International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-2/142-148
ISSN 2227-670X ©2015 IJACS Journal

Defining the metal ions contaminants in the leaves and growing place soil of cultivated A.triphylla plant with addition of humic substances to root

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ABSTRACT: Utilizing aerial parts and even the root of medicinal plant, followed by making a brewed juce is common among the native people of the region where the plant of quince grows naturally as a self – growing plant. Therefore, toxic metal ions should be carefully monitored. A.triphylla plant that has mere medicinal application was analyzed. After the addition of Sapropel to the root of its leaf, essential sample was prepared through wet method , then the amount of metal ions was determined applying Flame atomic absorption technique. The results of measurements are as follows: Mn(161.70 dry mg/kg) and Fe(123.80 dry mg/kg) in plant another sample of leaves which possesses 75 µm particles , was analyzed as pills of dried product by means of XRF technique and the results are identifying and defining the quantities of Sr (237.20 dry mg/kg) and Na_2O (0.57 dry mg/kg) in plant. The soil of growing place was carried out for percentage of humic substances. After the addition of Sapropel, the amounts of humic substances for the soil of growing place were 0.26% in June and 0.29% in September.
***Keywords**:* Cultivated A.triphylla, humic substances, growing place soil, metal ions, Flame atomic absorption technique, XRF technique

INTRODUCTION

Soil is one of the most important components of basic resources, so that it is considered as the main bed of plant cultivation and also as a unique environment for all kinds of live ecological movement of present time and fear of water and soil contamination by chemicals on one hand, and rapid growth of population, and more demands for foods on the other(Mogimi, 2007.,Halverson and Smith ,1997) . As a result, concentrated utilization of soils, on the other hand altogether have increased the necessity of organic material usage in soil more than ever since their productive effects on physical chemical and biological properties of soil organic materials are considered as important elements in soil fertility .Humic Substances are criteria for soil fertilization because along with supplying the plants needed nitrogen, they provide the est. physically structure for plants growing in soil without organic materials(Lu et al ,2000). Biological materials like herbs are highly in-homogeneous. They contain a number of organic substances of different steps of stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave digestion process may cause difficulty in transferring analyses into solution(Baranowska et , 2002., Blicharska et al ,2006). With an increase in soil moisture in surface layer, nitrogen increases exponently in a stable temperature. Additionally, with moisture stability, temperatural increase may cause nitrogen exponential decrease in surface layer. Desirable rate of moisture for nitrification is 50 – 67%(Halverson and Smith,1997., Lu et al,2000).

Humic substances are plant growth stimulating agents that have been applied in agricultural in recent years. However, detailed mechanisms of how these materials work in plants are still not well understood, due to complexhumic substances structures in nature. There are many reports of humic substances role in promoting plant biomass, stimulation of root, shoot, and flowering growth, and even direct effects on crop productivity and increases in crop yields. The humic substances consist of humic acids, fulvic acids and humin.They can be extracted from many natural sources such as peat, soil, and Leonardite ore. Municipal yard wastes, sewage sludge and composts also could be sources of these materials. Application of the humics in golf course and sports turf management has recently been rediscovered. Due to major environmental concerns, it is becoming a popular practice to use these materials as soil amendments (Chen et al,2004).In this research, we want to study of effect of humic substances on the growth of medicinal plants in environmental conditions.

MATERIALS AND METHODS

Sampling

Aerial parts of *A.triphylla* were collected from the greenhouse of the university in June and September, 2008. In order for the moisture of the sample to be removed, it was placed at shadow, and was exposed to dry air. Then dried plant sample was ground with a Molinx grinder so that it was covered to powder. The four soil of growing place of two species was prepared from surrounding half meter of the root.

Reagents and materials

For dilution, demineralized water provided by a Milli-Q Plus filter apparatus (Millipore, USA) was used. All chemicals were of analytical reagent grade (Merck, Germany). Element test solutions and calibration standards were prepared from commercially available (Titrisol, Merck, Germany) ml/g stock solution using adjustable micropipettes (Gilson, France). Standards were acidified to 1% with nitric acid. Sapropel -solvent water (pH 6.35), Pelosilt blank-humic acid :30% humic acids, Sapropel from HUMIN TECH Germany Company, for the extraction of humic acid use HCl, NaOH, KOH, KCl, HF, all of them were taken from Merck Germany Company.

Equipment

Flame atomic absorption apparatus model PU 9100X, made in England by Philips Company, VGA 77 made in Australia by Varian Company, XRF apparatus model D&Advance made in Germany by Broker Company, extraction of humic acid was performed agitator (ELM 1400 rpm, Germany). FT-IR apparatus model 460 plus, made in JASCO Company (Japan). Acidic functional groups of humic acid were determined using a digital pH meter CD620 with glacial calomel electrode (Zag shimi, Iran)**.**

Extraction of humic acid

We extracted humic acid from the soil of Naharkhoran forest of Gorgan in north of Iran, according to IHSS (International Humic substances society) protocol (Stevenson , 1994., Davies et al,2001) and then purified it. Since Sapropel has not more than 30% humic acid, our extracted humic acid has a stronger effect.

To sprinkle humic substances

We solved the Sapropel of a 20g package in 400 ml water then sprinkled them along with 3 litter water on the base of root a little on plant leaves in an area about half acre in Mars when the plants make up from winter sleep and start to grow. In this area, some plants did not receive Sapropel; accordingly, they were considered as blank samples.

Table 1. Identification of the amounts of mineral elements in the blank soil and soil of growing place by Flame atomic absorption

Metal ion	The amount of metal ion in blank soil of greenhouse university (dry mg/kg) in)	The amount of metal ion in growing place soil of greenhouse university (dry mg/kg) with humic substances	The amount of metal ion in blank soil (dry mg/kg) in condition of natural(Zahedan)	The amount of metal ion in growing place soil (dry mg/kg) in condition of natural(Zahedan) with humic substances	The amount of metal ion in blank soil (dry mg/kg(Zarand)	The amount of metal ion in growing place soil (dry mg/kg) in condition of natral(Zarand) with humic substances
Cr	1.67	0.97	1.05	1.40	1.30	1.35
Mn	230.82	161.70	144.15	144.17	120.55	195.67
Fe	147.88	123.80	123.78	124.08	123.88	124.48
Co	3.40	2.80	2.82	3.42	3.15	4.72
Ni	5.45	4.45	4.72	5.22	5.10	5.77
Cu	0.80	0.57	0.55	0.65	0.75	0.80
Zn	20.70	66.33	24.32	34.65	33.54	30.83
Ag	1.12	0.80	0.62	0.72	0.72	0.80
Cd	1.00	0.75	0.90	1.00	1.02	1.57
Pb	4.24	5.05	3.77	5.10	4.51	4.75

METHOD

Two samples of five gram leaf powder were weighed. The first sample was mixed in the environment of nitric acid and perchloric acid (on a ratio of 1;2) by wet approach and was heated at 200^0 C for four hours. After refining, the product was placed into a 50ml juju balloon and reached double-distillation volume by water. The pH of the solution was one existing mineral elements of solution except for Arsenic (VGA-AAs) which were measured using Flame atomic absorption technique. The second sample was put into the oven at 400 ^{0}C for

four hours then the obtained ash was measured and weighed as 0.80 g. The best temperature for providing the ash sample is 400 ^{0}C in which the measurement of metal ions of samples is performed best with the least error mineral elements and oxides contained in resulted ash were measured with the tablet by XRF apparatus.

Table 2. Identification of the amounts of mineral elements in the leaf of plant (*A.triphylla*) and growing place soil from greenhouse university by Flame atomic absorption

Metal ion	The amount of metal ion in blank soil (dry mg/kg)	The amount of metal ion in growing place soil (dry mg/kg)	The amount of metal ion in the leaf of plant (dry mg/kg)
Cr	1.67	1.30	2.75
Mn	230.82	211.80	238.72
Fe	47.78	38.18	281.58
Co	3.40	3.27	5.25
Ni	5.45	5.57	4.75
Cu	0.80	0.65	3.92
Zn	20.70	18.62	80.60
Ag	1.12	0.80	1.1 7
Cd	1.00	0.90	1.37
Pb	5.05	4.57	5.45

Table 3. Identification of the amounts of metal oxide in the leaf of plant and growing place soil using XRF technique

metal oxide	Weight percent of metal oxide in the leaf of plant	Weight percent of metal oxide in blank soil	Weight percent of metal oxide in soil of growing place soil
Na_2O	0.57	0.70	0.94
MgO	<0.21	1.39	2.42
Al_2O_3	<6.71	10.98	10.55
SiO_2	<33.81	46.77	45.66
K_2O	>5.19	2.41	2.31
CaO	>7.52	>7.52	>7.52
Fe_2O_3	<1.89	5.21	5.19
TiO_2	<0.23	0.70	0.68

Table 4.Identification of the amounts of mineral elements in blank soil and growing place soil using XRF technique

Metal ion	The amount of metal ion in the leaf of plant of greenhouse university (dry mg/kg)	The amount of metal ion in blank soil (dry mg/kg) in greenhouse university	The amount of metal ion in growing place soil (dry mg/kg)
Sr	237.20	518.20	514.3 0
Sn	4.40	4.00	4.20
Ba	<185	488.30	564.40

Table 5. Identification of the amounts of nitrogen in the leaf of plant and growing place soil

Sample	N% in June	N% in September
The leaf of plant of greenhouse university without humic substances(Sapropel)	-	1.78
The leaf of plant of greenhouse university with humic substances(Sapropel)	-	3.04
Blank soil of greenhouse university	0.002	-
growing place soil of greenhouse university without humic substances(Sapropel)	0.029	0.020
growing place soil of greenhouse university with humic substances(Sapropel)	0.027	0.019
growing place soil of greenhouse university with extracted humic acid	-	0.022
growing place soil of environmental condition(Zahedan) without humic substances(Sapropel)	0.036	0.016
growing place soil of environmental condition (Zahedan)with humic substances(Sapropel)	0.021	0.015
growing place soil of environmental condition (Zarand) without humic substances(Sapropel)	0.039	0.032
growing place soil of environmental (Zarand) with humic substances(Sapropel)	0.025	0.025

Table 6. Decomposition of blank soil and growing place soil

N_0	Type of soil	%humic substances
1	Blank sample of greenhouse condition of June 2010	0.19
2	Humic sample(growing place soil)from greenhouse condition of June 2010	0.26
3	Blank sample of greenhouse condition of September 2010	0.19
4	Humic sample(growing place soil) from greenhouse condition of September 2010	0.29
5	Exracted humic acid sample(growing place soil) of greenhouse condition of September 2010	0.35
6	Blank sample of Zahedan environmental conditions of June 2010	0.21
7	Humic sample(growing place soil) of Zahedan environmental conditions of June 2010	0.28
8	Blank sample of Zahedan environmental conditions of September 2010	0.20
9	Humic sample(growing place soil) of Zahedan environmental conditions of September 2010	0.27
10	Blank sample of Zarand environmental conditions of June 2010	0.24
11	Humic sample(growing place soil) of Zarand environmental conditions of June 2010	0.30
12	Bank sample of Zarand environmental conditions of September 2010	0.23
13	Humic sample(growing place soil) of Zarand environmental conditions of September 2010	0.32

Table7. Comparison longer of stem and leaf in find growing stage

N_0	Sampling	Max long of stem(cm)	Max long of leaf (cm)
1	Blank sample of greenhouse condition of June 2010	108	9
2	Humic sample(growing place soil) of greenhouse condition of June 2010	116	10
3	Blank sample of greenhouse condition of September 2010	113	10
4	Humic sample(growing place soil) of greenhouse condition of September2010	122	10.50
5	Extracted humic acid sample(growing place soil) of greenhouse condition of September 2010	125	10.50
6	Blank sample of (Zahedan)environmental conditions of June 2010	68	6.50
7	Humic sample(growing place soil) of (Zahedan)environmental conditions of June 2010	75	8
8	Blank sample of (Zahedan)environmental conditions of September 2010	53	5.50
9	Blank sample of (Zarand)environmental conditions of June 2010	56	10.50
10	Humic sample(growing place soil) of (Zarand)environmental conditions of June 2010	69	11
11	Blank sample of (Zarand)environmental conditions of September 2010	51	8
12	Humic sample(growing place soil) of (Zarand)environmental conditions of September 2010	64	9.50

RESULTS AND DISCUSSION

Given that the composing percentage of heavy metals of each region depends on the minerals of that region, determination and assessment of mineral contaminants which exist in the soil are hard and complicated. So, for measuring a part of heavy metals that is available to plants, the possibility of heavy metal existence in the humus is much more than other compounds existing in the soil. Since the leaves of this plant are used by native people as a medicine, the amount of its metal ions should be controlled. Considering the factors of soil and climate, we planted it under greenhouse and environmental conditions. Then we collected the leaves and the soil of plants growing place at two stages of growth i.e. the last month of spring (June), when the plant is fully matured and first month of fall season, when the plant flowers.Humic substances such as Sapropel and extracted humic acid from soil of Naharkhoran forest

of Gorgan as well as Sapropel made in Germany Company were used at different growth stages in Environments with different soil and climatic conditions.

Table 8. Increased or decreased long of stem or leaf (cm) compared to her leaf blank sample in find growing stage

N_0	Smapling	Increased long of stem or leaf (cm)	Decreased long of leaf or stem(cm)
1	Long of leaf humic sample(growing place soil) of greenhouse condition of June 2010 compared to her leaf blank sample	11.11%	
2	Long of stem humic sample(growing place soil) of greenhouse condition of June 2010 compared to her stem blank sample	7.41%	
3	Long of stem humic sample(growing place soil) of greenhouse condition of September 2010 compared to her stem blank sample	7.97%	
4	Long of leaf humic sample(growing place soil) of greenhouse condition of September 2010 compared to her l eaf blank sample	5%	
5	Long of stem extracted humic acid sample(growing place soil) of greenhouse condition of September 2010 compared to her stem blank sample	10.62%	
6	Long of leaf exracted humic acid sample(growing place soil) of greenhouse condition of September 2010 compared to her leaf blank sample	5%	
7	Long of stem of humic sample(growing place soil) (Zahedan)environmental conditions of June 2010 compared to her stem blank sample	10.29%	
8	Long of leaf of humic sample(growing place soil) (Zahedan)environmental conditions of June 2010 compared to her leaf blank sample	23%	
9	Long of stem of blank sample (Zahedan)environmental conditions of September 2010 compared to stem blank sample June 2010	22.06%	
10	Long of stem humic sample(growing place soil) of (Zarand)environmental conditions of June 2010 compared to her stem blank sample	23.21%	
11	Long of leaf humic sample(growing place soil) of (Zarand)environmental conditions of June 2010 has increased compared to her leaf blank sample	4.76%	
12	Long of stem humic sample(growing place soil) of (Zarand)environmental conditions of September 2010 compared to her stem blank sample	24.07%	
13	Long of leaf humic sample(growing place soil) of (Zarand)environmental conditions of September 2010 compared to her leaf blank sample	18.75%	
14	Long of stem of blank sample (Zarand)environmental conditions of September 2010 compared to stem blank sample June 2010		23.81%
15	Long of leaf of blank sample (Zarand)environmental conditions of September 2010 compared to leaf blank sample June 2010		8.93%
16	Long of leaf of blank sample (Zahedan)environmental conditions of September 2010 compared to leaf of blank sample June 2010	15.38%	

Table 1 shows that chelating property of humic substances, at early stage of plant growth, provent heavy metal ions such as Pb and cd that decreases the quality of effective substances of medicinal plants. By measuring the pH of the soil, it was proved that all conditions of the first and last stages of plant growth changed from 7.90 to 8.50. Table 2 shows the amounts of mineral elements in the leaf of plant and growing place soil and blank soil (the greenhouse of the university) by Flame atomic absorption. The identified 4.75 dry mg/kg for

Ni in plant and 5, 57 dry mg/kg for Ni in soil of growing place and 5.45 dry mg/kg for Ni in blank soil. The results show the quantities of metal ions in Table 1 being less than those of metal ions in table 2 that were used humic substances. Table 3 shows the identifying metallic oxides in the leaf of plant and growing place soil and blank soil by XRF technique. The identified 0.57 dry mg/kg for Na_2O in plant and 0.94 dry mg/kg for Na_2O in soil of growing place and 0.70 dry mg/kg for in blank soil. Table 4 shows the measures of heavy metal ions in different environments by XRF technique. The measurement of plant and soil nitrogen indicates (Table 5) that the addition of humic substances to the soil of root results in an increase in the amount of plant and soil nitrogen at the early stage of plant growth that implies the fertilizing property of humic substances in June for blank soil of greenhouse condition and in blank soil of greenhouse condition of September were 0.19%. The of humic substances in June for soil of growing place that after the addition of Sapropel, it was 0.26% and in September, it was 0.29%(Table 6).

The conditions in greenhouse are stable; this rule only stands for humic sample of greenhouse plants, picked up in September. Average temperature of Zahedan from March to June ranges between18.5°C and 2.76 ^{0} C, while from July to September, it varies between 30.2°C and 23.7°C i.e. the average temperature is less in September than that in June. The case is as the same in "khanok", where the average temperature is lower than that in Zahedan. The atmospheric moisture in khanok region ranges from35%in March to 26% in September, while in Zahedan, it ranges from 35% in March to15% in September. It means that "khanok"'s particular environmental conditions for quince growing are more suitable than those for Zahedan by sprinkling humic substances to the soil around the root of the plant.

The results of soil elemental analysis are exactly consistent with measurements of lengthes of stem and leaf in all conditions (Table 7), for example, in the sample of greenhouse plant. was exposed to extracted humic acid a yearly growth stage , stem with respect to proper climatic conditions for plant growth, June sample of Zahedan's environmental conditions grew more than that of khanok. However, because of unsuitable climatic conditions, samples of September grew less than khanok's samples. But in the case of university greenhouse samples, because of stable climatic conditions, samples of September grew better than samples of June.

CONCLUSION

Controller or monitoring metal ions contaminants in environment is very important for the health of humans and also other living organisms. Implies decrease in heavy metal ions in plant leaf(Sowa,2003).According to tables 1 and 2, addition of humic substances to root soil that results in humic substances of soil increase, is another reason for heavy metals removing and better growing. Measurement of the rate of humus of growing place soil leaf and soil mineral analysis is fully consistent with an increase in stem and leaf length in all planting environments. In the sample of greenhouse plant, to soil around the root with humic acid was sprinkled in late June, length of stem was 125cm in early September, by 2010 that had increase of 10.25 % comparing to the length of the control stem and had an increase of 15.74% comparing with control stem in June (Tables 7& 8). Soil of the greenhouse of the university which had been sprinkled by Sapropel nitrogen level decreased at the time of leaves collection. While it increased in leaves plant of the plant by 70.78%, relating to humic sample of Zahedan's environmental conditions. Decrease in nitrogen in soil and increase in its amount in the leaves are the result of the effects of humic substances in soil fertility and better growth of plant(increase in lengths of stem and leaf) comparing to blank sample. Anthor advantage of humic substances along with the sample around the root of plant is shortening of flowering stage. Nitrification is sensitive to hot degree and happens mostly at 5 to 40 °C. Its proper level is around 30 – 50 °C as table 7 shows, Sapropel increase results in humus of soil increase. Microbes like azetoloacter which raise the soil nitrogen are considerably sensitive to amount of soil carbone. The more the amount of soil Carbone, the more they activate. The relative standard deviations for all these elements in this method were between 0.60% and 8.40%**(** Tivy,1993)**.**

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