Examination of Correlation Between Lead and Histidine in *Eleagnus angustifolia* L., *Vitis vinifera* L., and *Nerium oleander* L. Using ICP-MS and HPLC-MS

Sukran Akkus Ozen and Mehmet Yaman*

Firat University, Faculty of Science, Department of Chemistry, Elazig, Turkey

INTRODUCTION

Over the past two centuries, anthropogenic and industrial activities have led to high emission of toxic metals into the environment at high concentrations. Because metals emitted into the environment are toxic and non-biodegradable (unlike organic compounds), the removal of excess metal ions from polluted sites is important. Lead is utilized in many industrial activities and is considered a major contaminant in most industrial factories. Lead, as with most of the heavy metals, can be harmful to humans and animals and tends to bioaccumulate through the food chain (1-4). Thus, numerous efforts have been undertaken to find methods for removing heavy metals from soil. Among those methods, conventional (excavation), chemical remediation, phytoremediation, soil washing, nano materials and remediation with bacteria are used (5-7). The excavation method is neither economical nor practical. Because chemical remediation methods involve the use of chemicals, this method is not in universal use, is very costly and may cause secondary pollution (8).

Phytoextraction (in other words, phytoremediation) is the removal of metals from soil using hyperaccumulator plants. Phytoextraction or phytoremediation is 1000-fold less costly than conventional remediation methods and is also environmentally friendly (8-9). The use of hyperaccumulator plants opens a new branch of phytoremediation technology that is scientific in its approach to remove, extract, or

ABSTRACT

In this study, ICP-MS and HPLC-MS methods were used for the determination of lead and histidine, respectively, in the leaves of the plant species *Eleagnus angustifolia* L., *Vitis vinifera* L. and *Nerium oleander* L., sampled at industrial areas, including the cities of Gaziantep and Bursa, Turkey. In the determination of histidine by HPLC-MS, all of the system parameters such as flow rate of the mobile phase, fragmentation potential, injection volume and column temperature were optimized and found to be 0.2 mL min⁻¹, 70 V, 15 μ L and 20 °C, respectively. Histidine was extracted from the plant sample by ultrapure water at 90 °C for 30 minutes.

The concentrations of histidine were found to be in the range of 3–23 mg kg⁻¹ for *Eleagnus angustifolia* L., 2–33 mg kg⁻¹ for *Vitis inifera* L., and 3–11 mg kg⁻¹ for *Nerium oleander* L. The concentrations of Pb were in the range of 1–45 mg kg⁻¹ for *Eleagnus angustifolia* L., 1–32 mg kg⁻¹ for *Vitis vinifera* L. and 1–53 mg kg⁻¹ for *Nerium oleander* L. A linear correlation between Pb and histidine for *Eleagnus angustifolia* L. at $r=0.77$ was found.

inactivate metal ions in the soil using plants (5, 10-11). In phytoremediation, the basic concept is as follows: Growing and harvesting of plants in the polluted soils, burning of plants and smelting or storing of the ash. Hyperaccumulators often exhibit higher metal concentrations in their tissues than are present in the soil and can tolerate high metal concentrations without symptoms of toxicity (12).

Plant ligands play a role in the sequestration of metals from soils, transport to the above-ground tissue, and finally storage (13). Nitrogen-donor ligands, and especially free amino acids including histidine and cysteine, organic acids, metallothioneins and phytochelatins, are assumed to play a role in hyperaccumulators (10-14). While there are many metal-binding biomolecules, this study focuses only on the histidine ligand which plays a role in sequestering, transporting or storing of the accumulated metal, and serves as a tridentate ligand via its carboxylate, amine, and imadazole functions. As a result, future discovery of new hyperaccumulator plants is of great importance. In terms of the known toxicity of elements, most concern to date has been focused on Cu, Cd, Pb, and Ni found in plants (15-20).

The aim of this study was, first, to examine the correlation between Pb and histidine in plant leaves, such as *Eleagnus angustifolia* L., *Vitis vinifera* L., and *Nerium oleander* L. Second, to establish the extent of Pb pollution in two industrialized cities in Turkey, namely Gaziantep and Bursa, using these three plant species. For this purpose, the ICP-MS and HPLC-MS techniques were applied. The representative locations were industrial zones, including an obsolete lead battery production site, a cement factory, and other industrial factories found within these cities.

EXPERIMENTAL

Instrumentation and Reagents

The concentrations of Pb were determined using a PerkinElmer® ELAN® 9000 inductively coupled plasma mass spectrometer (ICP-MS)

*Corresponding authors:
ijpacmy@gmail.com

(PerkinElmer, Inc., Shelton, CT, USA). The ICP-MS operating conditions were taken from the manual handbook and are listed in Table I. A CEM MARS-X-press microwave digestion system (CEM Corporation, USA) was used to digest the samples before analysis. Ultrapure water, obtained with a Direct-Q® water purification system (Millipore Corporation, USA) was used for the preparation of all samples and standards.

An Agilent 1200 HPLC-MS system was used for the determination of histidine (Agilent Technologies, USA). The instrument was equipped with an autosampler, a binary pump, a temperature-controlled column oven, and an Agilent 6110 MS detector, which was operated in the selected ion monitoring and scan mode equipped with positive ion electrospray ionization. The HPLC effluent entered the mass

spectrometer through an electro-spray capillary at 3000 V. Nitrogen was used as the drying and vaporizer gas at 300 and 500 °C, respectively. The drying gas flow rate was 11.0 L min⁻¹ (Table I). An Agilent Zorbax Eclipse XDB-C18 column (4.6 mm, 150 mm, and 5 µm particle sizes) was used. Unless otherwise stated, all chemicals used throughout the study were of high-purity reagent grade. Concentrated nitric acid (65%, Merck, Germany) was used in the digestion procedure. The lead stock solution (1000 mg L⁻¹) was prepared from its nitrate salt (Merck).

Sampling and Sample Preparation

Eleagnus angustifolia L., *Vitis vinifera* L. and *Nerium oleander* L. leaves were collected in Gaziantep and Bursa, cities with populations of 1,500,000 and 2,000,000, located in SE and NW Turkey, respectively,

with important industrial centers (see Figure 1). The locations of the plant samplings were also obtained by modification from Google Map. The control samples were collected away from urban and industrial areas in the summer of 2011. About 100 g of healthy, fresh leaves were taken from each site. The leaves were transferred to the laboratory in plastic bags, washed with tap water, and rinsed with ultrapure water. After drying at 70 °C, the samples were ground using an agate mortar and then homogenized.

ICP-MS Analysis

In the digestion step, a 0.3 g sample was transferred to a Teflon® vessel and 5.0 mL of concentrated nitric acid added. Then, the mixture was irradiated at 1600 W (80% power) and 200 °C for 15 minutes and at 1600 W (50% power) and 170 °C for 15 minutes in accordance with the instructions provided in the microwave oven manual. The cooled solution was heated until near dryness. After addition of 20 mL of 0.1 M nitric acid, the solution was filtered, if necessary (a precaution due to the high sensitivity of the ICP-MS with the particles), and the clear solution was analyzed for Pb by ICP-MS. Each sample was analyzed in triplicate.

HPLC-MS Analysis

The HPLC-MS method was optimized for the determination of histidine in *Eleagnus angustifolia* L., *Vitis vinifera* L., and *Nerium oleander* L. leaves considering the peak area, peak symmetry, and abundance. A suitable mobile phase was investigated, and the best results were obtained using a binary gradient elution of 10% formic acid (mobile phase A) and 90% acetonitrile (mobile phase B). The flow rate of the mobile phase, fragmentor potential, injection volume, and column temperature

TABLE I
Operating Conditions for Histidine and Pb Determination by HPLC-MS and ICP-MS

HPLC-MS Conditions	
Mobile phase	Formic acid 10%, acetonitrile 90%
Mobile phase flow rate	0.2 mL min ⁻¹
Column	Zorbax XDB-C 18 (4.6 mm x 150 mm, 5 µm)
Column temperature	20 °C
Fragmentor potential	70 V
Injection volume	15 µL
Nebulizer pressure	30 psi
Drying gas flow	11.0 L min ⁻¹
Drying gas temperature	300 °C
Vaporizer temperature	500 °C
Capillary voltage	3000 V
ICP-MS Conditions	
Plasma gas flow rate	15 L min ⁻¹
Carrier gas flow rate	0.9 L min ⁻¹
Sample uptake rate	1.0 L min ⁻¹
Nebulizer	Cross flow
Spray chamber	Ryton®, Double pass
Sample uptake rate	1.0 mL min ⁻¹
Plasma power (RF power)	1000 W

were optimized at values of 0.2 mL min⁻¹, 70 V, 15 µL and 20 °C, respectively. To extract histidine from 1.5 g of plant leaves, the following steps were taken: 0.10 M HCl and ultrapure water were heated from 20 to 90 °C and stirred from 15 to 60 minutes. The optimum conditions were found to be ultrapure water at 90 °C and a stirring time of 30 minutes. The volume of ultrapure water was optimized using a constant mass of plant material; the best results were obtained with 30 mL. To determine the best derivatization reagent, direct ultrapure water (underivatization), fenil isothio-

siyanat and dabsyl chloride were examined by using the scheme listed in Table II.

One-way analyses of variance (ANOVA) were conducted to test the equality of the mean values for each plant species of interest. One of the pairwise ANOVA comparison tests, the Tukey HSD, was carried out to find which of the plant means is different from each other depending on location. The IBM® SPSS (version 15, SPSS Inc., IL, USA) statistical program was used for all statistical computations. Statistical significance was considered when P was equal to or higher than 0.05.

RESULTS AND DISCUSSION

Analytical Performance

There are three methods to check the accuracy of the results obtained: (a) the use of Standard Reference Material (SRM), (b) comparison of the results with those obtained by independent methods for the same samples, and (c) the spike recovery test. In this study, the first method was used for the Pb determinations by ICP-MS and the third method was used for the histidine determination by HPLC-MS. The obtained concentration of Pb in SRM NCS DC73348 Bush Branches and Leaves - Trace Elements (China National Analysis Center for Iron and Steel, Beijing, P.R. China) was found to be 6.8 µg g⁻¹ which is close to the certified value of 7.1 µg g⁻¹. Because a recovery of 96% was achieved, the Pb determination in this study was considered to be accurate.

In the HPLC-MS determination for histidine, the recoveries from the plant leaves fortified with 3 mg kg⁻¹ of histidine were employed to check for accuracy. A recovery of 96% was obtained. The effects of contamination were eliminated by subtracting the blank levels from the obtained values.

LOD and LOQ Determination for Histidine and Pb

There are three ways of determining the limits of detection (LOD) and the limits of quantitation (LOQ): (a) based on visual evaluation, (b) based on the signal-to-noise ratio, (c) based on standard deviation of the response and slope. In this study, the third way was applied by using 3x the standard deviation of the low concentration/slope of the calibration line for the LOD and 10x the standard deviation of the low concentration/slope of the calibration line for the LOQ. The limits of detection and quantitation for histidine were found to be 0.025 mg L⁻¹ and 0.075

Fig. 1. Map of sampling locations. (a) B1: Bursa organized industrial zone, in city center; B2: Bursa city, Demirtas organized industrial zone; B3: Bursa city, Nilufer organized industrial zone; B4: Bursa city, Gursu organized industrial zone. G4: Around the obsolete lead battery center, distance 50 m; G1: Gaziantep city, 200 meters South direction away from G4; G2: Gaziantep city, 400 meters West direction away from G4; G3: Gaziantep city, 200 meters North direction away from G4; G5: Gaziantep city, 200 meters West direction away from G4; G6: Gaziantep city, 1000 meters Northwest direction away from G4.

mg L⁻¹, respectively. The linear dynamic range using histidine standards extended from 0.075 to 5.0 mg L⁻¹ using the optimized conditions with a correlation coefficient of 0.9999. Using ICP-MS, the LOD and LOQ for Pb were found to be 0.1 and 1 ng L⁻¹, respectively. Related to the derivatization reagent, it was found that dabsyl chloride is the best reagent.

The concentrations of Pb were found to be in the range of 1–45 mg kg⁻¹ for *Eleagnus angustifolia* L., 1–32 mg kg⁻¹ for *Vitis vinifera* L., and 1–53 mg kg⁻¹ for *Nerium oleander* L. The concentrations of histidine were found to be in the range of 3–23 mg kg⁻¹ for *Eleagnus angustifolia* L., 2–33 mg kg⁻¹ for *Vitis vinifera* L., and 3–11 mg kg⁻¹ for *Nerium oleander* L.

Kabata-Pendias (2011) (21) considered the Pb concentrations in plants at a value lower than 10 mg kg⁻¹ as normal or acceptable, while Allen (1989) (22) considered the much lower value of 3 mg kg⁻¹ Pb. Kabata-Pendias (21) also considered 30 mg kg⁻¹ of Pb as an excessive or toxic level, and reported that 43

mg kg⁻¹ of Pb is the threshold value causing death to trees.

Hyperaccumulation has been recognized as an extreme physiological response in heavy metal tolerance. In other words, hyperaccumulator plants can tolerate much higher metal concentrations without symptoms of toxicity (12, 23–25). However, the physiological processes involved in hyperaccumulation are not well understood. Plants must be able to store the metal ions in nonlabile complexes to eliminate toxic effects. The most likely areas for storage are the cell wall, the cytosol, and the vacuole. A number of steps are required for metal ions to reach the storage tissues: mobilization and uptake from soil, compartmentation and sequestration within roots, transfer to the xylem for transport, sequestration and storage in leaf cells (26). It must also be noted that the distribution between metals changes in above-ground tissue and each stage the accumulation of metals. The hyperaccumulator may be detoxifying the metal in the leaves via strong binding ligands. Thus, the

ligand including histidine, cysteine, and phytate may play a part in the sequestration within isolated compartments (10–14).

Kaya et al. (15, 19–20) reported that Pb concentrations in *Eleagnus angustifolia* L., *Vitis vinifera* L., and *Nerium oleander* L. leaves growing in areas around a lead battery factory were up to 2750 mg kg⁻¹ (in the range of 6.2–2750), 193 mg kg⁻¹ (in the range of 1.4–193) and 2820 mg kg⁻¹ (in the range of 1–2820), respectively. They obtained these results during the period from 2006 to 2007 when the battery factory was still in operation. Four years later, after that factory closed, our group collected plant samples from this area for the current study, and the obtained values are given in Table III and Figures 2 and 3. The correlation coefficient between Pb and histidine concentrations was $r = 0.77$ for *Eleagnus angustifolia* L., $r = -0.05$ for *Vitis vinifera* L. and $r = 0.44$ for *Nerium oleander* L. Hence, a significant linear correlation of $r = 0.77$ was found for *Eleagnus angustifolia* L.

It has also been reported that metal concentrations in plants change depending on the plant species, polluted source, and the wind direction. Onianwa and Fakayode (27) determined trace metal levels in topsoil and vegetation (*Cromonolona odorata*, a composite plant) sampled in the vicinity of a lead battery manufacturing plant located in Ibadan, the largest city in Nigeria. They found that the Pb concentrations in the soil and plants changed significantly depending on wind direction: 2010 and 1350 mg kg⁻¹ from NW, 4100 and 970 mg kg⁻¹ from SW, 950 and 3640 mg kg⁻¹ from NE, and 3010 and 1710 mg kg⁻¹ from W, respectively, while the Pb concentrations in the plant samples taken from the control site were 10 mg kg⁻¹ (27). They concluded that Pb levels in plants did not depend on the soil-Pb concentra-

Table II
Analytical Steps for Histidine Determination

-
- * Sample (1.5 g)
 - * Distilled water added (30 mL)
 - * Mixed at 90 °C for 30 min
 - * Filtered
 - * Dried at room temperature. for 24 h
 - * HCl added (5 mL of 0.1M)
 - * Placed in vortex mixer
 - * Reaction buffer (sodium hydrogen carbonate 0.15 M, pH 8.6)
 - * Placed in vortex mixer
 - * Placed in a water bath at 70 °C for 15 min
 - * Added – 8 mL dilution buffer (mixture of 50 mL acetonitrile + 25 mL ethanol + 25 mL sodium dihydrogenphosphate, 9 mM dimethylformamide 4% and triethylamine 0.15% [pH 6.55 with phosphoric acid])
 - * Centrifugation at 10,000 rpm for 10 min
 - * Filtered through (0.45 µm) nylon membrane
 - * Analysis with HPLC-MS
-

TABLE III
Pb and Histidine Concentrations in the Studied Samples (mg kg⁻¹)

Plant Species	Sampling Site	Histidine	Pb	Histidine Range	Pb Range	^a Pb Range 2006-2007
<i>Eleagnus angustifolia</i> L.						
	G1	23±2	45±6	3-23	1-45	6.2-2750
	G2	15±3	8±1			
	G3	14±2	38±4			
	B1	12±2	1.0±0.1			
	B2	11±2	2.0±0.2			
	B3	8±1	1.0±0.1			
	Control	3.0±0.3	1.0±0.1			
<i>Vitis vinifera</i> L.						
	G1	10±2	32±4	2-33	1-32	1.4-193
	G4	33±6	27±3			
	G5	11±2	29±3			
	B2	27±6	2.0±0.2			
	B3	25±5	1.0±0.1			
	Control	2.0±0.2	1.0±0.2			
<i>Nerium oleander</i> L.						
	G3	11±2	12±2	3-11	1-53	1-2820
	G4	9±1	53±8			
	G6	6±1	5±1			
	B2	8±1	2.0±0.2			
	Control	3.0±0.3	1.0±0.1			
SRM: Bush Branches and Leaves – NCSDC73348						
	Certified		7.1±1.1			
	Found		6.8±1.0			

^a These values were taken from references 15, 19, 20.

tion, but these results were attributed to the wind direction. Furthermore, hyperaccumulation in a number of species appeared to be the result of airborne contamination of the leaf surface, rather than root uptake and translocation.

Boyd (28) reviewed the interaction between heavy metal pollutants and chemical ecology but concluded that communities and ecosystems are difficult to study due to their complexity. However, a complete understanding of metal pollutant effect is important to help limit the harmful effects of anthropogenic heavy metal pollutants on the Earth's biota. Due to the high levels and toxic effects of Cu, Ni, Cd, and Pb, numerous studies have been carried out over the past 10 years to determine their concentrations in the environment, food and biological matrices (29-34). But much more research needs to be done.

CONCLUSION

Lead concentrations of up to 53 mg kg⁻¹ were found in the leaves of *Nerium oleander* L. Other plants

Fig. 2. Comparison of Pb and histidine levels in *E. angustifolia* L. depending on sampling location.

Fig. 3. Comparison of Pb and histidine levels in *V. vinifera* L. depending on sampling location.

such as *Eleagnus angustifolia* L. and *Vitis vinifera* L. were sampled from a 50-meter distance around an obsolete lead battery manufacturing plant and in other industrial cities in Turkey. The lowest Pb concentration in this plant species was 1.0 mg kg⁻¹. It was found that the ratio of highest to lowest Pb concentration for *Nerium oleander* L. was 53-fold, which implies that the leaves of this plant are a potential biomonitor and/or hyperaccumulator. The linear correlation between Pb and histidine for *Eleagnus angustifolia* L. was found to be $r=0.77$.

ACKNOWLEDGMENT

This study was financially supported by the Scientific Investigate Projects of Firat University, Turkey (Project Number: FF.11.19).

Received November 24, 2015.

REFERENCES

1. W. Mertz, Academic Press, New York, USA, Fifth Ed. (1987).
2. M. Yaman, Curr. Med. Chem. 13(21), 2513 (2006).
3. S.J.S. Flora, J. Environ. Biol. 23 (1), 25 (2002).
4. M.D. Mingorance, S. Rossini Oliva, Environmental Monitoring and Assessment 119, 57 (2006).
5. C.N. Mulligan, R.N. Yong, and B.F. Gibbs, Engineering Geol. 60, 193 (2001).
6. B. Gunawardana, N. Singhal, and A. Johnson, Plant Soil 329, 283 (2010).
7. E. Pilon-Smils and M. Pilau, Critical Reviews in Plant Sciences 21, 439 (2002).
8. M. Yaman, Ekoloji 23(90), 81 (2014).
9. U. Krämer and A.N. Chardonnens, Appl. Microbiol. Biotechnol. 55, 661 (2001).
10. M.J. Haydon and C.S. Cobbett, New Phytol. 174(3), 499 (2007).
11. D. L. Callahan, A. J. M. Baker, S. D. Kolev, and A. G. Wedd, J. Biol. Inorg. Chem. 11, 2 (2006).
12. L. Hunt, C.W.N. Anderson, N. Bruce, A.M. García, et al., Green Processing and Synthesis, 3(1), 3 (2014).
13. J. L. Hall, Journal of Experimental Botany 366, 1 (2002).
14. U. Krämer, J.D. Cotter-Howells, J.M. Charnock, et al., Nature 379, 635 (1996).
15. G. Kaya, N. Okumus, and M. Yaman, Fresenius Environ. Bull. 19(4), 669 (2010).
16. G. Kaya and M. Yaman, Trace Elements and Electrolytes 25(3), 156 (2008).
17. G. Kaya and M. Yaman, Talanta 75, 1127 (2008).
18. G. Kaya, C. Ozcan, and M. Yaman, Bull. Environ. Contam. Toxicol. 84(2), 191 (2010).
19. G. Kaya and M. Yaman, Spectrosc. Spectral Anal. 32(1), 229 (2012).
20. G. Kaya and M. Yaman, Instrumentation Science & Technology 40(1), 61 (2012).
21. A. Kabata-Pendias, Trace Elements in Soils and Plants, Fourth Edition, Taylor and Francis Group (2011).
22. S.E. Allen (Eds.), Chemical Analysis of Ecological Materials, Oxford: Blackwell Scientific Publications, UK (1989).
23. R. Bargagli, Plants as Biomonitors, in: Trace Elements in Terrestrial Plants: an Ecophysiological Approach to Biomonitoring and Biorecovery. Springer, Berlin-Heidelberg-New York (1998).
24. A. Mulgrew and P. Willeams, Biomonitoring of Air Quality Using Plants, Air hygiene report no:10 Berlin, Germany, WHO CC. 165, (2000).
25. J. Mertens, S. Luyssaert, and K. Verheyen, Environmental Pollution 138, 1 (2005).
26. S. Clemens, M.G. Palmgren, and U. Kramer, Trends Plant Sci. 7, 309 (2002).
27. P.C. Onianwa and S.O. Fakayode, Environ. Geochem. Health 22, 211 (2000).
28. R.S. Boyd, J. Chem. Ecol. 36(1), 46 (2010).
29. M. Yaman, G. Kaya, and H. Yekeler, World J. Gastroenterology 13(4), 612 (2007).
30. C. Er, B.F. Senkal, and M. Yaman, Food Chem. 137(1-4), 55 (2013).
31. O.A. Ozen, A. Songur, M. Sarsilmaz, M. Yaman, and I. Kus, J. Trace Elem. Med. Biol. 17(3), 207 (2003).
32. M. Yaman and N. Cokol, At. Spectrosc. 25(4), 185 (2004).
33. M. Yaman and S. Bakirdere, Mikrochim. Acta 141, 47 (2003).
34. I. Ugulu, Appl. Spectrosc. Reviews 50, 113 (2015).