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Use of soil chemical analysis to detect commercial wildlife game baits

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Abstract: Hunters and poachers often use commercially available, nutrient-rich baits to attract wildlife game animals. We used atomic absorption spectroscopy and ion selective electrochemical analysis techniques to determine whether 2 common proprietary baits (Deer Cane and Acorn Rage) would leave detectable chemical signatures in soil (i.e., $Na^{+,}$ Cl^{-}, and Ca^{+2}). Our goal was to evaluate low-cost tests that could be replicated by wildlife conservation officers in the field. To complete the evaluation, we randomly placed 2 commercial baits on 3 sites in the Millersville University Biological Preserve in Millersville, Pennsylvania, USA. We collected soil samples from each site over the course of 35 days after bait placement to conduct our soil chemical analysis. We found that baited soils consistently exhibited higher concentrations of Na^{+} and Cl^{-} compared to control soils. The levels of Na^{+} on baited soils for the first 3 weeks for both bait sites averaged 3,209 ppm and 4,056 ppm, and these levels were substantially higher than average and median concentrations of Na^{+} found on wild natural lick sites in North America. The simple low-cost techniques we used to test baited soils, NaCl Insta-TEST strips and acetic acid test, proved effective in detecting the higher concentrations of Na^{+} and Cl^{-}. These inexpensive field tests may provide wildlife conservation officers with a simple tool to verify the use of commercial wildlife baits in areas under investigation for illegal baiting. We recommend that future evaluations of commercial wildlife baits in soils include data on heavy rainfall events, soil type, bait placement, and duration.

Key words: baiting, commercial wildlife baits, conservation officers, evidence, game animals, Pennsylvania, sodium, soil chemical analysis, wildlife

Unlike piles of organic foods such as apples or corn, commercially available wildlife game baits (i.e., baits) contain minerals such as calcium (Ca^{+2}), chloride (Cl^{-}), and sodium (Na^{+}), that attract wildlife to a specific location. Additional ingredients of baits may include natural and artificial sweeteners, vitamins, and proteins (Shaw et al. 2007). These baits were developed to supplement natural sources of minerals for animal development and have been found to attract wildlife game animals during periods of physiological need (e.g., fawning of young, lactation, new antler development; Peterson et al. 2015).

Hunters may use baits to legally attract game animals to specific sites (Brown and Cooper 2006, Inslerman et al. 2006, Rudolph et al. 2006). However, poachers have also used the same baits (Martin 1992, Eliason 2003, MaMing et al. 2012). In the United States, baiting has resulted in widespread illegal harvest activities (Whitcomb 1999, Virginia Department of Game and Inland Fisheries 2014). In Minnesota alone, citations for illegal baiting reached record levels in 2012 (Col. K. Soring, Director, Minnesota Department of Natural Resources Law Enforcement, personal communication; Associated Press 2013).

Wildlife baiting may contribute to the spread of disease, including chronic wasting disease, bovine brucellosis, and ovine tuberculosis, and non-infectious diseases such as aflatoxin poisoning, rumenal acidosis, and enterotoxaemia (Brown and Cooper 2006, Inslerman et al. 2006, Ramsey et al. 2014, zu Dohna et al. 2014). These diseases may also impact other wildlife species

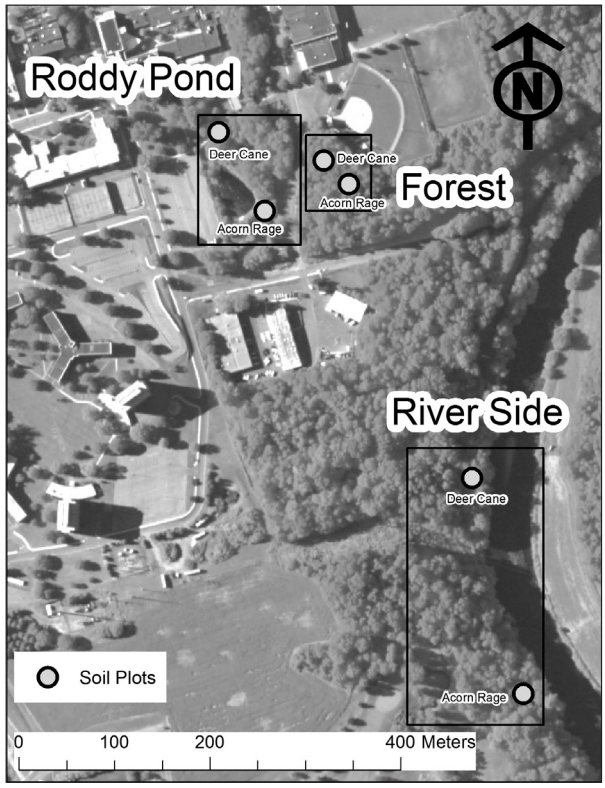

Figure 1. Locations of 6 baited soil plots baited with Deer Cane or Acorn Range commercial wildlife bait, found within 3 study sites on the Millersville University campus, Millersville, Pennsylvania, USA in 2013, including the Forest, River, and Roddy sites in the Millersville University Biological Preserve. Each soil plot was paired with a control (i.e., not baited) soil plot spaced 2 m apart.

(i.e., non-target species) and livestock (Campbell et al. 2013, Sorensen et al. 2014, zu Dohna et al. 2014, Milner et al. 2014), adversely impacting local economies (Patrek 2009). Baiting also causes an increase in negative human–wildlife interactions (Brown and Cooper 2006, Inslerman et al. 2006). In most of the United States and countries such as Sweden, Pakistan, and China, harvesting wildlife game species at baited sites is illegal or heavily restricted (Brown and Cooper 2006, Nawaz 2007, Bischof et al. 2008, MaMing et al. 2012, Selva et al. 2014). In Michigan, South Carolina, and Texas, the use of baits for harvesting wildlife game species is not restricted (Inslerman et al. 2006).

Some baits are mineral blocks and are easy to detect in the field. Many baits are liquids or powders mixed with water, which make them difficult to detect in the field, but they may still persist for many months in the soil (Peterson et al. 2015). In areas where baiting is illegal or heavily restricted, wildlife conservation officers seek to identify potential bait sites by looking for worn trails, heavy tracks, heavy fecal material, and urine (Inslerman et al. 2006).

These patterns are occasionally coupled with other techniques such as maps of poaching activity (Haines et al. 2012). Soil testing may confirm increased levels of chemical ions in areas exposed to commercial wildlife baits (Peterson et al. 2015).

We compared Atomic Absorption Spectroscopy (AAS) and Chloride Ion Selective Electrochemical (ISE) analysis to more user-friendly, less expensive LaMotte brand Sodium Chloride (NaCl) Insta-TEST strips (LaMotte Company, Chestertown, Maryland, USA) and an acetic acid test to determine if the less expensive tests could detect Cl^- and Na^+ signatures in the soils treated with commercial baits. In addition, we wanted to determine if chemical signatures in the soil changed in response to precipitation. Our goal was to evaluate if the less-expensive field tests could provide wildlife conservation officers with a quick and viable option to identify baited sites in areas where bait harvesting is illegal or heavily restricted. We hypothesized that soil testing techniques would indicate baiting activity when comparing baited soils to non-baited soils and that rainfall would not impact chemical ion levels in the soil.

Study area

We tested 2 commercial wildlife game baits: Acorn Rage (Wildgame Innovations, Grand Prairie, Texas, USA) and Deer Cane (Evolved Habitats Wildlife Nutritional Products®, New Roads, Louisiana, USA) on soil plots within 3 different sites (i.e., Forest, River, Roddy) in the Millersville University Biological Preserve in Millersville, Pennsylvania, USA. This preserve is an 8-ha strip of woodland located between the Millersville University Campus and the Conestoga River (Figure 1). All 3 sites were located ≥50 m from each other and along deciduous forest edges on silt loam alfisol soils (Custer 1985, USDA 2016). The dominant

tree species on the Roddy Pond site mainly consisted of silver (*Acer saccharinum*) and box elder maples (*Acer negundo*) with poison ivy (*Toxicodendron radicans*) and wildflowers on the undergrowth. The Forest site contained mainly tulip poplars (*Liriodendron tulipifera*) and sugar maples (*Acer* spp.) with patches of round leaf greenbriar (*Smilax rotundifolia*) along the forest floor. The River Side site had prominent American sycamores (*Platanus occidentalis*) and box elder maple trees with Paw Paw trees (*Asimina triloba*) at mid-canopy and limestone outcroppings dotting the surrounding area.

Based on the Köppen-Geiger climate classification system, Millersville, Pennsylvania, USA resides in a cold region with no dry season but a hot summer (Peel et al. 2007). The mean high and low temperatures for the month of October (our main study period) were reported as 18.89 °C and 5.55 °C, while average monthly rainfall was reported as 7.62 cm (Millersville University Weather Information Center 2017).

Methods

We administered both baits to soil in liquid form according to manufacturer directions. We cleared vegetation within a 1-m circle at 2 randomly selected plots within each site and applied bait over the cleared area (Figure 1). We defined these areas as soil plots. Each baited soil plot was paired with a non-baited or control soil plot. All soil plots were placed 2 m apart on areas with no slope to prevent run-off of bait chemicals. At each soil plot, we collected a soil sample and placed a 40-cm orange flag within the plot to denote sample removal. As sampling continued, the flag was moved accordingly to prevent repeat sampling of the same location; this ensured that all soil samples were independent of each other.

We collected 174 soil samples with 45 Acorn Rage and 45 Deer Cane bait samples and 84 control samples from all soil plots from September 20 to October 28, 2013. The first samples were taken on September 20, 2013 on baited plots before bait application (Day 0). After Day 0, we added the 2 commercial baits to the baited soil plots; we then collected soil samples from all soil plots (Day 1). We collected an additional 13 samples from each soil plot over 35 days. We collected soil samples at a depth of 6 cm to mimic a plausible sample collection method for a wildlife conservation officer. We stored soil samples at 40°F until all samples were collected. To prepare soil samples for analysis, we air-dried and then sieved samples into a fine powder. We placed 2 mL of each soil sample in a 14-mL test tube with 10 mL of deionized water and inverted it until the entire soil sample was suspended in the heterogeneous solution. We centrifuged the 14-mL test tubes at 905 rcf (or g-force) for 10 min. We removed 5 mL of the supernatant from the test tube and pipetted it into 6-mL glass vials for analysis. We then analyzed the supernatant's Na^{+} and Ca^{+2} concentrations in parts per million (ppm) using atomic absorption spectroscopy (AAS; PerkinElmer AAnalyst 800, Waltham, Massachusetts, USA). We used a Chloride Ion Selective Electrode (ISE) and the Logger Pro 3 software (Vernier Software and Technology Pamphlet 2014) to record Cl^{-} ion concentrations from the supernatant in ppm.

We used the AAS and ISE to measure ion concentrations for all soil samples and analyzed the samples using a General Linear Model (GLM) with a repeated measures design run in Minitab®17.2. A GLM is an ANOVA procedure to determine whether the means of ≥2 different predictor groups differ, and GLMs use a least squares regression approach to describe relationships between predictor and response variables. We defined mean chemical ion concentrations as our response variable and our predictor variables included Site (i.e., Forest, River, and Roddy), Treatment (i.e., Baited and Control) and Days (i.e., 0, 1, 7, 14, 21, 28, and 35). We used Days as our repeated measure. We also ran an interaction between Treatment and Days to compare changes in ion concentrations over time between baited and control soil. We conducted these analyses separately for chemical ion and bait type. A number of these measurements contained extreme outliers. Since we had no zero observations, we $\log_{10}$ transformed our data (O'Hara and Kotze 2010).

To determine the impact of rainfall on the amount of chemical ions that could be detected in our baited soil, we used data from the Millersville University Weather Information Center (2015) to obtain records of daily rainfall amounts during our baiting period. We used linear regression to evaluate if total rainfall amounts were related to greater declines in

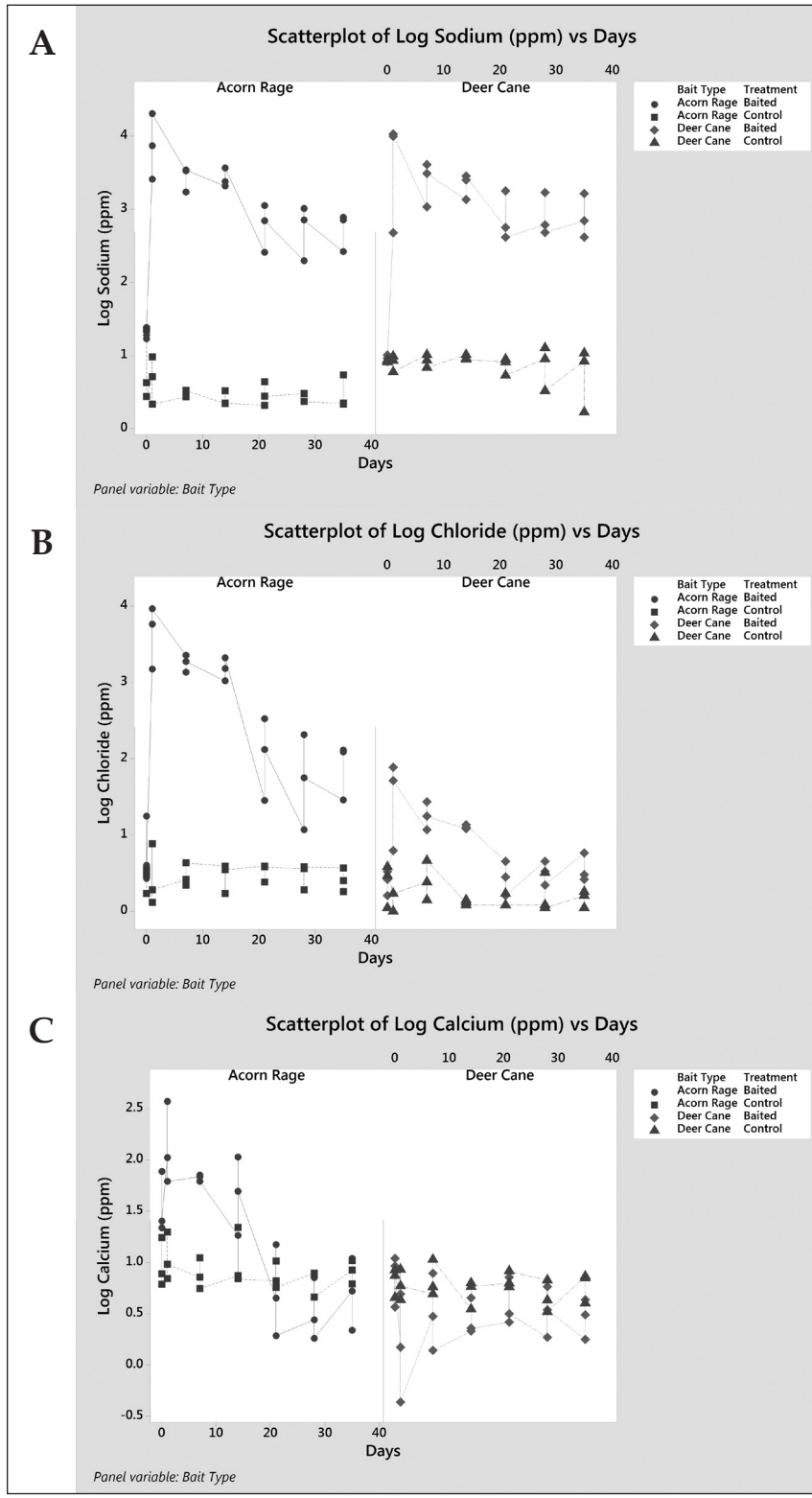

Figure 2. Mean $\log_{10}$-transformed ion levels of Na^{+} (A), Cl^{-} (B), and Ca^{+2} (C) in ppm in response to the number of days Acorn Rage and Deer Cane commercial wildlife bait had been placed on soil plots in comparison to control soil plots in Millersville, Pennsylvania, USA in 2013.

Table 1. General Linear Model (GLM) analysis of chemical ion concentrations obtained from soils baited with commercial wildlife baits (i.e., Acorn Rage and Deer Cane) and control soils in Millersville, Pennsylvania, USA, 2013.

Chemical ion	Bait	Comparisons of means	*F*-value	*P*-value	R^2
Na^+	Acorn Rage	Treatment: Control vs. Baited	46.64	<0.001*	0.96
		Site: Roddy vs. Forest vs. River	1.68	0.206	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	0.71	0.654	
		Interaction: Treatment vs. Day	18.20	<0.001*	
	Deer Cane	Treatment: Control vs. Baited	34.69	0.001*	0.94
		Site: Roddy vs. Forest vs. River	3.93	0.032	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	0.99	0.506	
		Interaction: Treatment vs. Day	14.87	<0.001*	
Cl^-	Acorn Rage	Treatment: Control vs. Baited	23.79	0.003*	0.93
		Site: Roddy vs. Forest vs. River	3.34	0.051	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	1.01	0.494	
		Interaction: Treatment vs. Day	17.01	<0.001*	
	Deer Cane	Treatment: Control vs. Baited	11.55	0.010*	0.74
		Site: Roddy vs. Forest vs. River	0.22	0.806	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	0.98	0.510	
		Interaction: Treatment vs. Day	5.53	0.001*	
Ca^{+2}	Acorn Rage	Treatment: Control vs. Baited	2.80	0.145	0.91
		Site: Roddy vs. Forest vs. River	28.69	<0.001*	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	1.49	0.320	
		Interaction: Treatment vs. Day	19.64	<0.001*	
	Deer Cane	Treatment: Control vs. Baited	12.29	0.013	0.46
		Site: Roddy vs. Forest vs. River	6.40	0.005*	
		Day: 0 vs. 1 vs. 7 vs. 14 vs. 21 vs. 28 vs. 35	1.34	0.367	
		Interaction: Treatment vs. Day	1.41	0.250	

* *P*-value ≤ 0.01 indicates a significant difference in the means of predictor variables.

chemical ions detected in baited soil samples between sampling periods.

We tested the effectiveness of inexpensive NaCl Insta-TEST strips in comparison to the AAS and ISE. We used the LaMotte Insta-TEST strips on the same prepared supernatant samples as were used for the AAS and ISE. We used Spearman rank correlations to compare the results of the NaCl Insta-TEST strips to values obtained using the AAS for Na^+ and the ISE for Cl^-. We only used the Acorn Rage wildlife bait samples because Acorn Rage contained high enough levels of both Na^+ and Cl^- to be detected by the NaCl Insta-TEST strips. We determined the significance for all statistical tests based on a conservative $P \leq 0.01$ to avoid a type 1 error. To determine the effectiveness of another low-cost test, we used a qualitative analysis to detect the presence of Deer Cane in soil by pouring acetic acid onto sites baited with Deer Cane to determine if there was a bubbling reaction when acetic acid reacts with bicarbonate salts (found in the Deer Cane) to produce carbon dioxide gas and water. This acetic acid test is similar to that used by soil scientists to search for carbonates in soils that have little to no weathering by water (Howland and Becker 2002). The NaCl Insta-TEST strips

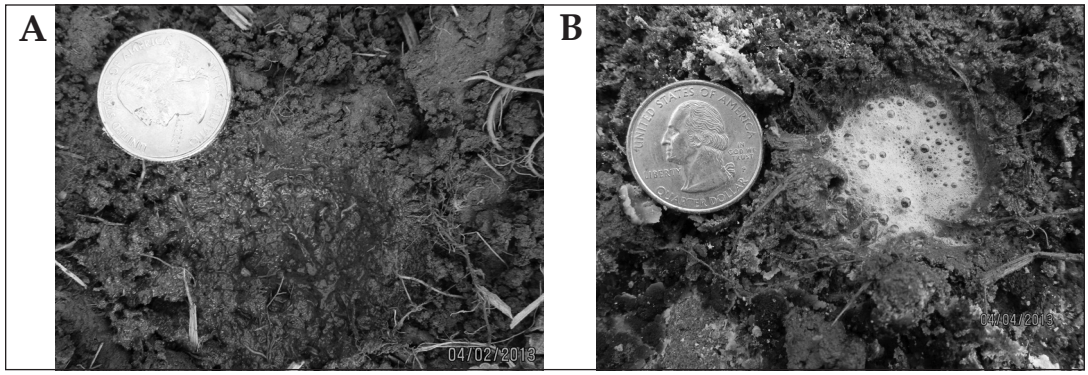

Figure 3. Comparison of a control soil plot (A; no reaction) to a soil plot baited with Deer Cane commercial wildlife bait (B; carbonation reaction) after adding 10 mL of 5% acetic acid in Millersville, Pennsylvania, USA in 2013. Pictures were taken after each plot was exposed to acetic acid for 5–20 seconds.

were used concurrently with the AAS and ISE analysis and the acetic acid test was conducted while soil samples were being collected.

Results

The GLM analysis for Na^+ and Cl^- differed between the baited and control soil plots for both baits ($F > 11.55$, $P \leq 0.01$), with the mean log concentrations on baited soil significantly higher than control soil (Table 1). This confirmed presence of a Na^+ and Cl^- chemical signatures in baited soil compared to control soil. For both bait types, we found no difference in the concentration of Na^+ and Cl^- ions between Sites ($F \leq 3.93$, $P > 0.03$) and Days ($F \leq 1.01$, $P > 0.49$; Table 1). The Na^+ and Cl^- concentrations for baited soil were higher compared to control soil for all days except for Day 0 (i.e., Pre-baiting) when concentrations between baited and control plots were similar (Figure 2).

Our study showed that there was a significant interaction between Treatment and Day for all chemicals and both bait types ($F > 5.53$, $P < 0.01$; Table 1) except for Ca^{+2} and Deer Cane ($F = 1.14$, $P = 0.25$; Table 1). The GLM analysis for Ca^{+2} did not differ between baited and control soil for both bait types ($F \leq 12.29$, $P > 0.01$; Table 1) and there was no difference in the concentration of Ca^{+2} ions between Days ($F \leq 1.49$, $P > 0.32$). There was a difference in Ca^{+2} concentration between Sites ($F \geq 6.40$, $P < 0.01$), suggesting that Ca^{+2} ion concentrations were impacted by where soil samples were collected rather than if soils were baited (Table 1).

Our results indicated that baited soil showed an immediate spike in the amount of Na^+ and Cl^- (Figure 2); soils that exhibited this spike showed gradual declines in ion concentration over time (Figure 2). We found no relationship between sum of rainfall events between sampling periods and the difference in ppm of Na^+, Cl^-, and Ca^{+2} detected in the soil for either bait ($F \leq 0.25$, $P > 0.63$; R^2 values ranged from 0.021 to 0.028).

For our more user friendly, less expensive tests, we found a correlation for both Na^+ ($Rho = 0.49$, $P < 0.01$) and Cl^- ($Rho = 0.92$, $P < 0.01$) when comparing the results of our AAS and ISE analyses to the NaCl Insta-TEST strips, with a stronger correlation to the Cl^- ion concentrations. When we applied acetic acid onto an area baited with Deer Cane, a bubbling reaction occurred, compared to the control (Figure 3), and this reaction still occurred after Deer Cane was in the soil >30 days, while the control continued to show no reaction.

Discussion

Our results supported our hypothesis and showed that Na^+ and Cl^- ion concentrations were higher in soil samples where baits were applied. Low Ca^{+2} ion concentrations indicated that Ca^{+2} is a weak indicator of baiting activity. Due to the low levels of Cl^- found on soil baited with Deer Cane, we recommend using Na^+ as an effective indicator to identify soils baited with commercial wildlife baits. Peterson et al. (2015) reported elevated mineral levels for both Na^+ and phosphorus (P) and low levels of Ca^{+2} in soils exposed to Deer Cane Black Magic bait mixed by Evolved Habitats Wildlife Nutritional Products®. Peterson et al. (2015) recorded Na^+

levels similar to our study and suggested that Na^+ cations replaced Ca^{+2} cations in the soil. Levels of Na^+ on baited soils for the first 20 days averaged 3,209 ppm for Deer Cane and 4,056 ppm for Acorn Rage. These levels were higher than average and median concentrations of Na^+ found on wild natural lick sites in North America: 285 ppm in South Dakota (Kennedy et al. 1995), 382 ppm in Indiana (Weeks and Kirkpatrick 1976) and 706 ppm in Yellowstone National Park (Tracy and McNaughton 1995). Because individuals using baits in the field may reapply bait to the same site to maintain wildlife activity in the area, the actual concentration of Na^+ to levels at active baited sites may be greater than reported in this study.

Besides the baits tested in this study and Peterson et al. (2015), many other baits have high levels of Na^+ listed in their ingredients (Shaw et al. 2007) because Na^+ is highly sought after by many wildlife game species, especially ungulates such as white-tailed deer, sika deer (*Cervus nippon*), moose (*Alces alces*), and mountain goats (*Oreamnos americanus*; Kennedy et al. 1995, Rice 2010, Ping et al. 2011, Rea et al. 2013). However, for baits that do not contain Na^+, other tests to determine their presence in soil would need to be identified.

For baited soils that had higher levels of chemical ions compared to control soils, these high ion levels occurred for ≈ 20 days from Day 0 and then a decrease in chemical ion levels were recorded. In support of our hypothesis, we did not identify a direct relationship between daily rainfall and the amount of chemical ions detected in our baited soil samples. However, during periods of heavy daily rainfall of 3.0–3.5cm, which occurred at Day 20 and 21, respectively, chemical ion levels recorded in baited soil samples declined (Figure 2). Peterson et al. (2015) found elevated mineral levels in the soil for 230 days after exposure to commercial wildlife baits, but they did not record rainfall amounts in their study areas. We recommend that future research quantify the amount of chemical ions left in the soil by wildlife baits after periods of heavy rain and determine if the concentration of chemical ions over time may vary based on soil type and duration.

Management implications

We found that our simple, low-cost techniques proved effective in testing soils for chemical ions. The NaCl Insta-TEST strips by La Motte fit well with the Acorn Rage results we found using the AAS and ISE, and we found that acetic acid readily reacted with the bicarbonate salts found in soil baited with Deer Cane. Bicarbonate salts are common chemicals found in other powdered commercial wildlife baits (Shaw et al. 2007). Our results suggest that soil testing for chemical ions could verify if a suspected area had been illegally baited. Wildlife conservation officers could use low-cost NaCl Insta-TEST strips by La Motte, or an acetic acid test, to verify high levels of chemical ions in the soil left by commercial wildlife baits in comparison to lower levels obtained from soil samples taken outside of a suspected bait area.

We recommend that future research explore other commercial wildlife baits tested on different soil types using blind examinations of baited vs. control sites to validate the findings of this study, an approach that is recommended in the forensics community (Saks and Koehler 2005). These tests should consider heavy rainfall events, soil types, bait placement, and duration. Additional work is needed to develop a consistent low-cost test for just Na^+ that produces similar results using an AAS. We found that commercial wildlife baits leave chemical signatures in the soil, mainly Na^+ ions. In addition, we identified inexpensive tests that could be used in the field to verify sites suspected of being baited. These tests would allow law enforcement officers to identify illegally baited sites that could be regularly patrolled to apprehend individuals suspected of poaching.

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