

## Specification of antimony and its inorganic species in grass samples along an Austrian motorway by HPLC-ID-ICP-MS

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### Abstract

*In this study the distribution of Sb concentrations and Sb species (Sb(III) and Sb(V)) in grass samples in a roadside profile were investigated. The Sb depositions on the grass surfaces are higher than the bioaccumulations of Sb in grass samples where it reaches two-fold concentrations at locations nearest to the traffic. The Sb species distribution, after a chromatographic separation, was determined using on-line isotope dilution (ID). The extraction of Sb species from contaminated grass samples was carried out using 100 mmol L<sup>-1</sup> citric acid (pH 2.08). The concentrations of Sb species in grass show decrease with increasing distance from traffic lane with a sharp drop within the first two meters. Sb(V) concentration is at least two folds higher than Sb(III) in grass samples near the road edge. The total and bioaccumulated Sb(V) species concentrations sharply decrease with distance from the road edge, while Sb(III) concentration also decrease with distance but less pronounced as for Sb(V). Sb(III) species which is the more toxic species, could be detected in all the grass samples (washed and unwashed) independent of the sampling distance from the edge of the road. It was also observed that the majority of inorganic Sb species can be washed out by applying water to the grass.*

**Keywords:** Antimony, Speciation analysis, Isotope dilution, Grass samples, Traffic roads

## Introduction

The antimony toxicity and its compounds have been a global concern (Mihucz V.G., Zaray G., 2016), Antimony is a non-essential element in plants, animals and humans but causes toxic effects. The toxicity of Sb depends on its molecular form, where inorganic Sb compounds are more toxic than Sb organic compounds. Antimony toxicity also depends on its oxidation state; Sb(III) compounds are ten times more toxic than Sb(V) compounds (Filella et al., 2009).

The major sources of environmental contamination of antimony are from smelters, coal combustion, and incineration of waste and sewage sludge. Additionally, soils from agricultural regions are often contaminated with antimony due to the use of phosphate fertilizers, which generally have high concentrations of toxic elements, such as cadmium, mercury, antimony, arsenic and others (Shtangeeva I. et al., 2014), accumulation of Sb and other potentially toxic elements (mainly As, Pb and Cu) in plants growing around a former Sb mine. Road traffic has been identified as a source of Sb release into the environment. Known sources are compounds used as fire retardants in brake linings and from Sb-containing additives employed during the vulcanization of tyres (Krachler et al., 1999). The close association of traffic emissions with Sb that shows significant decreases with distance from roadways in soil and grass (Dietl et al., 1996). (Helmets et al., 1996) explain the sharp decrease of Sb with increasing distance from the emission source, through the contribution of large Sb bearing particles, probably originating from the abrasion of motor vehicles surfaces, such as braking linings. The bioaccumulation of antimony in dry mass of standardized grass cultures after antimony deposition onto the grass surface was first observed in grass foliage in Munich (Peichl et al., 1996). Active biomonitoring at nineteen locations at varying distance to traffic suggested that antimony emissions seem to be closely associated with traffic impacts (Peichl et al., 1994). Correlation analysis revealed a positive linear dependency of antimony bioaccumulation vs. deposition, which was found to be significant (Dietl et al., 1996).

Many analytical methods have been described for the speciation analysis of Sb(III) and Sb(V). Much attention is focused on hyphenating a HPLC and ICP-MS (Lintschinger et al., 1998; Lindemann et al., 2000; Zheng et al., 2000b; Krachler et al., 2001). The capability of High Performance Liquid Chromatography- Inductive Coupled Plasma – Mass Spectroscopy (HPLC-ICP-MS) system to perform isotope ratio measurements and consequently isotope dilution mass spectrometry (IDMS) was used by (Amereih et al., 2005a) to determine Sb(III) and Sb(V) and they applied it to study antimony speciation in roadside soil samples (Amereih et al., 2005b).

Since there are few studies reporting species distribution of bioaccumulated Sb, grass samples that grew on the contaminated sites were studied in this work. In this work, an existing HPLC-ID-ICP-MS procedure (Amereih et al., 2005a) was modified to be applied for grass samples to study the environmental effect of vehicles emissions on the species distribution of bioaccumulated or deposited antimony in or on roadsides grass samples.

## Experimental

### Grass samples

Grass was sampled on June 2005 near the highway S36 at Knittelfeld-Austria. The sampling transect is located at the exit of the highway to a refueling station, hence the abrasion of brake lining through vehicles leaving the exit while slowing down will be emitted. The grass was cut just above soil surface. Three composite samples were collected. Each sample consisted of 30 portions taken along parallel lines at three different distances from the edge of the road (0.2, 2 and 10 m respectively). The subsamples represented a road section of about 50 m. The composite grass samples were transported in clean polyethylene bags and dried in the laboratory at ambient air temperature. The dried laboratory samples were grinded using a mixer mill and then split to test samples.

### Chemicals and reagents

For preparation of all solutions MilliQ system ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) water was used. Hydrogen peroxide (30 %m/m) and nitric acid (70 %m/m) of analytical grade obtained from Merck were used for digestion of grass samples

Extraction solutions were prepared from analytical grade reagents: citric acid monohydrate (99.5-100.5 %m/m, Merck), EDTA-disodium Salt (99.0-101.0 %m/m, Baker), methanol (99.8 %m/m, Merck).

Calibration solutions ( $0.2$  to  $50 \mu\text{g L}^{-1}$ ) for Sb were prepared daily by diluting aliquots of an ICP-MS calibration solution (High-Purity standards, Charleston, SC USA)  $10 \mu\text{g mL}^{-1} \pm 0.5\%$  in 2%  $\text{HNO}_3$  + trace HF. Indium ICP-MS solution (High-Purity standards) was used as internal reference in all measured solutions ( $20 \mu\text{g L}^{-1}$  in the final solution).

The HPLC eluents were prepared with adequate concentrations by dissolving appropriate amounts phthalic acid ( $\geq 99.5 \text{ %m/m}$ , Fluka) and EDTA disodium salt (99-101 %m/m, Baker). The pHs of these eluents were adjusted by the dropwise addition of ammonia solution (25 %m/m, Merck) and nitric acid ( $200 \text{ mmol L}^{-1}$ ).

A stock Sb(III) solution (1000 mg L<sup>-1</sup>) was prepared using potassium antimony(III) oxide tartrate hemihydrates (99.0 %m/m, Merck) and potassium hexahydroxoantimonate(V) (≥ 99 %m/m, Fluka) was used for the Sb(V) stock solution (1000 mg L<sup>-1</sup>). An isotopic labeled species specific Sb(V) stock solution with approximately 10 µg g<sup>-1</sup> Sb enriched (94.2%) in <sup>123</sup>Sb was prepared from antimony oxide (Sb<sub>2</sub>O<sub>5</sub>) (Product Number H3451) obtained from Euriso-Top France. The enriched isotope metal <sup>121</sup>Sb(III) (98.73% of 121) has been obtained also from Euriso-top (France) [lot number H2841]. The procedure describing antimony oxide as source material for enriched isotope solution illustrated elsewhere (Amereih et al., 2005a). All solutions containing Sb were stored in dark polymeric containers in a refrigerator at a temperature of 1-4° C.

### **Instrumentation**

A microwave sample preparation system (Multiwave, Anton Paar, Graz) was used for grass samples digestion. An ultrasonic bath obtained from Sonorex, an Eppendorf Centrifuge was used in the extraction procedure. HP4500 inductively coupled plasma quadrupole mass spectrometer (ICP-QMS, Agilent Technologies) using a Babington nebulizer (uptake 0.4 ml min<sup>-1</sup>). For the chromatographic module a quaternary HP 1050 (Agilent Technologies) series pump was used. Separation of Sb(III) and Sb(V) was carried out on a Hamilton PRP-X100 anion exchange column (150 mm x 4.6 mm i.d) with a flow rate of the mobile phases at 1 mL min<sup>-1</sup>.

### **Analytical procedure**

#### ***Sample preparation***

0.6 g of grass test portion sample was placed in high pressure transmitting medium (PTM) vessels together with 5 mL of subboiled conc. HNO<sub>3</sub> plus 2 mL of (30 %m/m) H<sub>2</sub>O<sub>2</sub> and digested using microwave for 35 min. The digestion conditions include a power of 800 W, a maximum temperature 300 °C and a maximum pressure 75 bar. The digested mixture was then transferred to 50 mL polyethylene bottle and diluted to 30 mL mark with highly purified water. To validate the digestion process of grass samples, Peach Leave Standard Reference Material (SRM 1547) a standard reference material was digested parallel with the digestion of grass samples. In order to determine the bio-accumulated Sb in grass samples, test portions of air dried grass samples were washed with highly purified water three times and then the washed grass was digested as described above. This value represents the bio-accumulated Sb

in grass samples. The deposited Sb values on grass surfaces was determined as the difference between the total Sb determined in unwashed grass portion and washed portion.

10 mL of one of the tested extractants (citric acid monohydrate ( $10 \text{ mmol L}^{-1}$ ) EDTA-disodium Salt ( $10 \text{ mmol L}^{-1}$ ), ethanol: water (9: 1 v/v) and water, respectively) were added to 0.5 g of grinded grass in a 50 mL screw-cap polypropylene centrifugation tube. Subsequently, the tube was sonicated for 60 min in an ultrasonic bath at room temperature. The solution was then centrifuged at a speed of 4000 rpm for 5 min and filtered using a  $0.45 \mu\text{m}$  syringe filter.

ICP-MS was used to determine total antimony concentrations in the filtered digested and extracted solutions using external calibration. Further dilutions in the range of 250 to 500-fold were done depending on the expected concentration range. Indium was used as internal reference in all measured solutions ( $10 \mu\text{g L}^{-1}$  in the final solution).

### *Speciation analysis*

Speciation analysis using the system HPLC-ID-ICP-MS was illustrated in detail elsewhere as well as the instrumentations and their operating conditions (Amereih et al., 2005a).

## **Results and discussions**

### **Variation of total Sb in grass samples with distance from roads**

Table 1 gives the results of Sb values in grass samples taken at different distances from traffic road after microwave digestion. The mean values of Sb obtained from 4 replicates for Peach Leaves (SRM 1547) (a NIST standard reference material) show good agreement of the reported value of Sb ( $0.02 \mu\text{g g}^{-1}$ ) (Becker et al., 1989), hence the digestion procedure is very effective in achieving complete digestion.

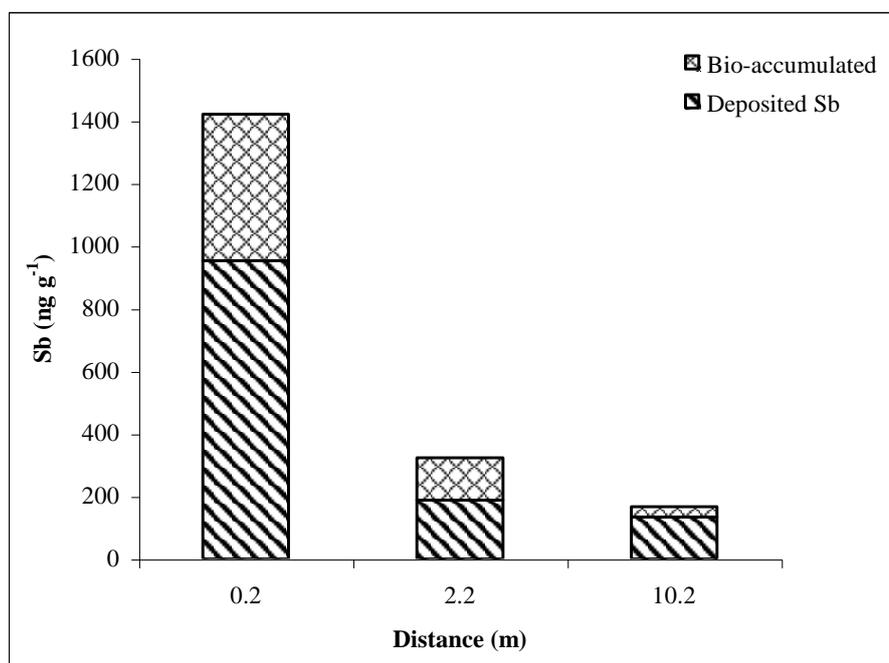
Table 1 and Fig. 1 represent a good correlation between the total Sb content in grass sample and the deposited Sb or the bio-accumulated Sb. All of Sb values (total, deposited and bioaccumulated) show the same distribution pattern. These concentrations sharply decrease with increasing distance from the traffic lane. For these results, similar observations were noticed by (Dietl *et al.*, 1996; Ratcliffe et al., 1984) in grass samples. Antimony impacts are at least partially due to abrasions of tyers and brake linings. The observed sharp decrease of Sb concentrations in grass samples with increasing distance from traffic road is an indication that Sb-bearing particles seemed to have settled out soon after emission and, as expected, are not submitted to meso- scale transport. The Sb depositions are much higher (two-fold) than the bioaccumulations of Sb in grass samples at locations nearest to traffic lane. As the traffic-

derived antimony was shown to be partially removable by washing (Figure 1), there is reason to believe that Sb bioaccumulation observed in this investigation was perhaps due to Sb deposition from ambient air or presumably due to Sb uptake from soil which is confirmed by the similar Sb distribution in the soil samples (Amereih et al., 2005b), where the analyzed grass samples were taken.

**Table 1. Variations of total Sb distribution in grass samples taken along a traffic road (Knittelfeld-Austria).**

Sample	Distance (m)	Mean Sb (ng g <sup>-1</sup> )	SD (ng g <sup>-1</sup> )	Mean Sb (bio-accumulated) (ng g <sup>-1</sup> )	SD (ng g <sup>-1</sup> )	Mean Sb (deposited) (ng g <sup>-1</sup> )
Knittelfeld-L1	0.2	1421.3	233.7	467.4	25.3	953.9
Knittelfeld-L2	2.2	323.7	11.5	135.9	3.3	187.9
Knittelfeld-L3	10.2	167.1	9.8	32.5	6.4	134.6
Peach leaves (SRM 1547)	-	24.6	1.8	-	-	-

- N= 4



**Figure 1. Distribution of deposited Sb and bio-accumulated Sb in grass samples taken along a traffic road (Knittelfeld-Austria)**

### Choosing a suitable extractant for Sb speciation in grass samples

Fig. 2 was constructed by changing the isotope ratio enriched with  $\text{Sb}^{121}$  isotope to mass using on-line isotope dilution technique, which was proposed in details elsewhere (Amereih et al., 2005a). The effect of the kind of extractant on antimony speciation in an unwashed grass sample (L1) taken near the edge (0.2 m) of the traffic road (S36). Different extractants (citric acid, EDTA, (1: 9 v/v) water: methanol and water) were tested in order to choose the suitable extractant, where the values of Sb species are maximum and also can have a preservation influence on Sb species. It is clear in Fig. 2 that 100 mmol  $\text{L}^{-1}$  citric acid (pH 2.08) is the most suitable extractant, since the recoveries of Sb(III), Sb(V) and even an unknown peak are the maximum among the recoveries of these species, which were obtained using other extractants. The values of Sb(III) and Sb(V) were  $36 \pm 2.5 \text{ ng g}^{-1}$  and  $113 \pm 3.9 \text{ ng g}^{-1}$ , respectively. This is in good agreement with results reported by (Miravet et al., 2005), who found that 0.1 mol  $\text{L}^{-1}$  citric acid was found to provide the best extraction efficiency while also giving a reliable antimony speciation information in terrestrial plants.

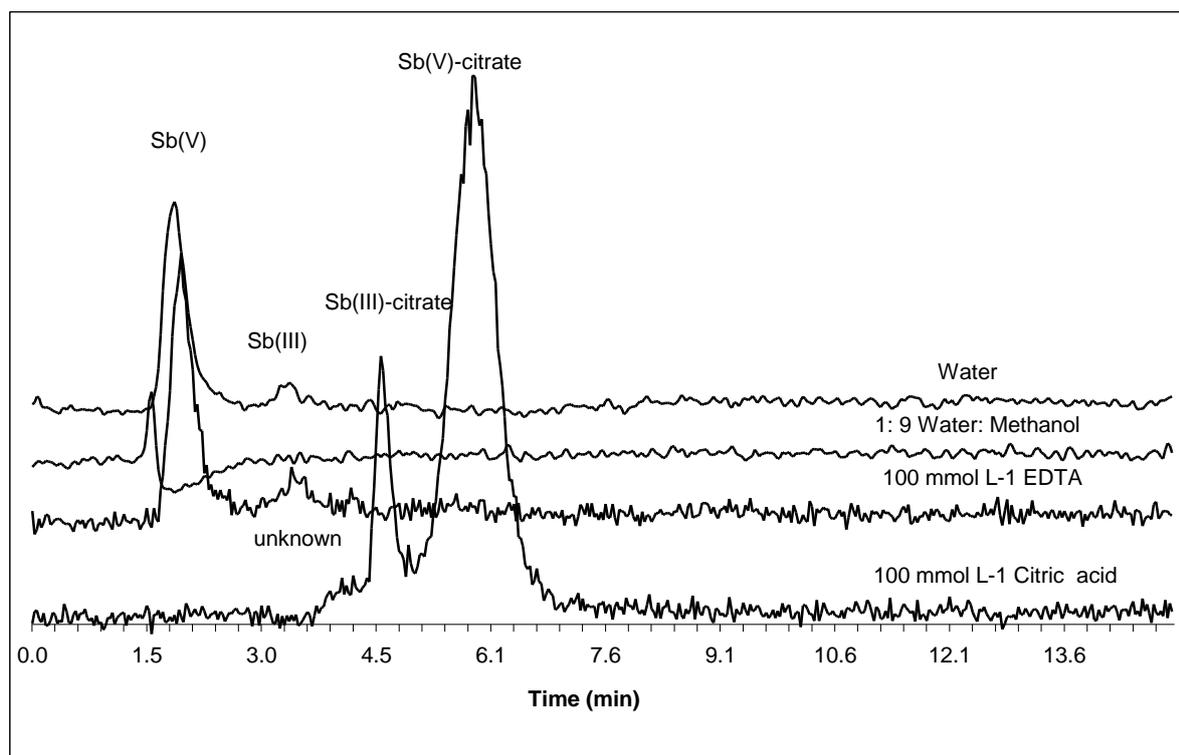


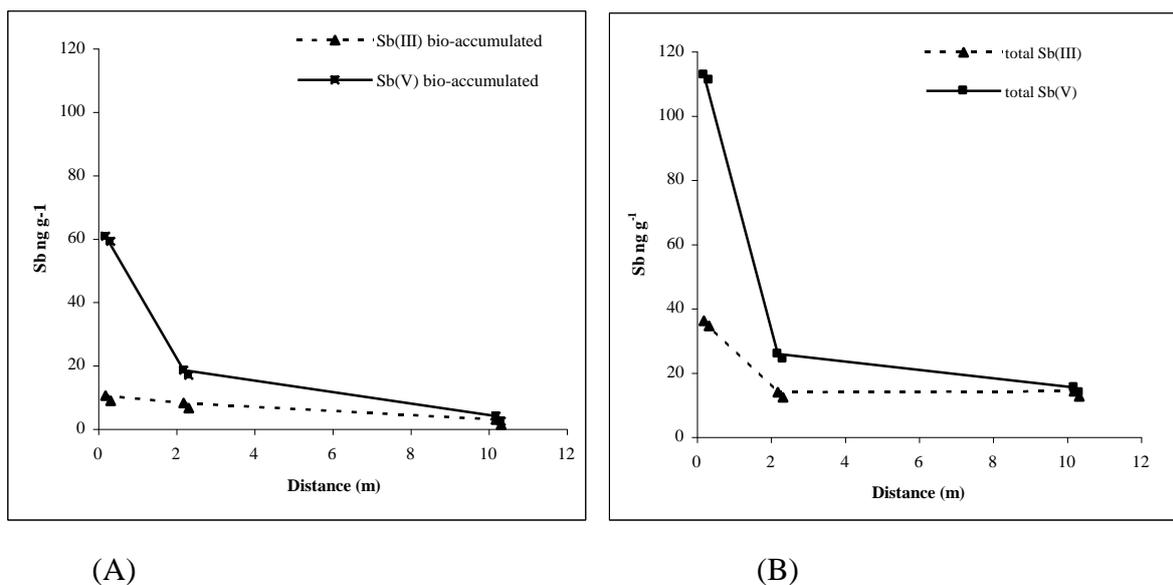
Figure 2. Effect of kind of extractant on antimony speciation in grass samples.

### Variation of extractable inorganic Sb species in grass samples with distance from roads

Speciation analysis of antimony in grass samples shows that Sb(III) species, in which the most toxic species was detected in all examined samples in spite of the sampling distance from the edge of the road, but in low concentrations. Sb(V) was found to be the major species in all the examined samples. Sb(V) concentrations are at least two folds higher than Sb(III). Total and bioaccumulated Sb(V) species concentrations sharply decreased with distance from the road edge, while Sb(III) concentration decreased with distance, but not sharply as Sb(V) (Fig 3 A and B). The results in (Table 2) show that the majority of inorganic Sb species can be removed by rinsing with water. This may decrease the toxicity impact of the presence of such toxic species, but there is an evidence of bioaccumulation of both species in low concentrations in all examined grass samples. These results agree with the results of a monitoring program in Munich (Peichl et al., 1996). Both inorganic Sb species have the same distribution pattern, which is similar to that obtained for the soil samples, which were sampled at the same site (Amereih et al., 2005b).

**Table 2. Results of antimony speciation in grass samples (unwashed and washed) taken at different locations faraway from a traffic road (Knittelfeld-Austria).**

Sample	Distance (m)	Sb(III) (ng g <sup>-1</sup> )	SD (ng g <sup>-1</sup> )	Sb(V) (ng g <sup>-1</sup> )	SD (ng g <sup>-1</sup> )
L1 (unwashed)	0.2	35.9	2.5	112.6	3.9
L2 (unwashed)	2.2	13.8	2.1	25.7	2.5
L3 (unwashed)	10.2	14.1	1.8	15.3	2.1
LW1 (washed)	0.2	10.4	2.7	60.7	4.2
LW2 (washed)	2.2	8.0	1.3	18.4	1.6
LW3 (washed)	10.2	2.8	1.2	3.9	0.9



**Figure 3. Sb(III) and Sb(V) distribution (A) bio-accumulated and (B) total with distance from a traffic road edge (Knittelfeld-Austria).**

## Conclusion

The grass samples taken near Knittelfeld site show nearly the same distribution pattern of Sb and Sb species as those in soil samples, which were taken exactly in the same locations. Total concentrations of Sb and its inorganic species found in grass decrease sharply as the distance from the traffic lane increases. The Sb (V) species is dominating the roadside grass samples, but also the more toxic Sb(III) species was detected in all the tested grass samples (washed and unwashed). This indicates that the grass which grows near the traffic roads can bioaccumulate Sb(III) and Sb(V). Thus Sb species needs to be routinely monitored in vegetation areas near heavy traffic.

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## التغير في التركيز الكلي لعنصر الأنتيمون ومركباته التي تحوي أرقام تأكسد مختلفة في

عينات من العشب على مسافات مختلفة من حافة طريق سريع في النمسا

باستخدام HPLC-ID-ICP-MS

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### الملخص

في هذه الدراسة تم استكشاف توزيع كل من تركيز عنصر الأنتيمون الكلي وكذلك أشكاله المتمثلة بأرقام تأكسده 3 و 5 في عينات عشبية أخذت من جانب طريق سريع. ووجدت الدراسة أن تركيز عنصر الأنتيمون المتموضع على سطح العشب أكثر من تركيزه المتراكم بيولوجيا داخل العشب بالضعف في المناطق المحاذية تماما للطريق السريع.

كما تم فصل أيوناته المختلفة  $Sb(III)$  و  $Sb(V)$  باستخدام طريقة الفصل الكروماتوغرافي عالي الكفاءة (HPLC) بعد استخلاصها من عينات العشب باستخدام محلول من حمض الستريك يحتوي على 100 ملليمول/لتر من الحمض بدرجة حموضة تساوي 2.08. ومن ثم تقدير تركيزها بعد عملية الفصل مباشرة باستخدام جهاز (ICP-MS). وقد أوضحت النتائج أن تركيز كل من أيون  $Sb(III)$  و أيون  $Sb(V)$  تقل كلما ابتعدنا عن حافة الطريق، وأن تركيز أيون  $Sb(V)$  تقريبا ضعف تركيز أيون  $Sb(III)$  في العينات المقاسة. الدراسة أظهرت أن أيون  $Sb(III)$ ، وهو الشكل الأكثر سمية، قد تم الكشف عنه في جميع العينات (المغسولة وغير المغسولة) ولا يعتمد تركيزه على بعد العينة عن حافة الطريق. كما تم ملاحظة إمكانية التخلص من مركبات الأنتيموني غير العضوية الموجودة على سطح العينات عن طريق غسل عينات العشب بالماء.

**الكلمات الدالة:** الأنتيمون، تحليل النفايات، تخفيف النظائر، عينات العشب.