

**Metal and PAH Concentrations in Fruit of
Vaccinium angustifolium Ait. (Lowbush Blueberry)**

A Comparison Among

Whitney Pier, North Sydney, and Supermarket

Final Report

To

Health Canada

On behalf of Government Partners

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EXECUTIVE SUMMARY

The concentrations of metals (arsenic (As), selenium (Se), thallium (Tl), vanadium (V), lead (Pb), chromium (Cr), copper (Cu), molybdenum (Mo) and zinc (Zn)) and PAHs (polycyclic aromatic hydrocarbons) (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene) in fruit from lowbush blueberries (*Vaccinium angustifolium* Ait.) from Sydney (Whitney Pier), Nova Scotia (NS), North Sydney, NS and supermarkets(etail), were quantified.

These concentrations were compared among the three sources, to determine whether the metal and PAH concentrations in the blueberries from Whitney Pier, are higher or lower compared to the reference(control) blueberries from North Sydney and from the supermarket. This question was answered by comparing mean values for each of the analytes using analysis of variance (ANOVA). The study determined whether washed berries have lower concentrations of contaminants than unwashed berries. Finally, the risk to human health of exposure to metals and PAHs through consumption of blueberry samples that were analysed was also calculated. The conclusions of this study are, using the most sensitive comparison, the concentrations of Se and Zn are elevated in blueberries from Whitney Pier as compared to berries from North Sydney, and the supermarket but are well within safe levels. The other elements (As, Tl, V, Pb, Cu and Mo) were similar for Whitney Pier, North Sydney; of these elements, only thallium was elevated in supermarket berries relative to those from Whitney Pier and North Sydney. Chromium was not detected in any of the samples. The potential for risk to human health from the nine metals (As, Se, Tl, V, Pb, Cr, Cu, Mo, Zn) in the blueberry samples that were analysed, was calculated. It was determined that there would be no risk to human health from exposure to any of these metals through the consumption of the blueberry samples that were analysed, regardless of whether they were Whitney Pier samples or reference samples (gathered in North Sydney and from the retail level).

Only two PAHs were detected in one of the unwashed samples from Whitney Pier; the concentrations of which were very close to the limit of quantitation. No PAHs were found in any of the other unwashed berries or any of the washed berries. From these data, it was also determined that there would be no risk to human health from exposure to any of these PAHs (both high molecular weight (HMW) and low molecular weight (LMW)) through the consumption of the blueberries samples that were analysed in this study. Washed and unwashed samples had similar metal concentrations, indicating that surface contamination did not occur, or, it did occur, and was not removed by simple rinsing. An alternate explanation is that a rainfall which occurred within 36 h before sampling may have removed surface contamination.

INTRODUCTION

The lowbush blueberry plant (*Vaccinium angustifolium* Ait.) has been a species of choice for monitoring the presence of metal element pollutants in soils. It readily colonizes disturbed sites, and is consumed by humans and wildlife, so may represent a dietary pathway of mammalian exposure to the pollutants. Most of the previously reported studies examine element concentrations in the foliage; fewer data on element concentrations in berries are available. Further, few of the elements most often reported for berries are the same as those measured in this study which were the chemicals of concern for this area reported by HSA Engineers & Scientists (2001). Bagatto and Shorthouse (1991) measured the concentrations of copper and nickel in blueberries and observed that concentrations of these metals were elevated in plants in proximity to the smelters. In a follow-up study, Bagatto *et al.* (1993) conducted a factor analysis on element analysis of blueberries in the Sudbury region and demonstrated that all parts of the blueberry plant except the berries 'contain a smelter sensitive (copper, nickel, zinc) factor'. This means that berry concentrations of these elements were independent of smelter emissions, whereas other parts of the plants did reflect emission patterns. This is not an unexpected observation, as fruit are physiologically the most distant from the roots (source of the contamination), and typically have the lowest concentrations of contaminants of any plant part. Sheppard (1991) measured element concentrations in blueberries growing in the Sudbury region. The data were expressed on a dry weight basis; using their stated loss on drying (88%), their reported concentrations of copper and zinc in the berries, expressed on a fresh weight basis, were 0.94 and 0.86 $\mu\text{g/g}$ fresh weight, respectively.

The presence of metals in soils does not necessarily mean that they are available for plant uptake; Bunzl *et al.* (2001) demonstrated that the plant-soil concentration ratios for arsenic, copper, lead and zinc, but not thallium, was smaller for slag-amended soils than for the soils alone. This suggests that the metals in the slag were bound more recalcitrantly than those in soil, so that total soil metals, in industrially contaminated soils, may not predict plant accumulation using relationships established on uncontaminated soils. Samsó-Peterson *et al.* (2002) demonstrated that contaminant (PAHs and metals) concentrations in fruits were generally low, and did not correlate with soil concentration, suggesting that the measured contaminants were deposited to the fruit from the air, rather than taken up by the roots.

PAHs in vegetation have been scantily studied, at least as reported in the literature. They have been detected in Chinese vegetables collected from markets, although leafy and stem vegetables had greater concentrations than root or fruit vegetables (Zhong and Wang, 2002). Studies of non-food crops have suggested that PAH accumulation by plants is due in part to the solubility of the PAH in soil solution, but also to the relative size of the lipophilic compartment (larger in oil producing plants), and to the ratio of leaf surface area to volume (Müller *et al.*, 2001).

PURPOSE AND OBJECTIVES OF THE TESTING PROGRAM

The first objective of the study is to quantify the concentrations of metals (arsenic (As), selenium (Se), thallium (Tl), vanadium (V), lead (Pb), chromium (Cr), copper (Cu), molybdenum (Mo) and zinc (Zn)) and PAHs (polycyclic aromatic hydrocarbons) (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene) in fruit from lowbush blueberry (*Vaccinium angustifolium* Ait.) from Sydney (Whitney Pier), Nova Scotia (NS), North Sydney, NS and the local supermarket (retail).

The second objective of the study is to compare these concentrations among the three sources, to determine whether there are more or less metals and/or PAHs in the blueberries from the Pier area, as compared to those blueberries from North Sydney and from the supermarket.

The third objective of the study is to determine whether washed blueberries have lower concentrations of contaminants than unwashed berries.

Since some samples were collected from private properties, risk assessments were also carried out and the conclusions included in this report. It was important to be able to state that the blueberries were safe to eat, not just similar or different from other sample locations. The risk to human health from exposure to PAHs and the nine metals through ingestion of blueberries samples analyzed in this study was carried out by the Chemical Health Hazard Assessment Division, Bureau of Chemical Safety, Food Directorate, Health Products & Food Branch of Health Canada.

METHODS

Field Sampling:

- Blueberries were sampled by hand on August 14 (Whitney Pier and local supermarket), August 15 (North Sydney), August 21 (Ontario supermarket) and August 22 (Quebec supermarket).
- The Whitney Pier location was Blueberry Hill. In order to collect a truly random sample from Blueberry Hill, the blueberry population density across the entire site would have to have been known, which it was not. Therefore, Blueberry Hill was divided into three strata determined by distance from the coke ovens site: very near (WP1 and WP2), close (WP3 and WP4) and far away (WP5 and WP6). This covers the "hill to plateau" gradient or distance well. Within each strata, two 500

- ml samples were collected from two separate sites covering 100 square metres each.
- The North Sydney locations were labeled in the table of results as NS1, NS2, NS3, NS4, NS5 and NS6.
 - Supermarket blueberries in Sydney were limited to one supplier from New Brunswick, so in addition to that supplier, samples were obtained from suppliers in northern Ontario, and northern Quebec.
 - The collected blueberries were placed in paper bags in the field, kept on ice, and transported back to the hotel.
 - Each of the field samples was split into two; one half was rinsed twice with distilled water, then both halves were sealed in glass jars supplied by PSC Analytical Services.
 - The blueberries were refrigerated until August 15, when they were packed into coolers with dry ice, and flown to Toronto with the researcher; the berries were delivered to PCS on August 16. At time of arrival, the loss on drying (% fresh weight) averaged 86%.
 - These methods of sample preservation are SOP for PAH's in plant tissues and exceed the sample preservation SOP for metals in plant tissues.

PAH Analyses

- The blueberries were analyzed for the following polycyclic aromatic hydrocarbons (PAHs): naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene. The samples (approx. 10 g) were mixed with sodium sulfate and extracted with dichloromethane using a Dionex ASE automated extractor. The extracts were purified using alumina column chromatography. Analysis was performed by gas chromatography/mass spectrometry using U.S. EPA Method 8270C (modified). Estimated Quantitation Limits (EQL) for all of the PAHs was 0.05 µg/g, based on wet weight. Surrogate recoveries from the blueberries ranged from approximately 50-60% for acenaphthene-d10, to 80-100% for benzo(a)pyrene-d12. The EQL is the concentration of an analyte that provides a signal on the detector that is ten times larger than the signal due to instrument noise.

As this relatively high EQL for the high molecular weight PAHs presented difficulties with the determination of risk of exposure through consumption of the

blueberries, the samples were re-analyzed using a method whereby deuterated PAHs are added to the samples as a measure of recovery. This approach results in a much lower quantitation limit (0.5 ng/g), but at a far greater cost per sample. Sample storage between the first and second analyses was at -10°C.

Metals Analysis

- The berries were analyzed for As, Se, Tl, V, Pb, Cr, Cu, Mo and Zn. Five (5) grams (fresh weight) of sample were digested with HNO₃ and H₂O₂ and brought up to 20 ml. The digestate was analyzed for trace metals by ICP-MS using a modified version of EPA method SW 6020.

Data Analysis

- The data were coded and entered into an Excel spreadsheet (Table 7); differences between subsamples and replicates, between washed and unwashed, and among sampling locations were determined using t-tests and analysis of variance (ANOVA) (SAS Inc. (Statistical Analysis System) Cary, NC)

PAH RESULTS

Only one sample had quantifiable quantities of PAHs: the unwashed blueberries from WP4 had 0.06 µg/g fluoranthene and 0.05 µg/g pyrene. It is prudent to remember that a total of 594 PAH analyses were carried out, and that two "quantifiables" might be expected to occur by chance alone. The fact that all the remaining samples were below the quantitation limit (recorded as non-detects by the laboratory) (EQL; 0.05 µg/g for each PAH) for the PAHs does not mean that there are no PAHs in or on the berries, but that if they are there, the concentrations are below 0.05 µg/g (50 ng/g). These data were used to calculate ingestion scenarios, by substituting the quantitation limit as the value (worst case, thus most conservative). This overestimates the risk of exposure, as the levels of PAHs typically found in foods are usually in the low ng/g range. While it is *possible* that PAH levels could be close to the quantification limit, the *probability* is very low, in consideration of past surveys for other foods (both published and the unpublished results). An exposure assessment was conducted for the two PAHs that were detected, and no unacceptable risk to human health through exposure to these two PAHs from the consumption of blueberries that were sampled in this analysis, was determined. As well, there would be no risk to human health from the low molecular weight (LMW) PAHs, for which the toxic equivalency factors (TEF) are negligible; the higher EQL for these PAHs was not a barrier to this calculation.

A method for analysis of HMW PAHs with a lower detection limit (0.5 ng/g) was developed which involved the addition of deuterated PAHs to the samples, so that recovery could be very accurately determined, thus improving the quantitation limit.

Unwashed blueberries were reanalyzed, the objective of which was to better quantify the concentrations of high molecular weight (HMW) PAHs in the samples; none were detected at concentrations greater than the EQL of 0.5 ng/g. Using these analytical results and the most current toxicological information available on this group of chemicals, the maximum quantity of blueberries that could be consumed on a daily basis before a potential health risk was likely, was calculated. From these data, it was determined that there would be no risk to human health from exposure to any of these PAHs (both HMW and LMW) through the consumption of the blueberry samples that were analysed in this study. Hundreds of kilograms of blueberries would have to be consumed on a daily basis in order for the LMW PAHs to pose a potential health risk. An average intake of approximately 300 grams of blueberries would have to be consumed each day (2.2 kg/week, every week of the year for 70 years) in order for the HMW PAHs to pose a potential health risk, based on an assumed concentration for each PAH of 0.5 ng/g, the EQL. It is very unlikely, however, that the HMW PAHs are present at these levels, considering that there were no quantitations of any HMW PAHs.

METAL RESULTS

There were a large number of non-quantifiable metals in the blueberry samples; these are indicated in Table 7 as ND for non detectable. For some of the metals (V, As, Pb and Mo), the distribution of the data were not normal, as required for analysis of variance. For As, transforming the data to the logarithm of the values achieved normality; however, the same outcome was arrived at for transformed and untransformed data. For V, Mo and Pb, transformation did not make the data normally distributed; the reasons varied. In the case of V, all data at one location had the same value, so there was no variance; in the case of Pb and Mo, the data distribution simply had some lack of smoothness. In other words, the lack of normality was not because the data were distinctly some other type of distribution. Because the differences between locations were so small relative to the means, a minor lack of normality as observed here would not unduly influence the outcome of the analysis of variance. For the first analysis of variance, the non-quantifiables (recorded as non-detects by the laboratory) were coded in the data set as the minimum quantitation limit as defined by PSC Analytical Services. The ramifications of this will be discussed within each metal, the results for which are discussed below. The analysis of variance for each metal partitioned the variability from the overall mean into the variability that could be attributed to location (Whitney Pier, North Sydney, supermarket), preparation (washed, unwashed), replication (three sampling sites at each of the locations) and sub-replication (two sampling areas at each of the three sampling sites). For each of the metals, the variance attributed to sub-replication was not different from the variation due to replication, meaning that the sub-replications within replication were not more similar to each other than the replications within locations ($P < 0.05$). Thus, the sub-replicates could be considered to be replicates, meaning that each location could be analyzed as having six replicates. There was also no interaction between preparation method and location, meaning that any difference between preparation methods was not dependent on sampling location. The next ANOVA partitioned the

variance among the locations, the preparation and the replicates (now numbering six for each location), for each metal. As the "replicate number" was assigned arbitrarily (unlike a laboratory experiment, where a block of experimental units is a clearly identifiable "replicate"), the significance of the variance attributed to replicates observed for some of the metals, in this ANOVA, is not great. It is likely that relabelling the replicates would cause the disappearance of significance for some of the metals, and the development of significance for others. Because of this, "replicate" in this study is not an attributable source of error in the traditional sense, rather it is the "within location" variation. It is important to this study to consider the relative sizes of the "within" and "among" location variations. Thus, for metals in which the within location variation was different in size from the overall error term, "within location" variation was used to test the significance of variation among locations. In the following description of results, any reported "effect" has a statistical significance of $P \leq 0.05$.

Arsenic (As)

There was no effect of washing on As concentrations of blueberries, and there was no difference between the replicate error and overall error terms. Using the overall error term to test the significance of location on As concentration in blueberries, we conclude that there was no difference among the three locations. There were a number of non-quantifiables for the As analysis (Table 7), and the EQL (.008) was substituted for these, for the ANOVA. As there were the same number of non-quantifiables for Whitney Pier and North Sydney, choosing an alternate value to 100% of the EQL is going to change the overall mean values, but will not have an effect on the comparison of the two locations. None of the berry samples had concentrations of As greater than Canada's Food and Drug Act (Part B, Division 15, Table I) most stringent tolerance, which is 0.1 $\mu\text{g/g}$ for fruit juice, fruit nectar, beverages when ready to serve, and water in sealed containers other than mineral or spring water. While this comparison to the Canadian standards is useful, it cannot be a direct one, as the tolerance limit may be set for a food, the consumption patterns of which are different from blueberries.

Selenium (Se)

There was no effect of washing on Se concentrations of blueberries, and the within location variation was significantly greater than the overall error, thus the within location mean square was used to test among locations. There was no difference among locations for Se concentration of blueberries. If an ultra-liberal approach to the data analysis was taken (namely using the smaller, overall error term) to test for differences among locations, the Whitney Pier berries would have more Se than the supermarket and North Sydney berries, which would have similar concentrations of Se. There were far more non-quantifiables for the North Sydney location than for Whitney Pier, so substituting a value lower than 100% of EQL, for the non-quantifiables, will lower the overall mean of North Sydney more than that for Whitney Pier, possibly changing the conclusion that Whitney Pier and North Sydney have blueberries with similar Se concentrations.

Thallium (Tl)

There was no effect of washing on Tl concentrations of blueberries, and within location variation was the same size as the overall error, thus the overall error was used to test the variation due to location. The effect of location was significant; separation of the means indicated that there was more Tl in the supermarket berries than in the berries from both North Sydney and Whitney Pier, which were similar to each other. There were a number of non-quantifiables for the Tl analysis (red data in Appendix A), and the EQL was substituted for these, for the ANOVA. As there were the same number of non-detects for Whitney Pier and North Sydney, choosing an alternate value to 100% of the EQL is going to change the overall mean values, but will not have an effect on the comparison of the two locations.

Vanadium (V)

There was no effect of washing on V concentrations of blueberries, and the within location variation was significantly greater than the overall error, thus the within location mean square was used to test among locations. When the within location mean square was used to test among locations, there was no difference among the three locations. If an ultra-liberal approach to the data analysis was taken (namely using the smaller, overall error term) to test for differences among locations, the supermarket berries would have more V than the Whitney Pier and North Sydney berries, which would have similar concentrations of V. However, there were far more non-quantifiables for the North Sydney location than for Whitney Pier, so substituting a value lower than 100% of EQL, for the non-quantifiables, will lower the overall mean of North Sydney more than that for Whitney Pier, perhaps leading to the conclusion that Whitney Pier has blueberries with more V than does North Sydney.

Lead (Pb)

There was no effect of washing on Pb concentrations of blueberries, and the within location variation was significantly greater than the overall error, thus the within location mean square was used to test among locations. When the within location mean square was used to test among locations, there was no difference among the three locations in Pb concentration of berries. If an ultra-liberal approach to the data analysis was taken (namely using the smaller, overall error term) to test for differences among locations, the supermarket berries would have the same concentration of Pb as the Whitney Pier and North Sydney berries. None of the berry samples had Pb concentrations in excess of Canada's Food and Drug Act (Part B, Division 15, Table I) most stringent tolerance, which is 0.08 µg/g for infant formula, when ready to serve. While this comparison to the Canadian standards is useful, it cannot be a direct one, as the tolerance limit may be set for a food, the consumption patterns of which are different from blueberries.

Chromium (Cr)

All of the Cr values were non-quantifiables, so there is no variation between treatments, or among locations.

Copper (Cu)

There was no effect of washing on Cu concentrations of blueberries, and the within location variation was significantly greater than the overall error, thus the within location mean square was used to test among locations. When the within location mean square was used to test among locations, there was no difference among the three locations in Cu concentration of berries. If an ultra-liberal approach to the data analysis was taken (namely using the smaller, overall error term) to test for differences among locations, the supermarket berries would have more Cu than the Whitney Pier and North Sydney berries, which would have similar concentrations of Cu.

Molybdenum (Mo)

There was no effect of washing on Mo concentrations of blueberries, and the within location variation was significantly greater than the overall error, thus the within location mean square was used to test among locations. When the within location mean square was used to test among locations, there was no difference among the three locations in Mo concentration of berries. If an ultra-liberal approach to the data analysis was taken (namely using the smaller, overall error term) to test for differences among locations, the Whitney Pier berries would have one half of the Mo concentration that was observed for North Sydney and supermarket berries, which would have similar concentrations of Mo.

Zinc (Zn)

There was no effect of washing on Zn concentrations of blueberries, and within location variation was the same size as the overall error, thus the overall error was used to test the variation due to location. The effect of location was significant; separation of the means indicated that there was more Zn in the Whitney Pier berries, than in the berries from both the supermarket and from North Sydney, which were similar to each other.

The potential for risk to human health from the nine metals (As, Se, Tl, V, Pb, Cr, Cu, Mo, Zn) in the blueberry samples that were analysed, was calculated. It was determined that there would be no risk to human health from exposure to any of these metals through the consumption of the blueberry samples that were analysed, regardless of whether they were Whitney Pier samples or reference samples (gathered in North Sydney and from the retail level).

DISCUSSION

The fact that only one sample out of the entire study had quantifiable LMW PAHs prevented meaningful comparison among sites, or between washed and unwashed blueberries for PAHs. In order to calculate risk for HMW PAHs, re-analysis of the samples using a lower EQL was carried out. The PAHs of interest in the re-analysis were

only those of high molecular weight (HMW), as the first analysis of the samples yielded data that were satisfactory for the determination of risk from exposure to low molecular weight (LMW) PAHs. HMW PAHs are very stable at room temperature, as their volatility is low, so the likelihood that significant quantities of them were lost through volatility from the samples during storage is nil. PAHs are highly reactive in the gaseous phase with atmospheric oxidants, and sulphur oxides; much less is known of their reactivity in solution (NAS, 1972). Any reactions that would take place in the plant tissues would be extremely slow or nil, at -10°C , and homogenized plant tissue is more reducing than oxidizing, so reactivity of the PAHs in these homogenized plant tissues during sample storage was not expected. The data describing concentrations for some of the metals in blueberries from the three locations can be compared, on a limited basis, to concentrations reported in the literature. The limitation is that concentrations for several of the elements measured in this study have not previously been reported in the literature. The data from a blueberry sampling campaign by the Ontario Ministry of the Environment are not yet available for comparison with this study.

The data can be compared among locations three ways: the arithmetic means without statistical analysis; the arithmetic means with the within location variance as the error term; and the arithmetic means with the residual variance as the error term. For some of the metals (Tl and Zn), the within location and residual variances were the same size, so for those elements, the latter two tests are the same. This also applies for As. The As data however were not normally distributed, so statistical analysis was carried out on the log transformed data, which were normally distributed. For other metals, the within location variance is larger than the residual variance, so the within location variance is the appropriate choice for testing, if one wishes to avoid falsely declaring a difference between means. If one wishes, on the other hand, to risk declaring a difference between means where one doesn't exist, then using the smaller (residual variance) error term is appropriate. The greatest likelihood of declaring a difference between means (and the greatest risk of declaring a difference where one doesn't exist) arises from simply comparing the arithmetic means with no statistical testing.

Using the method most likely to find a difference among locations (Table 4; column labeled "arithmetic"), Whitney Pier berries have elevated concentrations of Se and Zn, relative to North Sydney and supermarket berries. Se and Zn are essential elements to human health, and their concentrations in blueberries sampled in this study do not pose a risk to human health. This difference between Whitney Pier and North Sydney berries, in Zn concentration, is sustained for the other two more rigorous tests of differences, although in those two tests, North Sydney and supermarket have similar concentrations of Zn. The difference between Whitney Pier and North Sydney in Se concentration is not evident in the other two more rigorous statistical tests. By comparison of simple means, the supermarket berries are elevated in Tl, V, Pb and Cu; by the most rigorous statistical test, only Tl in supermarket berries continues to exceed the concentration found in Whitney Pier and North Sydney (Table 4). Arsenic and Mo are elevated in North Sydney berries relative to supermarket and Whitney Pier, based on the simple comparison of arithmetic means; however, neither of the statistical tests demonstrates any difference among locations.

The comparison of washed and unwashed samples was expected to demonstrate whether any elevated concentrations of contaminants on the berries were associated with particles on the outside of the berries. A simple interpretation of the data suggests that either there is no significant surface contamination of the blueberries, or the surface contamination was not removed by rinsing with distilled water, as there was no difference between the washed and unwashed berries in metal concentrations. However, there was a substantial rainfall the evening before berry collection day, so an alternate explanation is that all samples were “washed” within 36h prior to collection. So, the question of whether the berries acquire significant quantities of metal contaminants through surface adsorption of particles has been ambiguously answered by this study.

The question of whether the data are an adequate characterization of the population as a whole can be addressed from several aspects. Assuming that the means represent the true means of the population, it is clear from the comparison of the arithmetic means, without benefit of statistics, that increased precision from a larger sample size is not going to increase the number of metals for which Whitney Pier is elevated relative to North Sydney. The comparison of arithmetic means assumes a variance of zero, and no amount of sampling will result in a variance that small. The question of whether the means represent the true population means is more difficult to answer. The distribution of the values constituting each mean was examined using a box plot; for each of the elements except Cu and Zn, the distribution of their concentrations appears to be log normal, which has previously been reported to be typical (Shepherd, 1991) (Figure 1). Copper and Zn concentrations have a distribution which is closer to normal (Figure 2), likely because these are essential nutrients, thus more closely controlled by the organism. This suggests that the samples gathered for this study follow the distribution expected from a much larger sample size, and thus likely represent an unbiased sampling of the population.

A final consideration is the question of whether the means represent the true population means is the value substituted for the non-quantifiable elements. All the previously described analyses used 100% of the EQL in place of the non-quantifiables; as the distributions of concentrations above quantitation limit approximated log-normality, this is likely an overestimation of the concentrations, which are not quantifiable. To test how sensitive the conclusions of this study were to this assumption, the statistical tests were re-done, using one-half of the EQL in place of the non-detects. The arithmetic means from this exercise are reported in Table 5; the comparisons in Table 4 have been re-done (Table 6). A comparison of Tables 4 and 6 suggest that the conclusions of this study are not sensitive to the value chosen to replace the non-quantifiable values, between 50% and 100% of the EQL.

The general lack of elevated metal/PAH concentrations in the berries from Whitney Pier relative to those from North Sydney or the supermarket, may not be too surprising, for two reasons. The first is that the soil metals/PAHs are aged (≥ 13 years since coke ovens in operation) and in that time, those soil metals that are soluble (considered the main fraction of soil metals that are available for plant uptake) have likely been leached from the soil or become irreversibly bound (in a thermodynamic sense) to soil components

(Samsøe-Peterson *et al.*, 2002). Those metals remaining in the soils are not very available for plant uptake. Additionally, metals emitted from industrial processes, such as those found in slag, are less available for plant uptake than metals found in soil from geogenic sources (Bunzl *et al.*, 2001), and this may have been the case with the soil metals originating from the coking and steel-making operations. The second reason for non-elevated element concentrations in berries is that they are most distant from the roots, thus least reflective of soil elemental concentrations, as observed by Bagatto *et al.* (1993). For similar reasons, PAH concentrations in plants growing on soils with measurable PAHs may not be elevated, if the soil PAHs are well aged; those that are soluble, thus likely to be taken up by plants, are also most likely to be leached out of the soil. Those PAHs that remain are not soluble, or are complexed strongly with soil components, and not readily taken up by roots. The additional consideration with PAHs is that volatilization from the soil to the air, and then uptake by the leaves through stomates is a pathway by which plants accumulate these compounds (Bohme *et al.*, 1999). Kipopoulou *et al.* (1999) demonstrated that PAH mixtures in vegetable species were similar to the mixtures in air, suggesting that gaseous deposition was the more significant pathway of accumulation. Again, the age of the PAH contaminants in the soil suggests that those with a significant volatile phase would have already been reduced in concentration, thus not be 'available' for accumulation in plants.

CONCLUSIONS

The conclusions of this study are, using the most sensitive comparison (i.e. with no statistical separation), the concentrations of Se and Zn are elevated at Whitney Pier as compared to North Sydney but it is important to note that the concentrations in the Whitney Pier samples are well within safe levels. The other elements (As, Tl, V, Pb, Cu and Mo) were similar for Whitney Pier and North Sydney; of these elements, only thallium was elevated in supermarket berries relative to those from Whitney Pier and North Sydney. Using the statistically appropriate comparison, only Zn is elevated in Whitney Pier berries relative to those of North Sydney. Chromium was not detected in any of the samples. The limited comparisons with previously reported element concentrations suggest that Cu and Zn occur in the berries from both locations in Nova Scotia, and from the supermarket, in concentrations similar to those seen in other studies. Only two of the PAHs were detected, and that was only in one unwashed sample from Whitney Pier. The concentrations were very close to the limit of quantitation.

Washed and unwashed samples had similar metal concentrations, indicating that surface contamination did not occur, or, it did occur, and was not removed by simple rinsing. An alternate explanation is that a rainfall, which occurred within 36 h before sampling, may have removed surface contamination. Comparison among sites or between washed and unwashed samples could not be made for PAHs in blueberries since almost all the samples were non-quantifiables.

The potential for risk to human health from the nine metals (As, Se, Tl, V, Pb, Cr, Cu,

Mo, Zn) in the blueberry samples that were analysed, was calculated. It was determined that there would be no risk to human health from exposure to any of these metals through the consumption of the blueberry samples that were analysed, regardless of whether they were Whitney Pier samples or reference samples (gathered in North Sydney and from the retail level). Similarly, the risk of PAH exposure from consumption of blueberries was calculated. From these data, it was determined that there would be no risk to human health from exposure to any of these PAHs (both HMW and LMW) through the consumption of the blueberry samples that were analysed in this study.

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Figure 1: Box plots for all samples of As, Se, Tl, V, Pb, Cr and Mo.

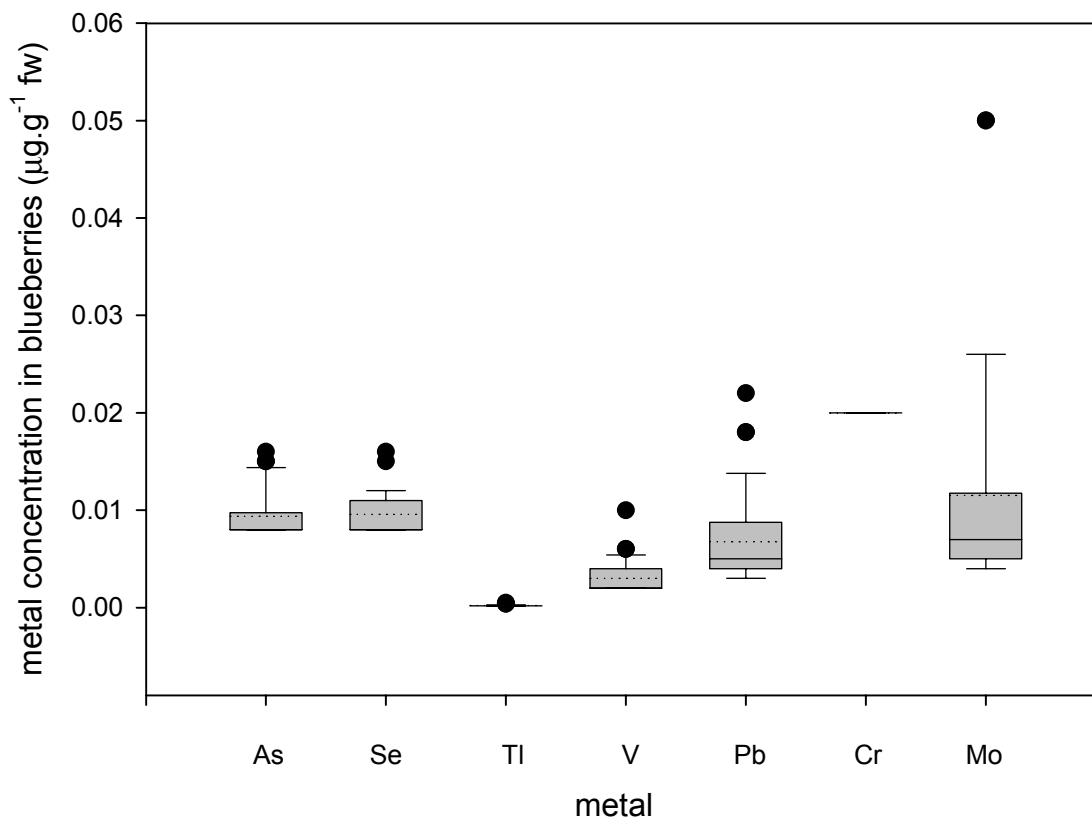


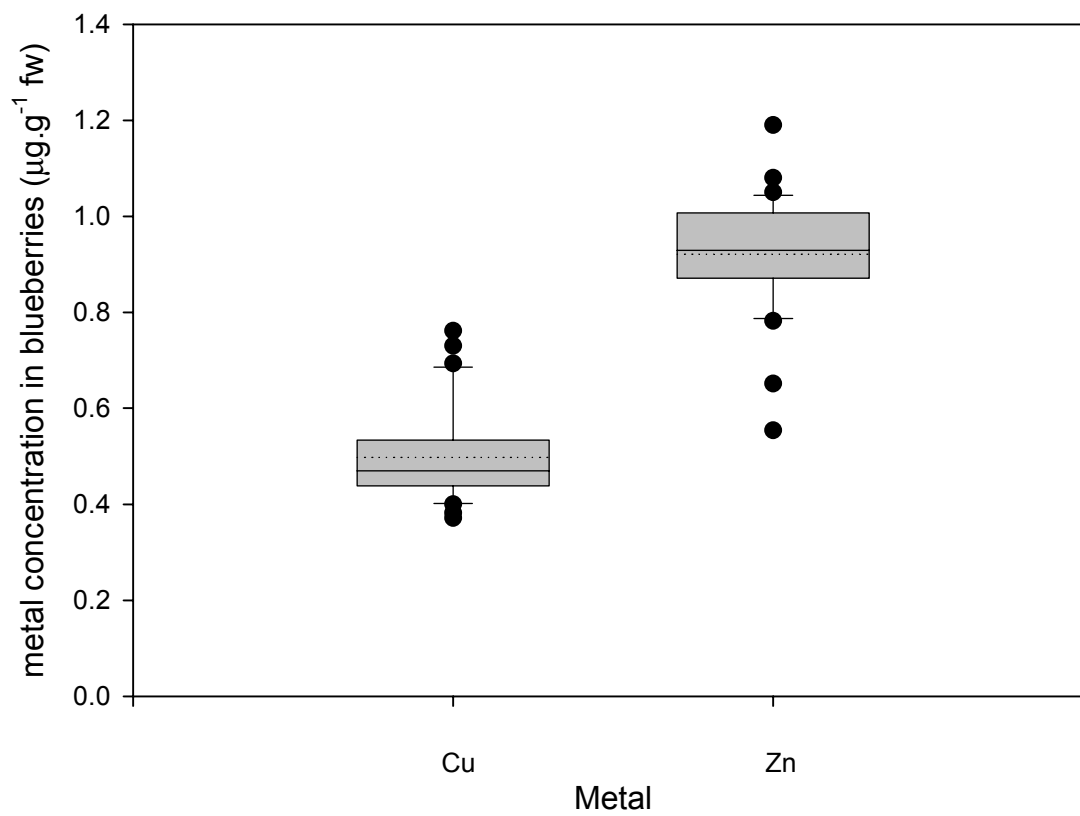
Figure 2: Box plots for all samples of Cu and Zn.

Table 1: Mean metal concentrations (n=6) and their standard deviations in blueberries from Whitney Pier: **a)** separately for washed and unwashed samples; **b)** pooled for washed and unwashed samples.

a)

	Unwashed		washed	
	mean ($\mu\text{g/g fw}$)	s.d.	mean ($\mu\text{g/g fw}$)	s.d.
As	8.67E-03	1.21E-03	8.33E-03	8.16E-04
Se	1.02E-02	1.33E-03	1.03E-02	1.86E-03
Tl	2.00E-04	0.00E+00	2.00E-04	0.00E+00
V	3.17E-03	1.60E-03	2.83E-03	9.83E-04
Pb	5.50E-03	2.43E-03	5.83E-03	2.71E-03
Cr	2.00E-02	0.00E+00	2.00E-02	0.00E+00
Cu	4.49E-01	4.17 ^E -02	4.44E-01	3.96E-02
Mo	6.83E-03	2.79E-03	6.67E-03	2.58E-03
Zn	9.83E-01	6.78E-02	9.88E-01	5.44E-02

b)

	washed and unwashed combined	
	mean ($\mu\text{g/g fw}$)	s.d.
As	8.50E-03	1.00E-03
Se	1.03E-02	1.54E-03
Tl	2.00E-04	0.00E+00
V	3.00E-03	1.28E-03
Pb	5.67E-03	2.46E-03
Cr	2.00E-02	0.00E+00
Cu	4.46E-01	3.89E-02
Mo	6.75E-03	2.56E-03
Zn	9.85E-01	5.86E-02

Table 2: Mean metal concentrations (n=6) and their standard deviations in blueberries from North Sydney: **a)** separately for washed and unwashed samples; **b)** pooled for washed and unwashed samples.

a)

	Unwashed		washed	
	mean ($\mu\text{g/g fw}$)	s.d.	mean ($\mu\text{g/g fw}$)	s.d.
As	1.03E-02	3.61E-03	1.02E-02	3.49E-03
Se	8.67E-03	1.21E-03	8.50E-03	1.22E-03
Tl	2.00E-04	0.00E+00	2.00E-04	0.00E+00
V	2.00E-03	0.00E+00	2.00E-03	0.00E+00
Pb	5.50E-03	3.08E-03	6.33E-03	5.79E-03
Cr	2.00E-02	0.00E+00	2.00E-02	0.00E+00
Cu	4.69E-01	6.15E-02	5.14E-01	1.06E-01
Mo	1.58E-02	1.86E-02	1.60E-02	1.87E-02
Zn	8.89E-01	7.60E-02	9.04E-01	1.04E-01

b)

	washed and unwashed combined	
	mean ($\mu\text{g/g fw}$)	s.d.
As	1.03E-02	3.39E-03
Se	8.58E-03	1.16E-03
Tl	2.00E-04	0.00E+00
V	2.00E-03	0.00E+00
Pb	5.92E-03	4.44E-03
Cr	2.00E-02	0.00E+00
Cu	4.91E-01	8.60E-02
Mo	1.59E-02	1.78E-02
Zn	8.97E-01	8.72E-02

Table 3: Mean metal concentrations and their standard deviations in commercially distributed blueberries: **a)** separately for washed (n=3) and unwashed (n=4) samples; **b)** pooled for washed and unwashed samples.

a)

	Unwashed		washed	
	mean ($\mu\text{g/g fw}$)	s.d.	mean ($\mu\text{g/g fw}$)	s.d.
As	9.50E-03	3.00E-03	9.33E-03	2.31E-03
Se	1.00E-02	4.00E-03	1.03E-02	4.04E-03
Tl	3.25E-04	1.50E-04	2.67E-04	1.15E-04
V	5.75E-03	2.87E-03	3.33E-03	2.31E-03
Pb	1.10E-02	7.53E-03	8.67E-03	8.08E-03
Cr	2.00E-02	0.00E+00	2.00E-02	0.00E+00
Cu	6.06E-01	1.26E-01	5.83E-01	1.56E-01
Mo	1.40E-02	8.29E-03	9.67E-03	3.21E-03
Zn	9.18E-01	2.20E-01	7.68E-01	1.88E-01

b)

	washed and unwashed combined	
	mean ($\mu\text{g/g fw}$)	s.d.
As	9.43E-03	2.51E-03
Se	1.01E-02	3.67E-03
Tl	3.00E-04	1.29E-04
V	4.71E-03	2.75E-03
Pb	1.00E-02	7.19E-03
Cr	2.00E-02	0.00E+00
Cu	5.96E-01	1.27E-01
Mo	1.21E-02	6.57E-03
Zn	8.54E-01	2.06E-01

Table 4: Summary of differences among locations, ranked for each metal element, using three bases of comparison, and the EQL for the non-quantifiables.

	arithmetic	using the residual variance as error term	using the within location variance as error term
As	NS > super > WP	NS = super = WP*	NS = super = WP
Se	WP > super > NS	WP > super = NS	WP = super = NS*
Tl	super > WP = NS	super > WP = NS*	super > WP = NS
V	super > WP > NS	super > WP = NS	super = WP = NS*
Pb	super > NS > WP	super = NS = WP	super = NS = WP*
Cr	all non quantifiable	all non quantifiable	all non quantifiable
Cu	super > NS > WP	super > NS = WP	super = NS = WP*
Mo	NS > super > WP	NS = super > WP	NS = super = WP*
Zn	WP > NS > super	WP > NS = super*	WP > NS = super

NS = North Sydney; WP = Whitney Pier; super = supermarket

* denotes appropriate statistical test for that element, based on F test of residual and within location error terms

Table 5: Mean metal concentrations and their standard deviations in blueberries from: **a)** Whitney Pier; **b)** North Sydney; and **c)** supermarket. One-half EQL was substituted for non-quantifiables, instead of the EQL, as for all preceding analyses.

a)

	mean	s.d.
As	5.50E-03	2.75E-03
Se	9.58E-03	2.84E-03
Tl	1.17E-04	3.89E-05
V	2.50E-03	1.73E-03
Pb	5.67E-03	2.46E-03
Cr	1.00E-02	0.00E+00
Cu	4.46E-01	3.89E-02
Mo	6.42E-03	3.03E-03
Zn	9.85E-01	5.86E-02

b)

	mean	s.d.
As	7.58E-03	5.33E-03
Se	5.92E-03	2.94E-03
Tl	1.08E-04	2.89E-05
V	1.00E-03	0.00E+00
Pb	5.92E-03	4.44E-03
Cr	1.00E-02	0.00E+00
Cu	4.91E-01	8.60E-02
Mo	1.53E-02	1.83E-02
Zn	8.97E-01	8.72E-02

c)

	mean	s.d.
As	6.57E-03	4.43E-03
Se	7.29E-03	5.62E-03
Tl	2.43E-04	1.81E-04
V	4.43E-03	3.10E-03
Pb	1.00E-02	7.19E-03
Cr	1.00E-02	0.00E+00
Cu	5.96E-01	1.27E-01
Mo	1.21E-02	6.57E-03
Zn	8.54E-01	2.06E-01

Table 6: Summary of differences among locations, ranked for each metal element, using three bases of comparison, and one-half of the EQL for non-detects.

	arithmetic	using the residual variance as error term	using the within location variance as error term
As	NS > super > WP	NS = super = WP	NS = super = WP*
Se	WP > super > NS	WP > super = NS	WP = super = NS*
Tl	super > WP > NS	super > WP = NS	super = WP = NS*
V	super > WP > NS	super > WP > NS	super = WP = NS*
Pb	super > NS > WP	super > NS = WP	super = NS = WP*
Cr	all non quantifiable	all non quantifiable	all non quantifiable
Cu	super > NS > WP	super > NS = WP	super = NS = WP*
Mo	NS > super > WP	NS = super > WP	NS = super = WP*
Zn	WP > NS > super	WP > NS = super*	WP = NS = super

NS = North Sydney; WP = Whitney Pier; super = supermarket

* denotes appropriate statistical test for that element, based on F test of residual and within location error terms

Table 7: Metal concentrations ($\mu\text{g/g}$ fresh weight) in blueberry samples

Site	As	Se	Tl	V	Pb	Cr	Cu	Mo	Zn	Treatment
NS1	0.016	ND	ND	ND	0.004	ND	0.403	0.026	0.813	washed
NS1	0.015	ND	ND	ND	0.003	ND	0.400	0.025	0.809	unwashed
NS2	0.013	ND	ND	ND	0.018	ND	0.427	0.050	0.791	washed
NS2	0.015	ND	ND	ND	0.004	ND	0.435	0.050	0.782	unwashed
NS3	ND	0.011	ND	ND	0.003	ND	0.450	0.005	0.892	washed
NS3	ND	0.011	ND	ND	0.003	ND	0.450	0.005	0.961	unwashed
NS4	ND	0.008	ND	ND	0.005	ND	0.681	ND	0.948	washed
NS4	ND	0.009	0.0002	ND	0.011	ND	0.575	ND	0.957	unwashed
NSS	ND	ND	ND	ND	0.003	ND	0.563	0.007	1.080	washed
NSS	ND	ND	ND	ND	0.007	ND	0.451	0.007	0.907	unwashed
NS6	ND	ND	ND	ND	0.005	ND	0.560	ND	0.901	washed
NS6	ND	ND	ND	ND	0.005	ND	0.502	ND	0.919	unwashed
WP1	ND	0.012	ND	0.003	0.006	ND	0.371	0.007	1.050	washed
WP1	ND	0.010	ND	0.003	0.005	ND	0.382	0.007	1.020	unwashed
WP2	0.010	0.012	0.0002	0.004	0.009	ND	0.438	0.008	1.010	washed
WP2	0.011	0.011	0.0002	0.004	0.006	ND	0.418	0.007	1.000	unwashed
WP3	ND	0.009	ND	ND	0.003	ND	0.475	0.005	0.914	washed
WP3	ND	0.010	ND	ND	0.003	ND	0.475	0.005	0.942	unwashed
WP4	ND	0.009	ND	ND	0.003	ND	0.461	0.011	0.965	washed
WP4	0.009	0.010	ND	ND	0.004	ND	0.495	0.012	1.040	unwashed
WP5	ND	0.012	ND	0.004	0.009	ND	0.440	ND	0.946	washed
WP5	ND	0.012	ND	0.006	0.010	ND	0.470	ND	1.030	unwashed
WP6	ND	ND	ND	ND	0.005	ND	0.479	0.005	1.040	washed
WP6	ND	ND	ND	ND	0.005	ND	0.452	0.006	0.864	unwashed
S1	ND	ND	ND	ND	0.004	ND	0.470	0.012	0.904	washed
S1	ND	ND	ND	0.004	0.009	ND	0.539	0.012	0.929	unwashed
S1	ND	ND	ND	ND	0.004	ND	0.518	0.011	0.847	washed
S1	ND	ND	ND	0.004	0.005	ND	0.463	0.011	0.903	unwashed
S2	0.012	0.015	0.0004	0.006	0.018	ND	0.761	0.006	0.554	washed
S2	0.014	0.016	0.0004	0.010	0.022	ND	0.693	0.007	0.651	unwashed
S3	ND	ND	0.0005	0.005	0.008	ND	0.730	0.026	1.190	unwashed

ND = non-detects (concentration below the laboratory detection limit / quantitation limit)