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Title: 2020 Results for Avian Monitoring of Inorganic and Organic Element Concentrations in Passerine Eggs and Nestlings Collected from Technical Area 16 Burn Grounds, Technical Area 36 Minie, and Technical Area 39 Point 6 at Los Alamos National Laboratory: Revision 2

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2020 Results for Avian Monitoring of Inorganic and Organic Element Concentrations in Passerine Eggs and Nestlings Collected from Technical Area 16 Burn Grounds, Technical Area 36 Minie, and Technical Area 39 Point 6 at Los Alamos National Laboratory: Revision 2

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A note from the authors regarding the revision: In report number LA-UR-21-22303, inorganic element results were reported on a wet weight basis when after further review, we have concluded that the results are consistent with dry weight values. This change does not affect the observations, the comparisons, or conclusions of the study.

A note from the authors regarding the second revision: In report number LA-UR-21-22303, authors discovered that egg samples collected from Los Alamos National Laboratory were inadvertently included into the regional statistical reference level calculations. This revision provides the corrected regional statistical reference levels calculations. These changes do not affect the conclusions of the study.

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ACRONYMS AND TERMS

ALS	Australian Laboratory Services
EPA	Environmental Protection Agency
LANL	Los Alamos National Laboratory
LOAEL	slowest observable adverse effect levels
mg/kg	milligrams per kilogram
pg/g	picograms per gram
PCBs	polychlorinated biphenyls
RSRLs	regional statistical reference levels
TA	Technical Area
TCDD	tetrachlorodibenzodioxin-2,3,7,8
TEF	toxic equivalent factors
TEQ	toxic equivalents
RCRA	Resource Conservation and Recovery Act
WHO	World Health Organization

1.0 SUMMARY

In 2020, non-viable avian eggs and two nestling were opportunistically collected at Los Alamos National Laboratory (LANL) near open detonation sites located at Technical Area (TA) 16 burn grounds, TA-36 Minie, and TA-39 Point 6. These samples were evaluated for inorganic elements (mostly metals), dioxins, and furans. A total of six eggs and two deceased western bluebird (*Sialia mexicana*) nestling samples were collected among the three locations of interest. Concentrations of inorganic elements observed in this study were compared with the regional statistical reference level (RSRL) which is the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Several inorganic elements were not detected in avian eggs. The majority of inorganic elements that were detected were below the RSRL and all concentrations were below the lowest observable adverse effect level (LOAEL), when available. One nestling collected from TA-39 contained detectable concentrations of two dioxin congeners. Heptachlorodibenzodioxin-1,2,3,4,6,7,8 and octachlorodibenzodioxin-1,2,3,4,6,7,8,9 concentration exceeded the RSRL, but did not exceed the calculated tetrachlorodibenzodioxin-2,3,7,8 (TCDD) toxic equivalent LOAEL. These data suggest that inorganic and organic element concentrations in eggs and nestlings are not of ecological concern. More data are needed to make a robust assessment and to evaluate trends over time.

2.0 INTRODUCTION

In support of the Resource Conservation and Recovery Act (RCRA) permit process, Los Alamos National Laboratory (LANL) began annual avian monitoring in 2013 around TA-16 burn grounds and at two firing sites, TA-36 Minie and TA-39 Point 6. Biomonitoring is an important tool for assessing environmental contamination by analyzing chemicals or their metabolites from biological tissues (Becker 2003). Avian eggs and nestlings are useful as bioindicators because different species occupy many trophic levels. Additionally, the collection of non-viable eggs and/or nestlings that die of natural causes is non-invasive and is non-destructive to populations. Inorganic elements and organic chemicals can pose risks of adverse effects to birds if exposed at high enough concentrations (Jones and de Voogt 1999). Levels of some constituents in biological tissues can also indicate whether adverse effects could be expected (Gochfeld and Burger 1998). Examining population parameters along with tissue concentrations provides a more comprehensive and robust assessment of potential impacts caused by environmental pollution.

Several congeners of polychlorinated biphenyls (PCBs), dioxins, and furans elicit similar toxic effects (i.e., immunotoxicity, carcinogenicity, and endocrine disruption) as those caused by tetrachlorodibenzodioxin-2,3,7,8 (TCDD), the most potent in this class of chemicals (Van den Berg et al. 2006). These congeners, like TCDD, have a high binding affinity to the aryl hydrocarbon receptor (Van den Berg et al. 2006). The World Health Organization (WHO) developed toxic equivalency factors (TEFs) for TCDD-like compounds that can be used to determine the relative potency, or toxic equivalents (TEQs), of dioxin-like compounds for different classes of animals (i.e., fish, birds, and mammals), as well as to facilitate risk assessment for TCDD-like exposure (Van den Berg et al. 1998).

Sources of inorganic elements include both anthropogenic and natural sources; birds can be exposed through a number of routes, including diet, ingestion of soil, drinking water, and inhalation. Inorganic elements (mostly metals), dioxins, and furans are of interest at open-detonation firing sites (TA-36 and TA-39) and at the burn grounds at TA-16 (Fresquez 2011).

3.0 OBJECTIVES

The objective of this ongoing study is to document chemical concentrations in eggs and nestlings collected near TA-16 burn grounds, TA-36 Minie, and TA-39 Point 6 and to compare concentrations of inorganic elements, PCBs, dioxins, and furans observed in this study with the upper-level bounds of background concentrations.

4.0 METHODS

4.1. Sample Collection

Eggs and nestlings were collected from nest boxes when they were determined to be non-viable, based on documented timing of known incubation periods for the species. We collected a total of six non-viable eggs and two deceased nestlings at LANL near the TA-16 burn grounds (Figure 1) and near open detonation sites TA-36 Minie (Figure 2) and TA-39 Point 6 (Figure 3). At TA-16, five non-viable western bluebird (*Sialia mexicana*) eggs and one deceased nestling samples were collected and submitted as one composite sample and four individual samples. At TA-36, one non-viable western bluebird egg was collected and submitted and at TA-39, one deceased western bluebird nestling was collected and submitted. All samples were collected May through July of 2020. Concentrations of chemicals in eggs and nestlings have been monitored annually at these locations since 2014.

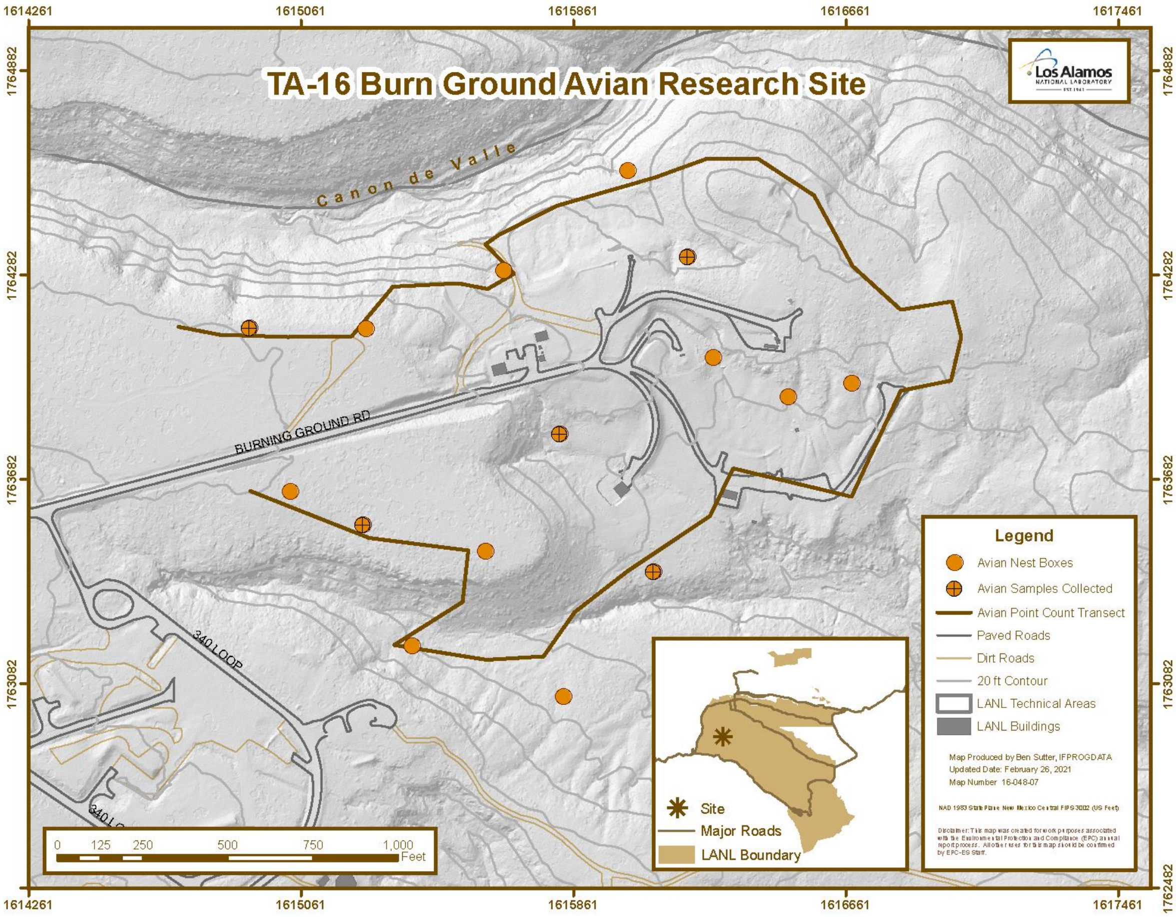

Figure 1. Avian nest box locations around TA-16 burn grounds.

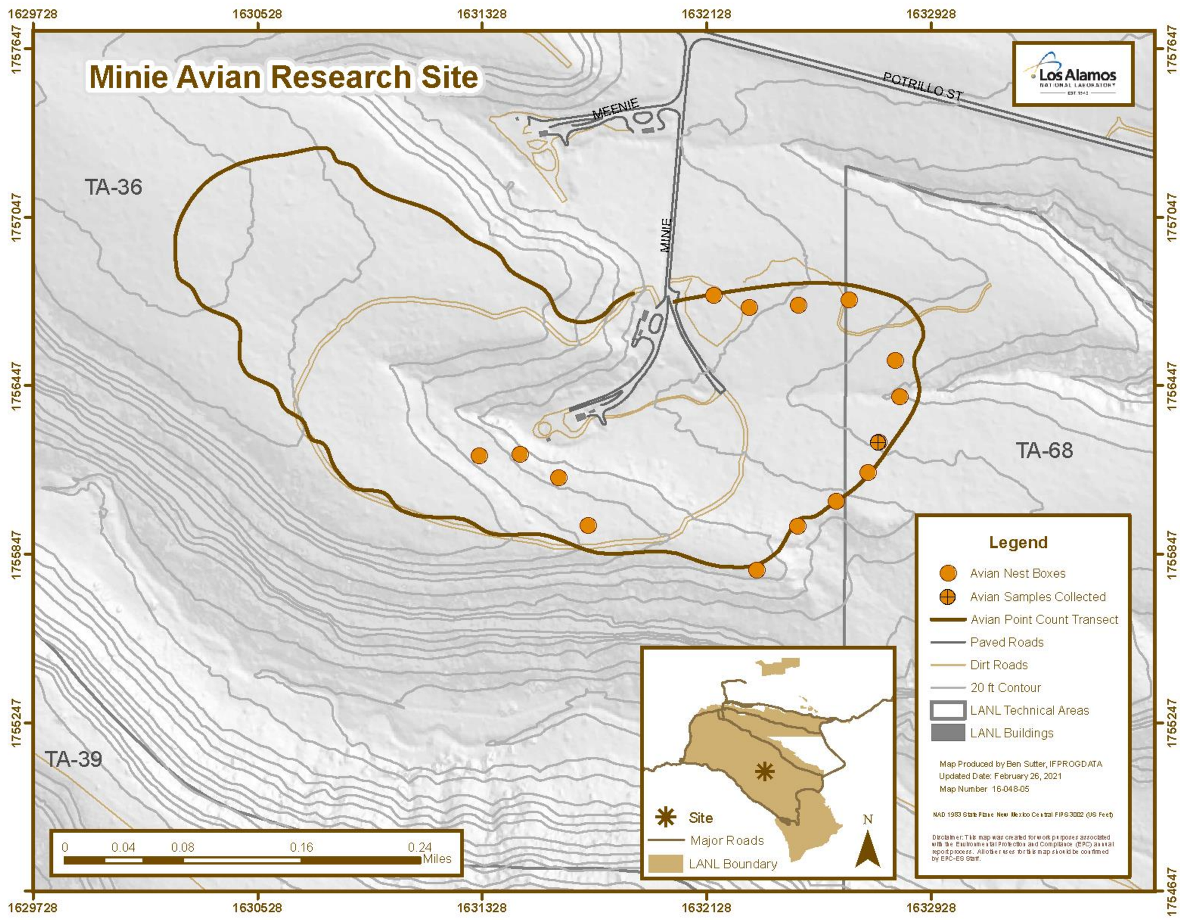

Figure 2. Avian nest box locations around TA-36 Minie.

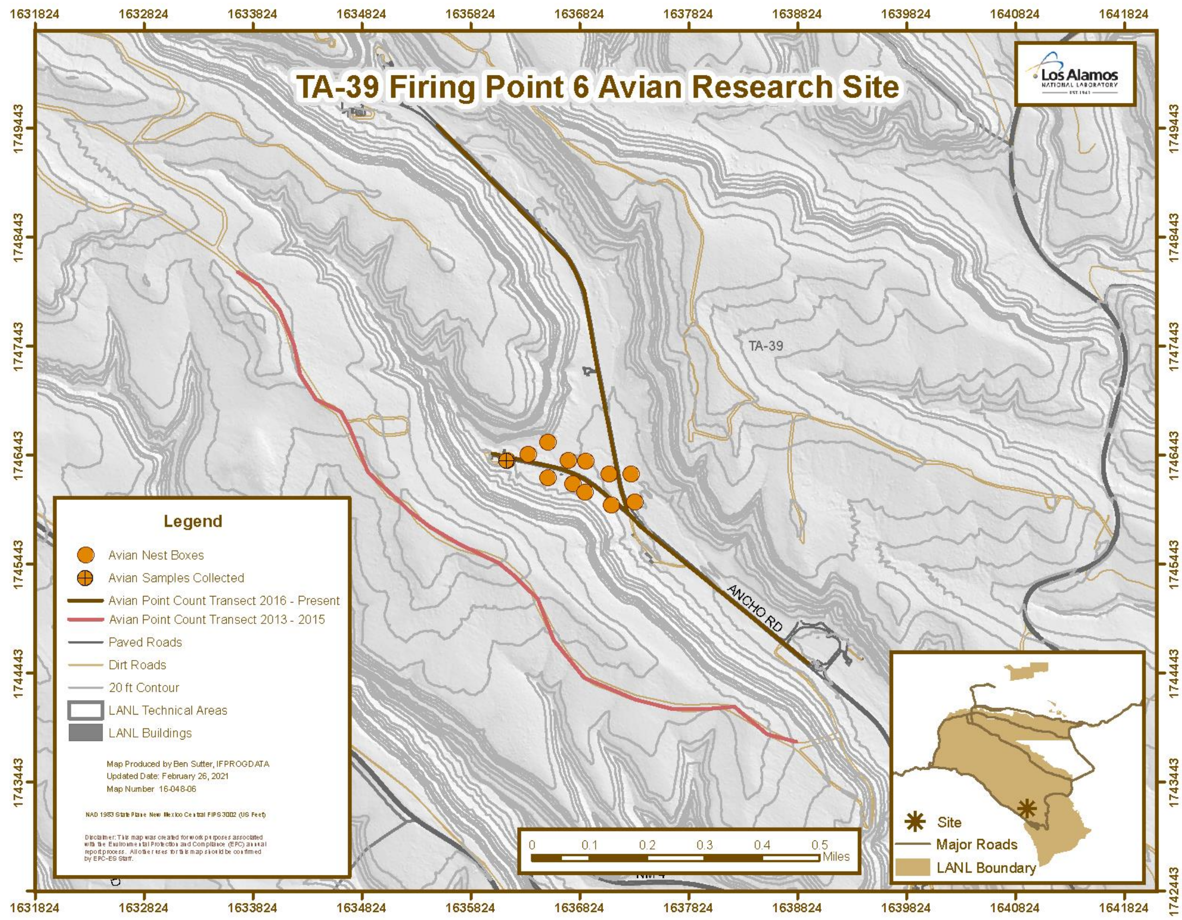

Figure 3. Avian nest box locations around TA-39 Point 6.

4.2. Chemical Analyses

Due to limited sample mass, non-viable eggs and one nestling sample were analyzed for total analyte list (mostly inorganic metals) only and were analyzed at ALS (Australian Laboratory Services, formerly Paragon Analytics, Inc.) in Fort Collins, Colorado. Antimony, arsenic, cadmium, lead, selenium, silver, and thallium concentrations were measured in egg samples by inductively coupled plasma mass spectrometry (Environmental Protection Agency [EPA] SW-846 Method 6020A), and aluminum, barium, beryllium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium, vanadium, and zinc were measured by inductively coupled plasma atomic emission spectrometry (EPA SW-846 Method 6010B). Mercury was measured by cold-vapor atomic absorption procedure (EPA SW-846 Method 7471A). All inorganic element results were reported on an mg/kg (milligram per kilogram) dry weight basis.

The non-viable nestling sample collected near TA-39 was analyzed for dioxin and furan congeners by EPA SW-846 Method 8290 at Cape Fear Analytical LLC, Wilmington, North Carolina. All organic chemical results are reported on a wet weight basis.

4.3. Statistical Methods

The 2020 results were compared with the regional statistical reference levels (RSRL), which represents natural and fallout levels of chemicals, and are the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Regional statistical reference levels were calculated from non-viable eggs of western bluebirds and ash-throated flycatchers (*Myiarchus cinerascens*) collected from Bandelier National Monument in 2020 (n = 7 samples). Non-viable egg results are also compared with the lowest observable adverse effect levels (LOAEL) from peer reviewed literature, when available.

Nestling sample results of dioxin and furans, were compared with RSRLs and LOAELs, when available. The nestling RSRL was calculated from non-viable nestlings of western bluebirds and ash-throated flycatchers at background locations from Bandelier National Monument in 2018 and 2019 (n = 5 samples). Nestling sample results of TAL were compared directly with one sample collected from background locations.

5.0 RESULTS AND DISCUSSION

Similar with previous years, many of the inorganic elements assessed in this study were not detected in passerine egg samples. Several elements are not (or very little is) maternally transferred into eggs or do not accumulate in eggs and include cadmium (Leach et al. 1979; Stoewsand et al. 1986), lead (Pattee 1984), vanadium (White and Dieter 1978), and silver (Schwarzbach et al. 2006; Seiler and Skorupa 2001), which may explain why these elements were mostly not detected.

Similarly, most dioxins and furans were not detected in the nestling sample collected from TA-39 burn grounds. Overall, most constituents that were detected in egg and the nestling samples were below RSRLs, and all constituents were below the LOAELs, when available.

5.1. TA-16 Burn Grounds

Western bluebird eggs collected from nest boxes at TA-16 burn grounds did not contain detectable concentrations of aluminum, arsenic, beryllium, cadmium, chromium, cobalt, nickel, silver, or vanadium. Barium was detected in all egg samples, three of which exceeded the RSRL (Table 1). Iron, lead, and mercury were also detected and slightly exceeded the RSRL in one egg sample each (Table 1). Detectable concentrations of antimony, calcium, copper, magnesium, manganese, potassium, selenium, sodium, thallium, and zinc were all below the RSRL (Table 1). Mercury and selenium concentrations were well below LOAELs (Ohlendorf and Heinz, 2011, Shore et al. 2011); no other LOAELs were available.

Many inorganic elements were not detected in the non-viable nestling sample from TA-16 burn grounds. Detections patterns and concentrations of inorganic elements between the nestling from TA-16 and the nestling sample from a background location are similar.

5.2. TA-36 Minie

The one western bluebird egg sample collected from TA-36 Minie, did not have detectable levels of several elements, including aluminum, arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel, silver, thallium, or vanadium. Antimony, calcium, and copper were detected and slightly exceeded the RSRL (Table 2). Detectable concentrations of antimony, barium, calcium, copper, iron, magnesium, manganese, mercury, potassium, selenium, sodium, and zinc were all below the RSRL (Table 2). Mercury and selenium concentrations were well below LOAELs (Ohlendorf and Heinz, 2011, Shore et al. 2011); no other LOAELs were available.

5.3. TA-39 Point 6

Most dioxins and furans were not detected in the nestling sample collected from TA-39. The sample contained detectable concentrations of 1,2,3,4,6,7,8,9-octachlorodibenzodioxin at 4.51 pg/g (picograms per gram) and 1,2,3,4,6,7,8-heptachlorodibenzodioxin at 1.83 pg/g, which exceeds the RSRL of 2.36 pg/g and 1.43 pg/g, respectively. Lowest observable adverse effect levels are not available for each dioxin and furan congener. However, TCDD, the most potent dioxin congener, induces toxic effects in avian eggs at concentrations between 1,000 to 10,000 pg/g wet weight (Harris and Elliott 2011). Toxic equivalent factors can be used to calculate the toxic equivalent values of dioxin-like compounds. The toxic equivalent factor for 1,2,3,4,6,7,8,9-octachlorodibenzodioxin and 1,2,3,4,6,7,8-heptachlorodibenzodioxin for avian species is 0.0001 and 0.001, respectively (Van den Berg et al. 1998). Multiplying the detectable concentration by the toxic equivalent factors yield values that are orders of magnitude less than the lowest observable adverse effect level for TCDD observed in avian eggs (Harris and Elliott 2011).

6.0 CONCLUSIONS

The overall results indicate that the levels of constituents detected in the eggs and nestlings are not likely to cause adverse effects in breeding bird populations. Several constituents were not detected in the non-viable egg and nestling samples collected near TA-16 burn grounds, TA-36 Minie, and TA-39. Most constituents that were detected were below RSRLs and all were below the LOAELs, when available. These results suggest that the detectable concentrations observed here were not of ecological concern. More data from non-viable eggs and nestlings are needed to make a robust assessment and to examine trends over time. Evaluating avian nestling samples for high explosives are also of interest for future work as those data become available.

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Table 1. Inorganic element concentrations (mg/kg dry weight) detected in single or composite egg samples collected near TA-16 burn grounds compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data from 2020 (n = 7).

Element	Western bluebird (n = 1) SFB-20-206077	Western bluebird (n = 1) SFB-20-206078	Western bluebird (n=1) SFB-20-206079	Western bluebird (n=2) SFB-20-206080	RSRL
Antimony	0.140	0.140	0.130	0.087	0.179
Barium[a]	**95**	**55**	8.4	**85**	17.4
Calcium	1,900	3,300	3,600	2,500	14,611
Copper	3.70	3.00	2.20	2.60	3.99
Iron	150	150	**280**	150	217
Lead[b]	**0.39**	ND	ND	ND	0.29
Magnesium	300	270	360	380	596
Manganese	1.80	1.50	1.70	2.00	4.03
Mercury	0.072	0.057	0.060	**0.180**	0.123
Potassium	9,700	9,100	7,900	7,800	10,411
Selenium	2.6	2.6	3.2	2.6	4.2
Sodium	9,400	10,000	8,300	8,300	11,028
Thallium	ND	ND	ND	0.008	0.033
Zinc	44.0	39.0	42.0	51.0	59.9

[a]A *bold value indicates that the element was detected and was above the RSRL.*

[b]ND = non-detect

Table 2. Inorganic element concentrations (mg/kg dry weight) detected in a western bluebird egg sample collected near the TA-36 Minie compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data from 2020 (n = 7).

Element	Western bluebird (n = 1) SFB-20-206081	RSRL
Antimony[a]	**0.190**	0.179
Barium	17.0	17.4
Calcium	**17,000**	14,611
Copper	**4.10**	3.99
Iron	150	217
Magnesium	530	596
Manganese	2.80	4.03
Mercury	0.021	0.123
Potassium	10,000	10,411
Selenium	2.0	4.2
Sodium	9,700	11,028
Zinc	55.0	59.9

[a]A *bold value indicates that the element was detected and was above the RSRL.*