International Journal of Latest Research in Science and Technology ISSN (Online):2278-5299
Volume 7, Issue 2: Page No. 22 –27 ,March-April 2018
https://www.mnkpublication.com/journal/ijlrst/index.php

CONTAMINATION FROM HEAVY METALS IN THE ERBORISTIC PREPARATIONS OF *MALVA SYLVESTRIS*

[1]Ilenia Bravo ,[2]Papetti Patrizia, [3]Spognardi Sara, [4]Carelli Angela, [5]Pelagalli Ester
[1,2]Department of Economics and Law University of Cassino and Southern Lazio , Italy
[3]Department of Business Studies University of Roma Tre , Italy
[4]University of Cassino and Southern Lazio - Department of Economics and Law - Italy
[5]University of Cassino and Southern Lazio - Department of Economics and Law - Italy
[1]Ilenia.bravo@libero.it,[2]papetti@unicas.it,[3]sara.spognardi@uniroma3.it,
[4]a.carelli@unicas.it,[5]ester.pelagalli@hotmail.it

Abstract – ***Malvasylvestrisis an annual plant native to Europe, North Africa and Asia. This herbaceous species has a long history, it has been used widely as food due to its therapeutic relevance. Some parts of this plant have been employed in traditional medicine, the leaves are perfectly safe and that there are no negative effects for human consumption, although some authors have reported harmful effects due to the growth of mauve in contaminated areas. In this study the content of heavy metals was analysed in various herbal products based on*** **M. sylvestris** ***obtained from different European countries. The analysis conducted included elements such as arsenic, cadmium, lead and mercury.The metals were found in various concentrations with the following ranges: 0.012-0.068 mg/kg^{-1} for arsenic, 0.116-0.339 mg/kg^{-1} for cadmium, 0.096-0.271 mg/kg^{-1} for lead, while the mercury concentrations are below the detection limits. According to our results, in all samples the arsenic and mercury levels are below both the permissible limits provided by European Commission on the maximum content of contaminants in foodstuffs and the international limits provided by FAO/WHO for medicinal herbs and plants. Instead, non-negligible concentrations of cadmium and lead were detected, especially in the samples from South Mediterranean areas. The relationship between the contaminants and the sampling sites was investigated using analysis of variance (ANOVA) and Tukey's HSD post-hoc tests. Finally, the Estimated Daily Intake (EDI) and the Health Risk Index (HRI) were calculated to evaluate the potential health risk from mauve products chronic consumption. Health Risk Index values resulted lower than 1, indicating that there were no risks to human health.***

Keywords— Malvasylvestris, Heavy Metals contamination, Herbal tea,Health risk assessment, Food safety

I. INTRODUCTION

Malvasylvestris is a member of family *Malvaceae* and its genus is represented by 40 taxa in all over of the world. It is an annual plant native to Europe, North Africa and South-west Asia, with lobed leaves and purple flowers which bloom in late spring, that grows spontaneously and prefers wetlands, such as meadows, the sides of the ditches and the banks of the rivers (Razavi*et al.*, 2011).

Commonly known as common mallow, *M. sylvestris*is a medicinal herb that has always been used in a traditional way, for its emollient and laxative properties (Barros *et al.*, 2010). Numerous surveys conducted in Europe highlighted the potential of a local resource often overlooked; the extracts of some parts of the plant of *M. sylvestris*, in particular leaves and flowers, have been compared for their nutraceutical potential, for their antioxidant properties and for their chemical composition (Ferreira *et al.*, 2006; Natali and Pollio, 2007; Guarrera*et al.*, 2007; Quave*et al.*, 2008; Leporatti*et al.*, 2009; Neves *et al.*, 2009). The mallow leaves have revealed very strong antioxidant properties, due to the presence of phenols, flavonoids, carotenoids and tocopherols, unsaturated fatty acids (α-linolenic acid) and mineral components.

For this reason mauve has been used for a long time (and still today) for medicinal applications and its roots, shoots, leaves, flowers are applied as infusions, decoctions, liniments, lotions, vapour baths and gargling.

These medicinal applications treat specific disorders of several systems of the human body, such as the digestive, the respiratory, the urinary, the muscular and skeletal systems, as well as skin disorders. Besides the anti-inflammatory properties, some pharmacological and clinical effects are recognized. It also has antioxidant and defense actions, essential for survival, in fact from epidemiological studies its use can protect against cancer and cardiovascular diseases (Mozaffarian, 2005; Geronikaki, 2006; Collins, 2009; Rackova, *et al.*, 2009; Della Greca*et al.*, 2009).

However, in addition to all uses for therapeutic purposes, M.*sylvestris* is also used for food purpose; specifically edible uses are concerned with folk gastronomy and with those uses generally included in "minor nourishment" (Guarrera, 2003; Carvalho 2005); while the young leaves are eaten raw in salads, the leaves and sprouts are consumed in soups and as boiled vegetables (Barros *et al.*, 2010).

The literature (Cooper and Johnson, 1984; Rivera and Obón de Castro, 1991) shows that the leaves are perfectly safe and that there are no negative effects for human consumption, although some authors have reported harmful effects due to the growth of mauve in contaminated areas.

Publication History
Manuscript Received : 16 March 2018
Manuscript Accepted : 24 April 2018
Revision Received : 9 April 2018
Manuscript Published : 15 April 2018

The plants are sensitive to the absorption of organic and inorganic molecules, increasingly widespread in the environment due to urban and industrial emissions.
In fact plants represent a sort of vehicle for these substances, playing an important role in the bioaccumulation and biomagnification processes (Shinwari*et al.*, 2009).

The contaminants that can accumulate in the soil are numerous and of different nature; in recent years particular attention has been paid to the following classes of organic and inorganic compounds: heavy metals, residues of plant protection products, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and mycotoxins. It is necessary to better understand the mechanisms by which contaminants are absorbed and accumulated by the plant species that are used for the production ofphytotherapics and food supplements.

In the last years, the contamination of food from metals has become a major problem, due to the rapid technological progress that has led to an increase in natural emissions of some elements such as lead, cadmium and mercury from production processes. The most important heavy metal contaminations that may affect medicinal plants concern the presence beyond the maximum limits of Cadmium (Cd), Mercury (Hg) and Lead (Pb) and Arsenic (As).

In medicinal plants, the accumulation of heavy metals is due to their presence in the areas where they grow, near the roads or in the places of metals extraction and melting. Furthermore, the contamination may also be due to anthropogenic processes involving the application of synthetic fertilizers, organic fertilizers and industrial wastes, or inadequate hygienic transport and storage conditions (Dual *et al.*, 2009).

On the basis of possible environmental and production contaminations that may affect the safety of plant products, many leader companies in the herbal sector have defined self-regulation, in according to the limits imposed by current national and international regulations, in order to offer to consumers the best possible guarantees. In the present study, the content of heavy metals was analysed in various herbal products based on *M. sylvestris*, sampled from various European countries. Data obtained have been processed and compared and, for each country, the Health Risk Index was calculatedin order to evaluate the potential health risk from mauve products chronic consumption.

II. MATERIALS AND METHODS

Sampling

Sixty samples from different areas of Europe, from France, Germany, Greece, Spain and Italy (Figure 1) were analysed to determine the presence of heavy metals in the herbal preparations of *Malvasylvestris*. The preparations consist mainly of dried plants, in the form of tisanes, either in filter or in bulk, consisting of leaves and flowers or only flowers. Each sample was weighed, mineralized and its content of heavy metals was determined by atomic absorption spectrophotometer (HG-AAS).

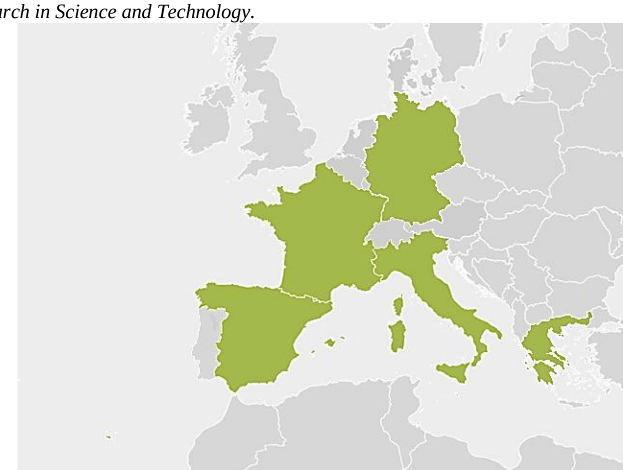

Fig.1 Sampling area

Standard and reagents

A mixture of concentrated nitric acid (HNO_3) 65% RS and a solution of hydrogen peroxide and 40% m/V in purely stabilized water supplied by Carlo Erba Reagents, were used for the mineralization. After the acid digestion, ultrapure distilled water HIGH PURITY 18 MΩ.cm 25 C was used for the recovery of the samples.

The TEs (Trace elements) calibration was made using appropriate dilutions of stock solutions of Pb, Cd, As, Hg (1000 ppm) (CPAChem). For the determination of hydrides, As and Hg, the following reagents were used: sodium borohydride ($NaBH_4$) at 98%, purchased from ACROS ORGANICS and hydrochloride acid (HCl) at 37% RPE from Carlo Erba Reagents.

To ensure the reproducibility and the accuracy of the method, the same analyses were conducted on certified control samples (NIST1570a Sigma Aldrich) according to Ababneh (Ababneh, 2017) with a mean recovery of about 96±2%.

Extraction procedures

0.2 - 0.3 grams of samples were weighed and mineralized through a wet digestion process, in presence of 3ml of HNO_3 and 0.5ml of H_2O_2 for about 7 hours. After mineralization and cooling, the mixture was brought to a final volume of 10 ml with deionized water(Papetti and Rossi, 2009).

The atomic spectrophotometer (AAS) determination

The levels of Cd, Pbwere analysed using the atomic absorption spectrophotometer AA-600 (Perkin Elmer, USA), while Hg and As have been determined through Hydride Generation Atomic Absorption Spectrometry (HGAAS). Instrument detection limits on the GFAA were 0.2 ppb for arsenic, 0.1 ppb for cadmium, 2.0 ppb for lead. In the graphite furnace 20μl of sample were introduced with the addition of 5μl of a specific matrix modifier composed of magnesium nitrate ($Mg(NO_3)_2$) and ammonium phosphate (NH_2PO_4) to reduce or eliminate interferences in the vapor phase and stabilize the matrix. Subsequently, the analyte was atomized in according to a specific temperature program. The TE concentrations were expressed in milligrams per kilo of dry weight (mg/kg dw).

Statistical analysis

Analysis of variance (ANOVA) tests were conducted for the concentrations of metals, using as independent variables the metals considered, the sampling areas, and the interactions. According to what resulted as significant, Tukey's HSD post-hoc tests were performed.

Estimated dietary intake of metals

The estimated daily intake of each TE depends on both the metal concentration in food and the daily food consumption. In addition, the human body weight can influence the tolerance of contaminants. EDI was calculated as follows:

$$\mathbf{EDI = (F_{IR} \times Cm)/W_{AB}}$$

Where F_{IR} is the mauve ingestion rate (g/person/day), which was considered to be 0.77 g/day, that is the average of European available data of the chronic consumption of mauve products such as tea and herbal infusions (Table 1)(Chamannejadian*et al.*, 2013; EFSA, 2015); Cm is the TE concentration in foodstuffs; W_{AB} is the average body weight (bw). The body weight was set to 60 kg in this study (Zazouli*et al.*, 2008).

TABLE 1.
Data of mauve chronic consumption from EFSA database.

Countries	**Mean** (g/day)	**SD**
Austria	1,47	4,13
Finland	1,40	3,14
Hungary	0,52	1,78
Ireland	0,24	1,34
Netherlands	0,82	3,28
Romania	1,81	3,54
Sweden	0,01	0,24
UK	0,16	0,96
UK	0,32	1,65
Germany	2,23	1,44
Italy	0,09	0,54
Latvia	0,16	0,59
Average	**0,77**	**0,76**

Health Risk Index

Health risk of consumers due to intake of TE contaminated mauve was assessed by using HRI (Health Risk Index). The HRI was calculated by using the equation:

$$\mathbf{HRI = EDI/RfD}$$

where RfD is the reference oral dose expressed in mg/kg/day and it represents an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. The RfD is 3.0×10^{-4} for As, 4.0×10^{-4} for Hg, 1.0×10^{-3} for Cd and 3.5×10^{-3} for Pb (Akoto*et al.*, 2014; USEPA, 2009).

The exposure to two or more pollutants may result in additive and/or interactive effects. The total HRI of heavy metals was treated as the arithmetical sum of the individual metal HRI: Total HRI (individual foodstuff) = HRI (toxicant 1) + HRI (toxicant 2) + HRI (toxicant n).

A Total HRI lower than 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a HRI above 1 means that there is a chance of non-carcinogenic effects, with an increasing probability as the value increases (Akoto*et al.*, 2014). HRI was determined for each sampling area.

III RESULTS AND DISCUSSION

The study was conducted on 60 samples from different European areas, in particular products based on mallow from different regions of Italy, from Germany, Greece, France, Spain and Mediterranean areas (in this last case the sampling sites are not explicitly specified in the labels of the products).

TABLE 2.
Origin of mauve and number of collected samples

SimplingSites	**Total n. of samples collected N=60**
France	11
Germany	8
Greece	9
Italy	15
Spain	9
Mediterranean areas	8

The table 3 shows the data of the average concentrations of As, Cd, Hg and Pb, obtained from 3 replicates performed on each sample, expressed in mg/kg (ppm).

TABLE 3.
Mean concentrations of heavy metals and Standard deviation (mg/kg^{-1})

Sites	**Pb**	**Cd**	**As**	**Hg**
France	0,096±0,019	0,339±0,083	0,012±0,003	n.d
Germany	0,167±0,044	0,116±0,048	0,068±0,019	n.d
Greece	0,132±0,024	0,290±0,060	0,063±0,019	n.d
Italy	0,136±0,019	0,177±0,029	0,014±0,005	n.d
Mediterraneanareas	0,271±0,060	0,311±0,131	0,014±0,011	n.d
Spain	0,116±0,057	0,102±0,036	0,022±0,008	n.d

From the data obtained, the highest concentrations were found for cadmium, particularly in samples from France, Greece and in the Mediterranean areas, where the cadmium concentration varied from 0.303 to 0.513 ppmon a dry weight basis.Overall, among the 60 samples analysed for cadmium, about 27% of the samples contained high levels of cadmium exceeding 0.3 and 0.2 ppm, respectively the permissible limits set by FAO/WHO for medicinal herbs and plants, and the EU Regulation for foodstuffs.Similar results of high levels of cadmium in Egyptian and Iranian medicinal herbs and plants have been reported in earlier studies (Dghaim*et al.*, 2015; Ziarati, 2012).

The level of lead was higher in the samples from the European Mediterranean area and Germany, with a range in between a minimum of 0.10 and 0.347 ppmon a dry weight basis.

The results related to the samples obtained from Mediterranean areas, are in line with the data on themost common medicinal herbs reported in literature, which showed the highest average concentrations of lead and cadmium in samples found in the Egyptian, Iranian and Jordanian regions (Ziarati, 2012; Abou-Arab *et al.* 2000). The FAO/WHO maximum permissible limit of lead in consumed medicinal herbs is 10 mg·kg^{-1}, therefore all the values found were below the allowed limits. According to Dghaim*et al.*, (Dghaim*et al.,* 2015), cadmium is the metal present at the highest concentrations in the traditional herbs, while arsenic and especially mercury are present in lower quantities.

In some cases, the concentration of heavy metals and in particular of cadmium has exceeded the permitted international levels. According to previous studies, the different metal concentrations detected in the products that have been analysed in this work could be attributed to differences in the environmental pollution, the growth stage, the type of soil and the sampling site (Dghaim*et al.* 2015 and Orisakwe*et al.* 2012).

Besides Chizzola*et al.*, (2003), in their research on the monitoring of micronutrient and heavy metals in herbs, spices and medicinal plants from Austria, found that some medicinal herbs have a greater tendency to accumulate cadmium. In fact, higher levels of cadmium were detected in medicinal plants similar to mauve, such as chamomile*(Matricaria chamomilla)*, yarrow*(Achillea millefolium)*, mint*(Mentha spicata)* and sage*(Salvia officinalis)*.

The arsenic contamination levels are less than 0.001 ppm for all samples under examination, in particular higher concentrations were detected in the samples from France, Italy and Greece. Mercury levels in all the samples analysed were below the detection limits.

The results obtained are in line with previous works and reflect the level of soil contamination in the sampling areas, in relation to natural and anthropogenic contributions (Tóth*et al.*2016). From the literature, the arsenic is more abundant in the substratum of the mountainous areas such as the Alps, the Carpathians, the Massif Central and the Pyrenees, and in the fluvial areas (PianuraPadana), while, high concentrations of cadmium have been found in the Bavarian Alps and in the south-eastern Alps.

High levels of lead can be found in many regions of Europe, mostly the most industrialized ones; such cases are evident in southern Saxony, in the North Rhine in Germany, in Bristol and Manchester in England and in Rome in Italy (Tóth*et al.*, 2016).

On the average metal concentrations obtained, statistical analysis were conducted. According to ANOVA analysis results, related to metals concentrations, only the variable "Metal" was found significant; instead both "Area" variable and the interaction between the two variables, weren't found significant. Hence, Tukey's HSD post-hoc test was performed for the variable "Metal" and results were showed in figure 2.

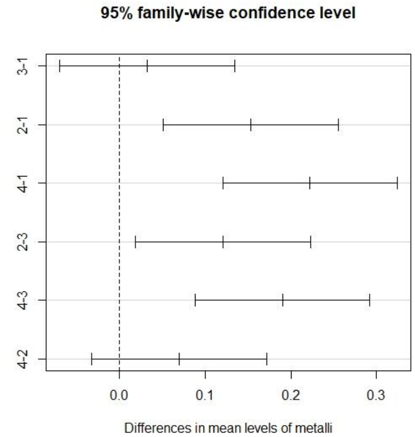

Fig.2 Tukey's Test Plot (95% family-wise confidence level) – Differences in mean levels of metals.
A number is assigned for each metal: Hg (1); Pb (2); As (3); Cd (g).

As showed in figure 2, only two pairs did not show any significant difference: 3-1 (As-Hg), 4-2 (Cd-Pb), for which the corresponding 95% family-wise confidence intervals include zero.

Regarding the risks for human health, the EDI (Estimated daily intakes) and the HRI (Health risk Index) values of trace elements in mauve products, considering data of chronic consumption for adults, were calculated and summarized in Table 4.

TABLE I
Mean concentration, EDIs and HRIs of trace elements in mauve products, from different European areas, considering data of chronic consumption for adults.

	Pollutant	Mean (mg/kg^{-1})	EDI (mg/kg b.w./day)	Reference Oral doses (mg/kg b.w./day)	HRI
ITA	Cd	0,176	2,25867E-06	0,001	0,0023
	Pb	0,136	1,74533E-06	0,0035	0,0005
	As	0,014	1,79667E-07	0,0003	0,0006
	Hg	-	-	0,0004	0,0000
					0,0034
SPA	Cd	0,102	1,31E-06	0,001	1,31E-03
	Pb	0,115	1,48E-06	0,0035	4,22E-04
	As	0,022	2,82E-07	0,0003	9,41E-04
	Hg	-	-	0,0004	0,00E+0
					2,67E-03
GER	Cd	0,116	1,49E-06	0,001	1,49E-03
	Pb	0,167	2,14E-06	0,0035	6,12E-04
	As	0,068	8,73E-07	0,0003	2,91E-03
	Hg	-	-	0,0004	0,00E+0
					5,01E-03
FRA	Cd	0,339	4,3505E-06	0,001	0,0044
	Pb	0,096	0,000001232	0,0035	0,0004
	As	0,012	0,000000154	0,0003	0,0005
	Hg	-	-	0,0004	0,0000

					0,0052
GRE	Cd	0,29	3,72167E-06	0,001	0,0037
	Pb	0,132	0,000001694	0,0035	0,0005
	As	0,063	8,085E-07	0,0003	0,0027
	Hg	-	-	0,0004	0,0000
					0,0069
EU Med	Cd	0,311	3,99117E-06	0,001	0,0040
	Pb	0,271	3,47783E-06	0,0035	0,0010
	As	0,014	1,79667E-07	0,0003	0,0006
	Hg	-	-	0,0004	0,0000
					0,0056

All the data obtained were compared with the limits established by European and International Regulations (Reg.EU 4020/2011 and FAO/WHO) and compared with those proposed by *ASSOERBE*, a national association representing Italian companies in the field of medicinal and aromatic plants, spices, plant extracts, essential oils and their derivatives (*www.assoerbe.eu*). The health risk due to the contamination of metals depends on the daily average dietary intake, and is not negligible.

From a study conducted in 2008 by Shen and Chen on the risk assessment of heavy metals in tea infusion, the HI values were very low and were within the limits of safety. However, to date there is still no systematic way to compare the health risk of heavy metals in tea infusions with tea leaves or flowers (Li *et al.*, 2015); many authors reported that EDI values of tea infusions decreased with the increase of the infusion times (Karimi *et al.*, 2008, Li *et al.*, 2015; Martin and Griswold, 2009). Experimental results of tea infusions show that about 40-52.8% of the total Cd has been released by some varieties of black tea (Shen and Chen, 2008), and the transfer ratio (release rate) of Cr (VI) in the green tea leaves by infusion was reported to 37.8% from Li *et al.* (2013).The percentage of Pb and Hg, which were released form black tea to infusions, were 2,6% and 70%, respectively (Karimi *et al.*, 2008).

The tea derived from the leaves of Camellia sinensis is one of the most popular beverages in the world; according to a study of Shekoohiyan*et al.* about 18-20 billion cups of tea are consumed every day in the world. Therefore, the risk associated with the assimilation of heavy metals through the consumption of tea could be quite high. The health aspects related to tea consumption are very important and consumers should be very confident about the absence of any pollutants, especially for imported products. Differences in metal concentrations may depend on conservation methods, processing of leaves and concentrations of metals in soil. For safety, it is recommended to prepare tea with water free of heavy metals and water with a low content of fluorine and preferably contained in the bottle (Shekoohiyan*et al.*, 2012).
Finally,the sum of HRI values determined for each metal are less than 1 for all sampling area, indicating that the consumption of the studied *M. sylvestris* samples do not constitute a health risk for tea consumers.

IV .CONCLUSION

The purpose of this work was to determine the levels of heavy metal contamination in *Malvasylvestris* products, sampled from different European countries, in order to compare them and evaluate the potential health risk from mallow chronic consumption in adults.

The study was carried out on tisanes and mauve based preparations, widely used on the market, and the levels of heavy metals detected were compared with the European and International limits and the guidelines proposed by *ASSOERBE*.

The arsenic and mercury levels are below the current limits provided by European Commission and FAO in all samples, despite the not negligible concentrations of cadmium and lead especially in samples from South Mediterranean areas.
Finally to evaluate the potential risk for human health in the case of mallow products chronic consumption the "Estimated Daily Intake" (EDI) and the "Health Risk Index" (HRI) were calculated. Total Health Risk Index values resulted lower than 1, indicating that there were no risks to human health for each sampling area.

It is certainly important to carry out studies and chemical analyses aimed at ensuring the quality of commercialized herb products, especially if they are used in tea infusion, for their aromatic and medicinal properties. Particular attention must be paid to good practices, from collection to consumption, in particular for imported products. Further studies are necessary for determining the presence of toxic metals, chemicals and other dangerous pollutants for assessing the cumulative long-term risk to tea consumer health.

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