



Arthropod communities in a selenium-contaminated habitat with a focus on ant species[☆]



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ABSTRACT

The selenium contamination event that occurred at Kesterson Reservoir (Merced Co., CA) during the 1970–80s is a frequently cited example for the negative effects of contamination on wildlife. Despite the importance of arthropods for ecosystem services and functioning, relatively little information is available as to the impacts of pollution on arthropod community dynamics. We conducted surveys of the arthropod community present at Kesterson Reservoir to assess the impacts of selenium contamination on arthropod diversity, with a focus on ant species richness, composition and density. Trophic groups were compared to determine which arthropods were potentially receiving the greatest selenium exposure. Plant samples were analyzed to determine the selenium content by site and by location within plant. Soil concentrations varied across the study sites, but not across habitat types. Topsoil contained higher levels of selenium compared to core samples. Plants contained similar concentrations of selenium in their leaves, stems and flowers, but flowers contained the greatest range of concentrations. Individuals within the detritivores/decomposers and predators accumulated the greatest concentrations of selenium, whereas nectarivores contained the lowest concentrations. Species composition differed across the sites: *Dorymyrmex bicolor* was located only at the site containing the greatest soil selenium concentration, but *Solenopsis xyloni* was found at most sites and was predominant at six of the sites. Selenium concentrations in ants varied by species and collection sites. Nest density was also found to differ across sites, but was not related to soil selenium or any of the habitat variables measured in our study. Selenium was not found to impact species richness, but was a significant variable for the occurrence of two out of the eight native species identified.

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1. Introduction

Understanding of the responses exhibited by arthropods to habitat variation or stress can be important for predicting changes that may occur within arthropod communities following disturbance. In particular, changes that occur among ant populations may reveal potentially negative consequences for ecosystem functioning due to the critical roles ants play in various ecological processes (Folgarait, 1998; Del Toro et al., 2012). In Australia, ants serve as useful biological indicators of ecosystem changes following such disturbances as fires, mining, deforestation, urbanization and

pollution (Hoffmann and Andersen, 2003; Andersen and Majer, 2004). The impacts of heavy metal pollution on ant populations have also been extensively investigated in Europe (Grześ, 2010). Such investigations have reported pollution-induced effects on abundance (Bengtsson and Rundgren, 1984; Eeva et al., 2004), colony size (Eeva et al., 2004), species diversity (Bengtsson and Rundgren, 1984; Grześ, 2009), behavior (Sorvari and Eeva, 2010), and health (Sorvari et al., 2007). However, there is a lack of similar studies available for ant populations in North America, despite the existence of both natural and anthropogenic sources of environmental contamination.

Selenium is a naturally occurring element that enters the environment through the weathering of Cretaceous sedimentary rock, but can be concentrated and mobilized following human activities such as mining, smelting, coal burning and irrigation (Haygarth, 1994). Selenium is globally widespread, but present in varying concentrations across regions within a given country (Oldfield,

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2002). In the United States, selenium is particularly abundant in soils of several western states (Brown et al., 1999), where about 414,400 km² of land are considered susceptible to rain or irrigation-induced selenium contamination (Seiler et al., 1999). Selenium is an essential dietary requirement for animals (including insects), and several reports have linked regions with high incidences of dystrophy, cardiovascular disease, and certain cancers to selenium deficiencies (NRC, 1983; Oldfield, 2002). Previous studies suggest selenium may also play a similar important role for some insects (Martin-Romero et al., 2001; Popham et al., 2005). However, exposure to excess amounts of Se can also cause negative effects, such as vomiting, hair loss and yellowing of nails in humans and blind staggers and hoof deformations in animals (NRC, 1983). In insects, selenium exposure can increase mortality, decrease reproduction, and modify behaviors (Hladun et al., 2013; De La Riva and Trumble, 2016; Burden et al., 2016).

The potential for selenium toxicity in wildlife became evident in the 1980s when high concentrations of selenium at Kesterson Reservoir were found responsible for the deaths and deformities exhibited by birds breeding in the reservoir's evaporation ponds (Ohlendorf et al., 1986). The holding ponds of Kesterson Reservoir, located in Merced Co., CA, were originally meant to act as both a wetland habitat for migrating birds and a location to divert excess agricultural drainage water from the San Joaquin Valley's irrigated fields. However, damage to avian and fish populations occurred when subsurface drainage waters carrying selenium from the valley's selenium-abundant soils concentrated at the reservoir (Garone, 1998). The use of the reservoir as a repository for drainage water was then terminated and the ponds were filled in and the vegetation plowed in an effort to prevent further exposure to wildlife. Scientists have continued to monitor wildlife in the area, with most studies focusing on birds and small mammals (Ohlendorf et al., 1988; Santolo, 2009, 2007). A few publications have reported on the accumulation of selenium by invertebrates at

Kesterson (Ohlendorf, 2002; Ohlendorf et al., 1988; Santolo and Yamamoto, 1999; Santolo, 2007), but no published information is available as to the impact on ants, pollinators, or terrestrial insects at the population or community level.

The objectives of this survey were as follows: 1) document the ant populations present; 2) determine whether selenium concentrations present in the environment are impacting ant species composition and abundance; 3) compare bioaccumulation levels of selenium across ant species and ant functional groups; 4) identify other insect taxa residing at Kesterson Reservoir; 5) compare selenium levels across different insect trophic groups.

2. Materials and methods

2.1. Study sites

Surveys were conducted during the spring of 2013 and 2014 at Kesterson National Wildlife Refuge, at the site of the former Kesterson Reservoir (37° 13' 53" N, 120° 53' 26" W, ~8 km east of Gustine, CA). The original 12 holding ponds no longer remain after the drying and filling or disking of the habitat; monitoring in the area has since been conducted based on three trisections of the entire 2100-ha land (Ohlendorf and Santolo, 1994). Our surveys were conducted on the southern end of the reservoir (Fig. 1) in sites at TriSection 1 (previously ponds 1–4) and the southern end of TriSection 2 (previously ponds 5–7, 9) in the three main habitat types (filled, open and grassland). Filled habitats were previously lower elevation areas that were filled in with soil, open habitats were the lands that formerly contained cattails that were disked, and grassland habitats were the upland areas that existed before the reservoir ponds were filled (Ohlendorf and Santolo, 1994). Collections were conducted in habitats at the southern end of Kesterson because we expected soil selenium concentrations to be greatest in the south (TriSection 1) and decrease northward,

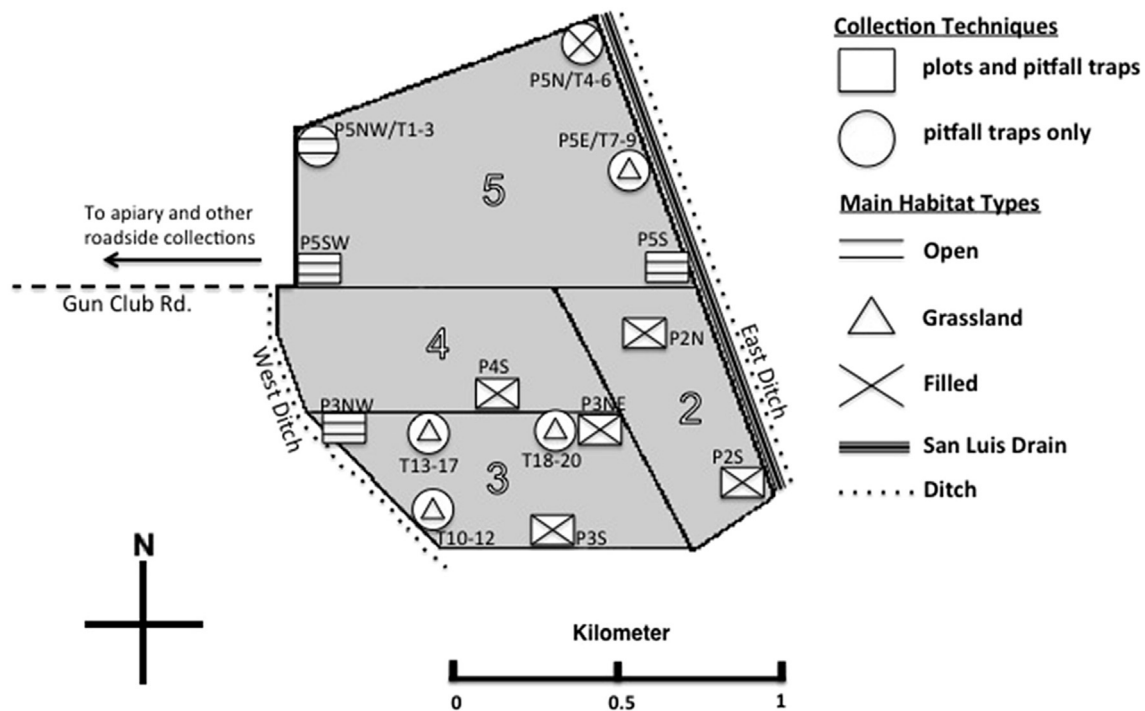


Fig. 1. Collection site locations within Kesterson Reservoir that were previously ponds 2, 3, 4 and 5. Rectangles represent sites that contained six replicate 4-m x 4-m plots containing pitfall traps. Circles only are those sites that only contained additional pitfall traps. Main habitat types were determined using previously established maps of Kesterson (Ohlendorf and Santolo, 1994).

because the reservoir previously received drainage water that flowed from the southern to the northern ponds (Wahl et al., 1994).

2.2. Data collection

At each of the main collection sites (represented by squares in Fig. 1), six collection locations were arbitrarily chosen encompassing a sampling area of ~1000 m². A 4-m x 4-m plot was established at each of the six collection locations within each site. A sugar/carbohydrate ant bait (crumbled Pecan Sandies, Keebler™) for foragers was evenly distributed throughout each plot (Agosti et al., 2000). Baiting allowed for easier location of individual foraging ants, as well as helping to determine nest locations when foragers were followed back to their nest. Two people conducted collections within each plot for a total timed duration of 15 min. Hand collections (blunt featherweight forceps, BioQuip Products, Inc., Rancho Dominguez, CA) and aspirator collections were made of foraging ants, individuals exiting each ant nest, and other wandering ground insects. Samples were stored in coolers containing ice in 49-mm x 85-mm plastic vials. Due to the presence of potentially high selenium levels at the southernmost collection site, we used a small shovel to scoop the top ~10-cm of each nest (ants and soil), rather than aspirating directly from the nest. All samples were bagged in 1-L Ziploc® bags and placed on ice. To determine the occurrence of species that may have had foraging activity periods at times other than when hand collections were made, we used pitfall traps (Agosti et al., 2000). Two pitfall traps (118-mL cups, 7-cm dia.) were placed (one per plot corner) diagonally from each other. These were filled ¾ with water and mixed with a small amount of dish soap. Blue and yellow pan traps (355-mL, 18-cm dia., one of each color per plot) were placed on opposite edges of each plot. Pan traps were also filled with a mixture of water and dish soap (Dawn®, Procter & Gamble Co., United States) and a small rock was placed at the center of each pan trap to prevent tipping from wind. Pitfall traps and pan traps were left out for ~72-hr. before collecting and emptying the contents into plastic collection bags (118-mL). These were also stored in ice coolers. Pitfall traps and pan traps were also individually placed in other sites around Kesterson (represented by circles in Fig. 1) to sample insects at sites from which we were unable to conduct hand collections. Sweep netting for insects on flowering vegetation was conducted within the reservoir and at several sites just outside the reservoir (roadside, near a local honey bee apiary, a ditch located on the east side of the San Luis Drain, and a ditch on the west edge of the reservoir, also known as “Kesterson Ditch” on Google Earth). Upon returning to the laboratory, insect samples were removed from the ice and stored in vials containing 80% ethanol before species identification. Once identified, insects were placed either individually or with other nest members into 1.5-mL centrifuge tubes. Bagged samples that contained topsoil and ants from the southernmost site were emptied into separate trays. All ants belonging to a single nest were removed from the soil, rinsed with double distilled water to remove soil debris, and placed into a 1.5-mL centrifuge tube. A portion of the remaining soil was taken for selenium analysis. Voucher specimens of collected ants were submitted to the UC Riverside Entomology Museum collection: UCRC ENT 461246–461263.

Soil samples also were taken at each plot using a metal soil core sampler (~1.5-cm dia.). Three core samples were taken to a depth of ~40-cm within each plot. Each of the entire three 40-cm cores were mixed together in a bucket using a small shovel. A composite sample from the mixture was bagged and stored on ice. Soil moisture, soil pH, soil type, soil description (bare with small rocks/gravel, bare with detritus/organic material, salt crusted), ground cover (0–25%, 26–50%, 51–75%, or 76–100%) and a description of the vegetative type (grass, small shrubs, large shrubs, weeds) were

recorded for each plot. Data for temperature, % relative humidity, and precipitation were taken from the online California Irrigation Management Information System (CIMIS) using recordings from the Kesterson weather station (#92). Flowering plants were sampled in to assess selenium exposure levels to pollen- and nectar-visiting insects. Most flowering plants were located along ditches on the eastern and western edges of the reservoir. Plant samples from these locations, as well as a few from within the habitat and near a local apiary and roadside, were collected by taking cuttings along the stalk of flowering sections and by taking leaf clippings. These samples were placed in separate Ziploc® bags and stored on ice until further separations could be made in the laboratory.

2.3. Selenium analysis

Insect and plant samples were freeze dried (Labconco Corp., Kansas City, MO) at –40 °C and –25 psi for 48-hr and 72-hr, respectively. Dried samples were then weighed on a microbalance before microwave digestion. Insect and plant samples were digested in 110 mL Teflon-lined vessels in 5-mL of HNO₃ for 20 min at 200 °C, 300 psi and 1200-W in a microwave oven (CEM Corp., Matthews, NC). Quality control was conducted using Se spikes, blanks, NIST Standard Reference Material 8436 (durum wheat flour) for plant samples and NIST 1566b (oyster tissue) for insect samples. Selenium reference recoveries averaged over 90%, with lower and upper limits ranging from 40 to 100%. Solid soil samples were submitted on ice to Test America (Irvine, CA), where analysis of selenium was conducted using method 6020-ICP/MS (reporting limit = 1.00 mg/kg; minimum detection limit = 0.5 mg/kg). Selenium spike recoveries for soil averaged >80%, with lower and upper limits of 62–93%.

2.4. Data analysis

All statistical analyses were performed using R version 3.2.2 (The R Foundation for Statistical Computing, 2015). Normality and checking for equal variance were conducted using Shapiro Wilk's Test and Bartlett's Test, respectively. A non-parametric Kruskal-Wallis test or Welch Test was used to perform multiple comparisons when data could not be normalized by transformation, whereas an ANOVA was used if data met requirements.

To determine whether selenium concentrations in soil differed across habitats (filled, open, grassland) or by collection sites we performed multiple comparisons using Kruskal-Wallis tests. For analysis of plant concentrations across the factors of family, habitat, and plant part (flowers, stems, leaves, and whole samples for those that were too small to separate) we first compared linear models containing interactions and no interactions between three factors. An initial comparison of models indicated that there were no significant interactions among the three factors (ANOVA, $F = 1.26$, $P = 0.3$), so we analyzed each factor separately using a Welch Test. All arthropod samples were categorized into five trophic groups based on their general feeding patterns (Predators (P), Herbivores (H), Nectarivores (N), Omnivores (O), Detritivores/decomposers (D)) and Ant. Although ants fall under the category of omnivores, we were interested in comparing their selenium accumulation to those of the other arthropod categories. A Kruskal-Wallis test with post hoc Dunn Test and Bonferroni adjustment were used to compare selenium concentrations across trophic groups and habitats. Extra habitat categories were included in the analysis for the insects that were collected at locations in the surrounding vicinity of the reservoir (road, apiary, west and east ditch).

A comparison of selenium body burdens in ants across ant species and collection sites was conducted using a Kruskal-Wallis

Test and post hoc Dunn Test. This was followed up with a simple linear regression to test for a correlation between ant body concentrations and soil concentrations. To determine which habitat variables had an impact on ant species richness and nest density we first conducted a principal component analysis (PCA, package *FactoMineR*) of all measured habitat variables (soil selenium concentration, % vegetative cover, soil type, % large shrub, % small shrub, % weeds, % grass, moisture, pH, elevation, and habitat type). The first principal component explained ~28% of the variation and the second principal component explained ~17% of the variation. The four habitat variables with the highest significant contributions to the first dimension were soil type (18%), grass (14%), moisture (15%), and pH (17%). A Spearman analysis of correlations between factors indicated that those variables were also highly correlated with each other. In addition, “cover” was highly correlated with “grass” at 81%. This suggests that the first principal component distinguishes between two broad habitat types: 1) higher % grass and cover, lower soil moisture, higher pH, and bare or organic soil type vs. 2) lower % grass and cover, higher moisture, lower pH, and salty soil. We then used the individual principal component coordinates within the first dimension as a proxy in analysis against species richness, nest density, and the occurrence of each individual ant species. Generalized linear models with Poisson probability distribution were used to analyze species richness and nest density, whereas binomial distribution was used in the analysis for the presence or absence of each ant species. Soil selenium concentration loaded highest on the fourth principal component (28%), which explained only 10% of the total variation. A Spearman correlation of selenium against the other factors indicated that it was not highly correlated to any other variable. We, therefore, analyzed it as a separate factor. To determine whether ant species composition might have also been influenced by the presence or absence of other ant species across the different collection sites, we conducted a Co-Occurrence Analysis (*EcoSimR* package 0.1.0, *SIM9* algorithm) with a C-score metric to indicate aggregation vs. avoidance (Gotelli, 2000; Parr and Gibb, 2010).

3. Results

Selenium concentrations for soil core samples did not differ across the established filled, open, and grassland habitat types (Kruskal-Wallis: $X^2 = 5.6$, $df = 2$, $P = 0.06$), but they did differ across collection sites (Kruskal-Wallis: $X^2 = 51.8$, $df = 12$, $P < 0.0001$,

Fig. 2). Core samples from the southernmost site (P2S) had the highest concentrations ranging from 16–35 mg Se kg⁻¹ (post hoc Dunn Test P values for comparisons against 11 out of 12 other sites ranged <0.05–0.0001). Topsoil concentrations from ant nests collected at this location were much higher than core samples and ranged from 7–200 mg Se kg⁻¹ (Table 1). In contrast, soil cores from collection sites P3S and P4S were among the lowest concentrations at ranges of 0–2.8 and 0 mg Se kg⁻¹, respectively.

During both years, most of the flowering plants were found at the surrounding edges of each pond as well as in the surrounding vicinity of the reservoir, rather than inside the habitats. We identified five plant families (Brassicaceae, Azoaciae, Amaranthaceae, Apiaceae, and Asteraceae; Supplemental Table 1) from our collected samples. Selenium contained in plants did not vary as a result of family (Welch Test: $F = 3.5$, $df = 4,4.2$, $P = 0.12$), collection site (Welch Test: $F = 3.9$, $df = 3,8.8$, $P = 0.051$) or plant part (Welch Test: $F = 4.09$, $df = 3,3.4$, $P = 0.09$). However, flowers contained the greatest range of concentrations (0–27 $\mu\text{g Se g}^{-1}$), whereas the stems, leaves, and whole samples contained concentrations below 5 $\mu\text{g Se g}^{-1}$.

Arthropods across 13 different orders and 29 families were analyzed for selenium accumulation (Supplemental Table 2). Arthropod concentrations were found to differ across habitat types and additional collection locations (Kruskal-Wallis: $X^2 = 13.18$, $df = 6$, $P = 0.04$). Except when compared to arthropods collected from plants along the ditch on the east end of the reservoir (post hoc Dunn test: $P = 0.12$), arthropods collected from the grassland habitat accumulated significantly greater concentrations compared to other habitat sites (open, filled) and additional habitat locations, such as plants along the roadside, apiary, and west ditch (post hoc

Table 1

Selenium concentrations in topsoil from ant nests with a comparison of accumulated selenium between two closely nesting ant species.

Nest sample ID	Soil conc. (mg Se/kg)	Ant species	Ant conc. ($\mu\text{g Se/g}$)
P2-1-1 N1	7	<i>S. xyloni</i>	21
		<i>D. bicolor</i>	10
P2-1-1 N2	99	<i>S. xyloni</i>	29
		<i>D. bicolor</i>	15
P2-1-1 N3	87	<i>S. xyloni</i>	29
		<i>D. bicolor</i>	25
P2-1-1 N4	200	<i>S. xyloni</i>	35
		<i>D. bicolor</i>	10
P2-1-6 N1	16	<i>D. bicolor</i>	10
P2-1-6 N2	52	<i>S. xyloni</i>	18

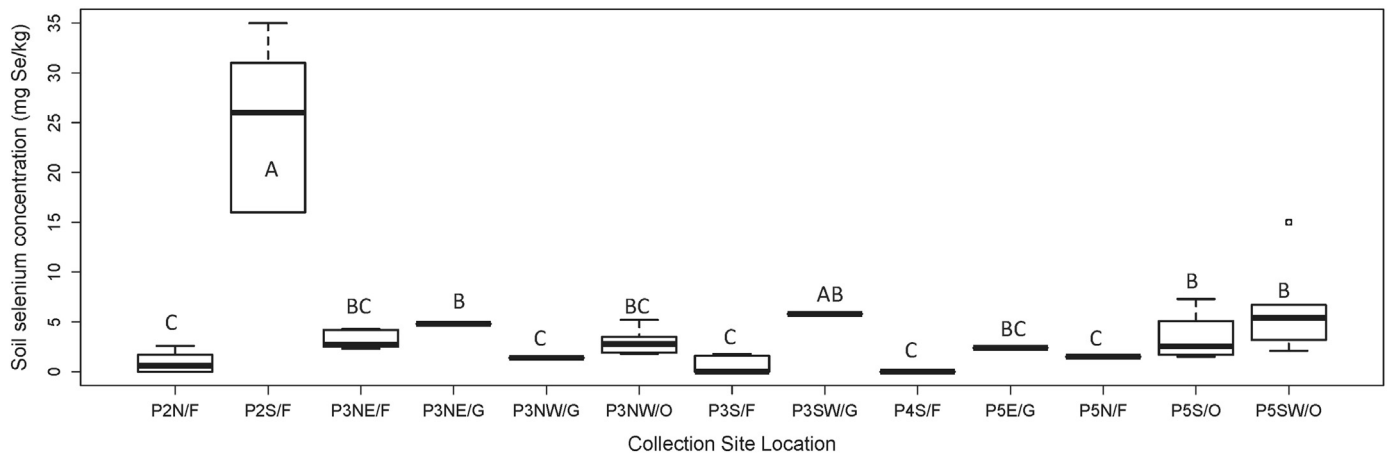


Fig. 2. Soil selenium concentrations across collection sites. There was no soil sample for “P5NW”. The letter following the dash refers to the collection sites’ respective habitat type (open, filled, grassland). Different letters above each boxplot represent samples that contained statistically different concentrations in soil (Kruskal-Wallis, post hoc Dunn test, $\alpha = 0.05$).

Dunn test: all P values = 0.04–0.005). Insects collected from the flowering plants near a local apiary and the ditch on the west edge of the reservoir contained the lowest average concentrations of 0 and $0.3 \mu\text{g Se g}^{-1}$, respectively. Selenium concentrations accumulated by arthropods were also found to differ across trophic groups (Kruskal-Wallis: $X^2 = 32.7$, $df = 5$, $P < 0.001$, Fig. 3), where nectarivores contained the lowest body concentrations ($0\text{--}14 \mu\text{g Se g}^{-1}$) of selenium compared to all other trophic groups (post hoc Dunn test with Bonferroni adj.: all P values < 0.01). Detritivores/decomposers had the greatest range of concentrations from $0\text{--}179 \mu\text{g Se g}^{-1}$ followed by predators with $0\text{--}123 \mu\text{g Se g}^{-1}$. A few herbivorous insects also contained high selenium levels such as $73 \mu\text{g Se g}^{-1}$ in a stink bug and $35 \mu\text{g Se g}^{-1}$ within a caterpillar. Ants did not differ in accumulated selenium compared to other omnivorous insects (post hoc Dunn test: $P = 0.49$).

Eight native ant species were found residing at Kesterson; these included *Solenopsis xyloni*, *Dorymyrmex bicolor*, *D. insanus*, *Pheidole hyatti*, *Forelius mccoocki*, two species of *Formica*, and *Tapinoma sessile*. Species composition differed across collection sites (Fig. 4). For instance, the ant species *D. bicolor* was found nesting only in the southernmost habitat containing the highest soil selenium concentrations. In contrast, the fire ant species *S. xyloni* was present at 10/14 sites, followed by *P. hyatti* at 50% of the sites. Species richness did not differ across collection sites (GLM with Poisson: $Z = -0.2\text{--}1.0$, $df = 12$, 67, all P values > 0.33), nor did it vary as a factor of selenium concentration (GLM with Poisson: $Z = -0.4$, $df = 7,47$, $P = 0.7$) or habitat factors (GLM with Poisson: $Z = -0.7$, $df = 7,47$, $P = 0.5$). Nest density did vary across collection sites with the greatest total number of nests located at P5S (15 nests, GLM with Poisson: $Z = 2.7$, $df = 12,67$, $P < 0.01$), P2S (12 nests, GLM with Poisson: $Z = 2.1$, $df = 12,67$, $P = 0.03$) and P4S (11 nests, GLM with Poisson: $Z = 2.7$, $df = 12,67$, $P = 0.04$). However, neither soil selenium concentrations (GLM with Poisson: $Z = -0.5$, $df = 7,47$, $P = 0.6$) nor the habitat factors (Dim 1) measured in our survey (GLM with Poisson: $Z = -0.8$, $df = 7,47$, $P = 0.4$) was found to explain this variation.

We tested whether soil selenium concentrations and/or our measured habitat variables might explain the presence or absence of each ant species across the different sites (Table 2). Soil selenium was a significant factor for *D. bicolor* and *Formica* sp. 2 and habitat

factors (Dim.1 from PCA) were significant for *S. xyloni*, *Formica* sp. 1 and *Formica* sp.2.

To determine whether competition might have played a role for ant species where habitat factors and selenium were not predictors, we conducted a co-occurrence analysis. The observed distribution of co-occurrence between all ant species pairs was not different from the simulated distribution (Co-Occurrence Null Model, Algorithm SIM9, $P = 0.4$, C-score = 3.14), indicating that their co-occurrence with each other was neither a result of aggregation nor avoidance between pairs. Finally, we found that selenium concentration differed across ant species (Kruskal-Wallis: $X^2 = 20.8$, $df = 7$, $P < 0.001$, Fig. 5) with the highest median accumulated selenium occurring in *D. bicolor* and the lowest in *F. mccoocki* and *Formica* spp. The pyramid ant species, *D. insanus* was omitted from the test due to the low sample size of one nest. The variation in concentration among *P. hyatti* and *S. xyloni* might be explained by the location at which those samples were collected, as ant concentrations also differed as a factor of collection site (Kruskal-Wallis: $X^2 = 30.9$, $df = 7$, $P < 0.001$). Ants collected at collection site P2S contained greater selenium body burdens compared to sites P3NE, P3NW, and P4S (post hoc Dunn test with Bonferroni: P s = 0.004–0.03). Selenium concentrations in ants were also highly correlated with the soil concentrations ($F = 37.14$, $df = 1,89$, $R^2(\text{adj.}) = 0.28$, $P < 0.001$). However, species nesting closely together in the same habitat (*D. bicolor* and *S. xyloni* were found together in several of the same soil nest samples taken from P2S) were still found to accumulate different levels of selenium (Table 1).

4. Discussion

Soil selenium concentrations differed across collection sites and ranged from 0 mg Se kg^{-1} in plots at sites P4S and P3S to 35 mg Se kg^{-1} in site P2S. Topsoil samples ($\sim 13\text{-cm}$ depth) directly from ant nest mounds contained greater levels of selenium than core samples ($\sim 40\text{-cm}$ depth), with a maximum concentration of $200 \text{ mg Se kg}^{-1}$ at one nest site. This supports previous reports that stated the highest selenium concentrations in soil at the reservoir are contained in the top 15-cm and decline with increasing depths (Wahl et al., 1994 [and references therein]). The morphology and depth of ant nests is species specific. For instance, nests of harvester ants, genus *Pogonomyrmex*, have depths ranging from 2 to 3.5-m (Tschinkel, 2003), whereas ant species within the genus *Dorymyrmex* commonly nest in depths no deeper than 10–15-cm (Cuezzo and Guerrero, 2011). This suggests that the native ant *D. bicolor* has high tolerance to selenium because it was found only in the topsoil at the site containing the highest soil selenium concentrations. Depending on the depth and structure of the ant nests for other species residing at Kesterson, there is a possibility that some of the other species may be escaping selenium contact by modifying the depth of the nests. However, initial excavation and periodic maintenance of those nests is likely a source of selenium transfer to workers. Nest depth, and thus exposure to concentrated soils, may change with season, as some ant species have been reported to adjust their distance from the surface in response to ambient temperature (Bollazzi et al., 2008). In contrast to previous studies which found the highest soil selenium concentrations in open habitats, compared to filled and grassland (Wahl et al., 1994; CH2M HILL and Lawrence Berkley National Laboratory, 2000; CH2M HILL, 2015), soil concentrations in our study did not vary. However, this is not surprising because our study was conducted predominantly at the heavily contaminated southern end of the reservoir, rather than across the entire reservoir.

Plant samples collected during our study were analyzed to assess selenium levels available to herbivores as well as insect

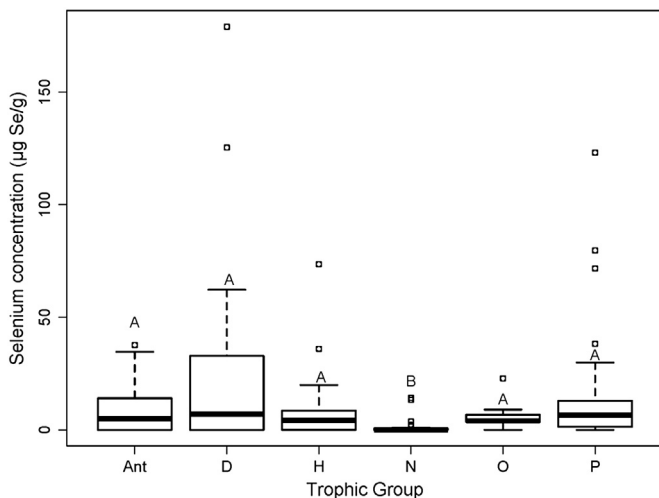


Fig. 3. Selenium concentrations compared across trophic groups, where (D = detritivores/decomposers, H = herbivores, N = nectarivores, O = omnivores, P = predators and Ant = ants). Ants are often considered omnivores, but were separated to compare their accumulation to all other groups. (Kruskal-Wallis, post hoc Dunn test with Bonferroni p. adj., $\alpha = 0.05$).

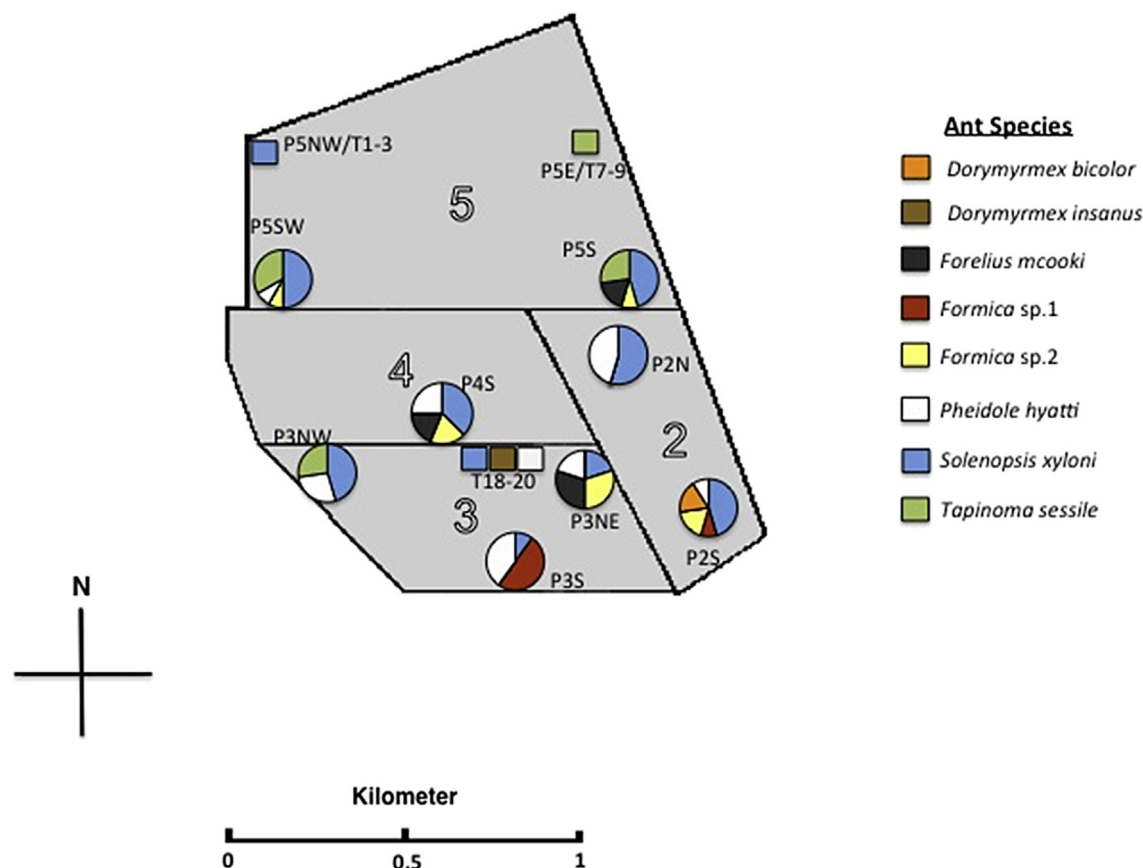


Fig. 4. Ant species composition at each collection site. The size of each slice in the pie graphs represents the relative proportion of each species at that location. Small squares represent the occurrence of a species in “pitfall trap only” sites.

Table 2

Individual ant species occurrence as a factor of soil selenium concentration (Se) or habitat factors (Dim1) from the PCA analysis. Test method: GLM with binomial distribution, $df = 47$, $\alpha = 0.05$. Dashed lines represent analysis that could not accurately be performed due to low occurrence across different sites.

Ant species	Factors	Z	P value ($\alpha = 0.05$)
<i>Solenopsis xyloni</i>	Se	0.23	0.82
	Dim1	−2.48	0.01
<i>Dorymyrmex bicolor</i>	Se	1.98	0.05
	Dim1	—	—
<i>Dorymyrmex insanus</i>	Se	—	—
	Dim1	—	—
<i>Forelius mcooki</i>	Se	−1.45	0.15
	Dim1	0.92	0.36
<i>Formica sp.1</i>	Se	−1.97	0.06
	Dim1	2.85	0.01
<i>Formica sp.2</i>	Se	2.28	0.02
	Dim1	−2.31	0.02
<i>Pheidole hyatti</i>	Se	−1.80	0.07
	Dim1	0.36	0.72
<i>Tapinoma sessile</i>	Se	0.20	0.84
	Dim1	−1.95	0.06

pollinators/nectarivores. Our findings indicated that selenium levels accumulated by plants did not statistically differ across plant families or collection site. However, most of the flowering plants during our spring surveys (predominantly Brassicaceae) were located at the surrounding edges rather than within the habitat. Total selenium concentrations also did not differ by plant structure, but flowers contained the greatest range of concentrations, exceeding $20 \mu\text{g Se g}^{-1}$ in some *Brassica sp.* samples, whereas other

structures did not contain levels above $5 \mu\text{g Se g}^{-1}$. Previous experiments have described the ability for selenium accumulating species within Brassicaceae to concentrate levels exceeding $100 \mu\text{L Se mL}^{-1}$ FW in the nectar and $1000 \mu\text{g Se g}^{-1}$ in the pollen (Hladun et al., 2011; Quinn et al., 2011). It was therefore surprising that nectarivores (native bees, syrphid flies, and honey bees) in our study contained the lowest selenium body concentrations compared to other trophic groups. This suggests the possibility that arthropods seeking nectar sources may be experiencing different levels of exposure throughout the year with the variation in location and timing of flowering plants.

A comparison of accumulated levels across trophic groups revealed that trophic groups were not statistically different except when compared to nectarivores, as described above. This suggests that biomagnification does not appear to be occurring among trophic groups within this habitat. Nevertheless, several samples among detritivores/decomposers, herbivores, and predators contained notably high selenium concentrations. The wide range in concentrations observed for detritivores/decomposers was mostly due to the concentrations accumulated by Isopods, which ranged from $29\text{--}179 \mu\text{g Se g}^{-1}$. Although we did not sample detritus, these findings support recent conclusions for dead/organic plant material serving as an important entry pathway for selenium into trophic food webs in this habitat (CH2M HILL, 2015). Despite previous reports for the greatest concentrations in soil, plant, and detritus samples occurring in the open habitat compared to filled and grassland habitats, our study indicated that arthropods collected from the grassland habitat contained the greatest body burdens. However, grassland habitat in our sampling area was sparse

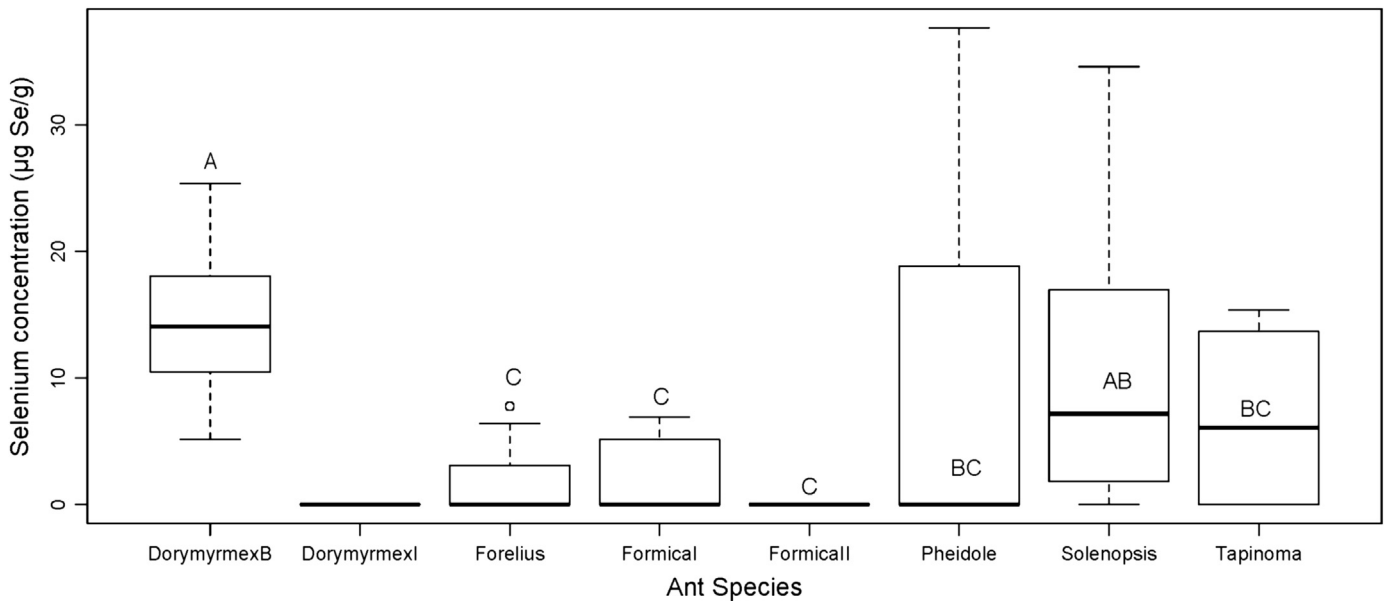


Fig. 5. Accumulated selenium across ant species. The ant species *Dorymyrmex insanus* (*DorymyrmexI*) was graphically represented, but was not included in the analysis due to the small sample size. (Kruskal-Wallis, post hoc Dunn test, $\alpha = 0.05$).

compared to the northern end of the reservoir, and was largely surrounded by both filled and open habitat types. Except for nesting or burrowing arthropods, such as ants and some spiders, which remain relatively sedentary over longer periods, it is very likely that several insects analyzed may have been travelling between habitats in search of food. Overall concentrations for arthropods reported in our study are similar to those found in previously sampled invertebrates from this habitat (Santolo and Yamamoto, 1999; Santolo, 2007).

Several studies have investigated the impacts of disturbance on ant diversity (Hoffmann and Andersen, 2003 [and references therein]). The majority of research exploring the effects of pollution on ants has come from Europe, where findings include impacts on abundance (Bengtsson and Rundgren, 1984; Eeva et al., 2004), colony size (Eeva et al., 2004), species diversity (Bengtsson and Rundgren, 1984; Grześ, 2009), behavior (Sorvari and Eeva, 2010) and health (Sorvari et al., 2007). Currently, there is a lack of comparable information available for North American ant species. In our study, species composition differed across collection sites, but selenium contamination as a possible factor was significant only for the occurrence of two species, *D. bicolor* and *Formica* sp. 2. In addition, selenium appears to have little impact on ant species richness and density. Taken together, this suggests that these native species have a high tolerance to selenium. This is in contrast to experiments that have shown detrimental impacts of selenium on the survival (De La Riva et al., 2014) and reproduction (De La Riva and Trumble, 2016) of the invasive Argentine ant, *Linepithema humile*. According to an updated document mapping the global distribution of selenium (Oldfield, 2002), there is a high incidence of selenium-deficient soils in the native range of Argentine ants. This might help to explain the previously reported susceptibility of Argentine ants to selenium as well as lead to important predictions for the response of other invasive species from the same area, such as *Solenopsis invicta*, the red imported fire ant. Sub-lethal impacts of selenium on native species, which were not captured by our measurements, are also possible. For instance, Eeva et al. (2004) found that populations of wood ants *Formica* sensu stricto were able to nest and reproduce in habitats containing high concentrations of metals, but exhibited smaller colony sizes compared to those

nesting in non-polluted sites. Additional research is necessary to elucidate the reasons behind these differences in tolerance.

Selenium concentrations accumulated by ants were influenced by species and nesting site. For example, *D. bicolor* was among the highest accumulating species and was found nesting in soil with the highest level of selenium contamination. However, diet preferences and possible differences in regulation of selenium between species should not be ruled out, as was evidenced by the different concentrations accumulated by *D. bicolor* and *S. xyloni*, despite their identical locations. The low R^2 value of 0.28 from the linear regression analysis further indicates that although ant body selenium concentrations were highly correlated to soil concentrations, other factors might also be responsible.

This study demonstrates the ability of arthropods to bioaccumulate potentially toxic compounds in varying concentrations depending on their activity within their environment. For this reason, arthropods may serve as a potential pathway for transfer of contaminants to higher trophic groups. More work is necessary to explore the physiological mechanisms and/or evolutionary reasons behind tolerance of pollutants among different arthropods. Knowledge of existing differences in metalloids and metal regulation ability between native and invasive arthropod species can lead to important predictions and management decisions for areas susceptible to invasion. Future research on arthropods at Kesterson Reservoir should consider comparing differences in trophic groups with vegetation and flowering changes throughout the year. A greater sampling effort is necessary to compare arthropod communities across the entire reservoir area to elucidate the existence of additional species or changes in community dynamics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.09.052>.

References

- Agosti, D., Majer, J.D., Alonso, L.E., Schultz, T.R., 2000. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Smithsonian Institution Press, Washington and London.
- Andersen, A.N., Majer, J.D., 2004. Ants show the way down under: invertebrates as bioindicators of land management. *Front. Ecol. Evol.* 2, 291–298.
- Bengtsson, B.G., Rundgren, S., 1984. Ground-living invertebrates in metal-polluted forest soils. *Ambio* 13, 29–33.
- Bollazzi, M., Kronenbitter, J., Roces, F., 2008. Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf cutting ants. *Oecologia* 158, 165–175.
- Brown, G., Foster, A., Ostergren, J., 1999. Mineral surfaces and bioavailability of heavy metals: a molecular-scale perspective. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3388–3395.
- Burden, C.M., Elmore, C., Hladun, K.R., Trumble, J.T., Smith, B.H., 2016. Acute exposure to selenium disrupts associative conditioning and long-term memory recall in honey bees (*Apis mellifera* L.). *Ecotoxicol. Environ. Saf.* 127, 71–79.
- CH2M HILL, 2015. Kesterson Reservoir 2013/2014 Biological Monitoring Report. Prepared for the U.S. Bureau of Reclamation Mid-Pacific Region.
- CH2M HILL and Lawrence Berkeley National Laboratory (CH2M HILL and LBNL), 2000. Ecological Risk Assessment for Kesterson Reservoir. Prepared for the U.S. Bureau of Reclamation Mid-Pacific Region.
- Cuezzo, F., Guerrero, R., 2011. The ant genus *Dorymyrmex* Mayr (Hymenoptera: Formicidae: Dolichoderinae) in Colombia. *Psyche A J. Entomol.* 2012, 1–24.
- De La Riva, D.G., Trumble, J.T., 2016. Selenium exposure results in reduced reproduction in an invasive ant species and altered competitive behavior for a native ant species. *Environ. Pollut.* 213, 888–894.
- De La Riva, D.G., Vindiola, B.G., Castañeda, T.N., Parker, D.R., Trumble, J.T., 2014. Impact of selenium on mortality, bioaccumulation and feeding deterrence in the invasive Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Sci. Total Environ.* 481, 446–452.
- Del Toro, I., Ribbons, R.R., Pelini, S.L., 2012. The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). *Myrmecol. News* 17, 133–146.
- Eeva, T., Sorvari, J., Koivunen, V., 2004. Effects of heavy metal pollution on red wood ant (*Formica* s. str.) populations. *Environ. Pollut.* 132, 533–539.
- Folgarait, P., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodivers. Conserv.* 7, 1221–1244.
- Garone, P., 1998. The tragedy at Kesterson Reservoir: a case study in environmental history and a lesson in ecological complexity. *Environ. Envtl. Law Policy J.* 22, 107–144.
- Gotelli, N.J., 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81, 2606–2621.
- Grześ, I.M., 2010. Ants and heavy metal pollution – a review. *Eur. J. Soil Biol.* 46, 350–355.
- Grześ, I.M., 2009. Ant species richness and evenness increase along a metal pollution gradient in the Boleslaw zinc smelter area. *Pedobiol. (Jena)* 53, 65–73.
- Haygarth, P.M., 1994. Global Importance and Global Cycling of Selenium. In: Frankenberger Jr., W., Benson, S.M. (Eds.), *Selenium in the Environment*. Marcel Dekker, Inc., New York, pp. 1–27.
- Hladun, K.R., Kaftanoglu, O., Parker, D.R., Tran, K.D., Trumble, J.T., 2013. Effects of selenium on development, survival, and accumulation in the honeybee (*Apis mellifera* L.). *Environ. Tox. Chem.* 32, 2584–2592.
- Hladun, K., Parker, D., Trumble, J., 2011. Selenium accumulation in the floral tissues of two Brassicaceae species and its impact on floral traits and plant performance. *Environ. Exp. Bot.* 74, 90–97.
- Hoffmann, B.D., Andersen, A.N., 2003. Responses of ants to disturbance in Australia, with particular reference to functional groups. *Austral Ecol.* 28, 444–464.
- Martin-Romero, F.J., Kryukov, G.V., Lobanov, A.V., Carlson, B.A., Lee, B.J., Gladyshev, V.N., Hatfield, D.L., 2001. Selenium metabolism in *Drosophila*. *J. Biol. Chem.* 276, 29798–29804.
- National Research Council, 1983. *Selenium in Nutrition*, Revised Edition. The National Academies Press, Washington, D.C.
- Ohlendorf, H.M., 2002. The birds of Kesterson Reservoir: a historical perspective. *Aquat. Toxicol.* 57, 1–10.
- Ohlendorf, H.M., Hoffman, D.J., Saiki, M.K., Aldrich, T.W., 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. *Sci. Total Environ.* 52, 49–63.
- Ohlendorf, H.M., Kilness, A.W., Simmons, J.L., Stroud, R.K., Hoffman, D.J., Moore, J.F., 1988. Selenium toxicosis in wild aquatic birds. *J. Toxicol. Environ. Health* 24, 67–92.
- Ohlendorf, H.M., Santolo, G.M., 1994. Kesterson reservoir-past, present, and future: an ecological risk assessment. In: Frankenberger Jr., W.T., Benson, S.M. (Eds.), *Selenium in the Environment*. Marcel Dekker, Inc., New York, pp. 69–117.
- Oldfield, J.E., 2002. *Selenium world atlas* (updated edition). Selenium-Tellurium Developmental Association, Belgium.
- Parr, C.L., Gibb, H., 2010. Competition and the Role of Dominant Ants. In: Lach, L., Parr, C.L., Abbott, K. (Eds.), *Ant Ecology*. Oxford University Press Inc., New York, pp. 77–96.
- Popham, H.J.R., Shelby, K.S., Popham, T.W., 2005. Effect of dietary selenium supplementation on resistance to baculovirus infection. *Biol. Control* 32, 419–426.
- Quinn, C., Prins, C., Freeman, J., Gross, A.M., Hantzis, L.J., Reynolds, R.J.B., Yang, S.I., Covey, P.A., Bañuelos, G.S., Pickering, I.J., Fakra, S.C., Marcus, M.A., Arathi, H.S., Pilon-Smits, E.A.H., 2011. Selenium accumulation in flowers and its effects on pollination. *New Phytol.* 192, 727–737.
- R Development Core Team, 2015. *R: a Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>.
- Santolo, G.M., 2009. Small mammals collected from a site with elevated selenium concentrations and three reference sites. *Arch. Environ. Contam. Toxicol.* 57, 741–754.
- Santolo, G.M., 2007. Selenium accumulation in European Starlings nesting in a selenium-contaminated environment. *Condor* 109, 862–869.
- Santolo, G.M., Yamamoto, J.T., 1999. Selenium in blood of predatory birds from Kesterson Reservoir and other areas in California. *J. Wildl. Manage.* 63, 1273–1281.
- Seiler, R., Skorupa, J., Peltz, L., 1999. Areas Susceptible to Irrigation-induced Selenium Contamination of Water and Biota in the Western United States, US Geological Survey. U.S. Geological Survey, Carson City, Nevada.
- Sorvari, J., Eeva, T., 2010. Pollution diminishes intra-specific aggressiveness between wood ant colonies. *Sci. Total Environ.* 408, 3189–3192.
- Sorvari, J., Rantala, L.M., Rantala, M.J., Hakkarainen, H., Eeva, T., 2007. Heavy metal pollution disturbs immune response in wild ant populations. *Environ. Pollut.* 145, 324–328.
- Tschinkel, W.R., 2003. Subterranean ant nests: trace fossils past and future? *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 192, 321–333.
- Wahl, C., Benson, S., Santolo, G., 1994. Temporal and spatial monitoring of soil selenium at Kesterson Reservoir, California. *Water, Air Soil Pollut.* 74, 345–361.