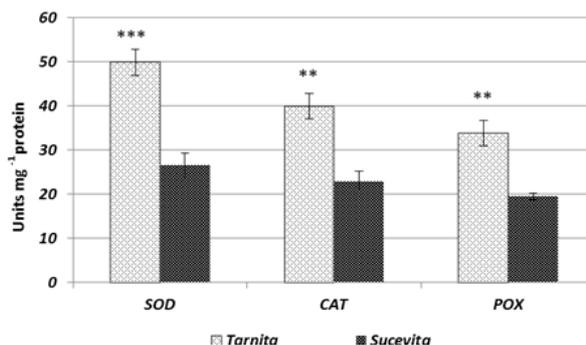

Fig. 6. SOD, CAT and POX activities in *Rosa canina* L. leaves

Experimental data regarding the POX activity indicates the same trend as SOD activity, with slightly lower mean values of this oxidoreductase by 19.4±0.8 units mg^{-1} protein in the reference lot and 33.7±2.8 units mg^{-1} protein in the Tarnita mining site.

Student-T statistical analysis indicated the existence of highly significant differences ($p < 0.001$) in the SOD case and significant for 99% confidence interval ($p < 0.01$) for CAT and POX.

As can be seen from figure 7, the dehydrogenase activity of the Krebs cycle oscillates within fairly wide limits, with maximum values also observed in experimental variants in the Tarnita area. Thus, the isocitrate dehydrogenase activity (I-DH) was measured to be between 23.2±1.6 µg formazan/g fw (Sucevita) and 30.7±1.8 µg formazan/g fw (Tarnita), while the á-ketoglutarate-dehydrogenase (K-

DH) displayed closer values (23.3±1.8 µg formazan/g fw in the foliar tissue of the *Rosa canina* species taken from the Sucevita area, and 27.4±1.4 µg formazan/g fw in the Tarnita area, respectively). The activity of succinate dehydrogenase (S-DH) had comparative values in the two lots (20.3±1.8 µg formazan/g fw in the reference lot and 19.1±2.8 µg formazan/g fw within the Tarnita samples), the succinic acid being generated or catabolized by other metabolic processes of Krebs cycle, as well [38].

The rate of malate mobilization under the action of malate-dehydrogenase (M-DH) is is different from one sampling area to another, the higher average activity being recorded in the plant material harvested from Tarnita (16.2±1.5 µg formazan/g fw) compared to those taken from Sucevita (11.9±1.2 µg formazan/g fw).

Heavy metals and metalloids found in the environment as a consequence of mining activities may generate ROS in living cells, being able to cause major imbalance of metabolism by destroying the antioxidant potential of the plant body [39].
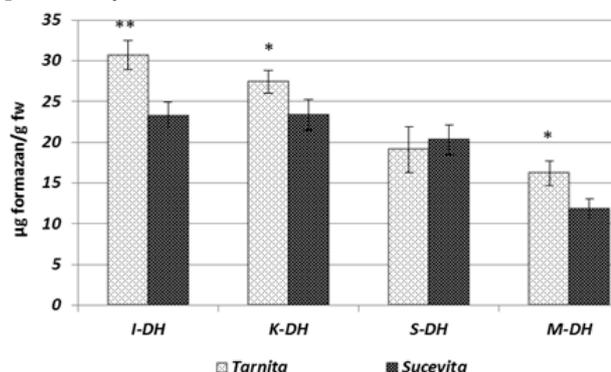

Fig. 7. Cycle Krebs dehydrogenase activity in *Rosa canina* L. leaves: isocitrate dehydrogenase (I-DH); α-ketoglutarate-dehydrogenase (K-DH); Succinate dehydrogenase activity (S-DH); malate dehydrogenase (M-DH).

The massive pollution with heavy metals, aluminum and arsenic of the soil around the closed mine of Tarnita has resulted in the absorption of these agents at root level and, from here, at the leaves level, determining an increase in the oxidative stress and, implicitly, in the antioxidant enzymes activity, CAT being a key barrier against the free radicals [40-42].

The literature indicates that POX exhibits activity against small amounts of H_2O_2, while CAT is responsible for the excess of peroxides, whereas POX is known for its polyfunctional role in plants [43]. At the same time, an iron excess in the soil of Tarnita is able to double CAT activity [44].

Therefore, the investigated plant species showed drastically decrease in useful food components alongside with an increase in the activity of oxidoreductive enzymes. *Rosa canina* L. plants could be an effective marker of heavy metal pollution, but they also show the high pollution level of Tarnita closed mine area.

Conclusions

Our results highlight the remarkable antioxidant potential of the *Rosa canina* species and the richness in vitamins, provitamins and nutrients of this species.

Noteworthy is the profoundly negative impact of environmental pollution on vegetal organisms, translated, both by a low content of bioactive compounds and by a high level of oxido-reductase enzyme activity in response to the oxidative stress produced by the free radicals, ROS, induced in plants by heavy metal contaminants.

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