



## Review Article

# Advanced Strategies for Detecting Chemicals and Pollutants Using Sentinel Systems in Nature

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## Abstract

A review of advanced strategies used to detect chemicals and pollutants in sentinel species in nature is presented along with recent research to discover chemicals and pollutants in new sentinel species. The health, nutrition, and environment of several sentinel species were monitored by ICP - OES to reflect normal and abnormal levels of trace elements. Levels of trace elements in these species were correlated with trace element concentrations in the surrounding environment (air, soil, and water). We present extensive studies of the metal content in: (a) deer hooves from various counties in Alabama, (b) exotic moth orchid petals, (c) Asian lady beetles, and (d) Monarch butterflies obtained during 2014-2016 from overwintering locations adjacent to the Monarch Butterfly Biosphere Reserve in Mexico. Our results demonstrate that metal content in these selected species can be used as sentinels of health, nutrition, and the environment. The usefulness of advanced techniques and strategies for biomonitoring programs is demonstrated. The challenges, limitations, and future directions in the detection of chemicals and pollutants in sentinel species found in nature are presented.

## Introduction and Background

Interest in the chemistry and biochemistry of sentinel species that can serve as biosensors of the environment as well as report on the species' health, environment, and nutrition received heightened awareness when Chemical & Engineering News published a cover story on the topic [1]. For a species to serve as a sentinel, the species should be readily available, easily handled, and have consistent and regularly measurable responses to health, nutrition, and changes in the air, water, and soil in the surrounding environment. Various sentinel species have been used to monitor the environment as well as to gauge the health and viability of a species. These include representative sentinel species such as salmon, honeybees, liches, seagulls, mussels, and caribou [1]. Lead levels in salmon were monitored concomitant with deteriorating water quality and high lead levels in the waters where the salmon were collected [2]. Honeybees have been analyzed for neonicotinoids via GC-MS to determine the source of these compounds, which have been implicated in the decline in the U.S. honeybee population by 30-40% in the last decade [3]. Table 1 summarizes the results from the determination of organic and inorganic compounds and elements in current research involving some representative sentinel species. One must take caution in relating concentration of a chemical or

element to cause of loss of species. A chemical or pollutant or its metabolite may cause a more specific impairment in a species that requires further investigation. A case in point is the topic of DDT (dichloro-diphenyl-trichloroethane) and the decline in the bald eagle population [4]. In the early 1970s, DDT was suspected to play a role in the decline of the bald eagle and other bird-of-prey populations (e.g., ospreys, brown pelicans). There was co-occurrence of the declining bird populations and the chemical, DDT. There was also evidence of a complete exposure pathway to birds based on body burden of DDT. Extensive toxicity testing of DDT on adult bird mortality revealed no relationship. Field observations noted eggshell thinning in nests of bald eagles and eventually revealed a potential mechanism of reproductive failure due to eggshell thinning among bald eagles and other birds of prey. Laboratory experiments showed that DDE (dichloro-diphenyl-ethane) could cause eggshell thinning. Field studies showed that field exposures to DDE, a metabolite of DDT, were sufficient to cause effects in many species of birds based on the stressor-response relationship. Together these findings provided lines of evidence by which DDT might cause eggshell thinning and reduce reproductive success, a more specific impairment than declines in bird population.

Sentinel	Part	Analytes	Method	Reference
Arctic caribou Rangifer tarandus	Blood Antlers	As, Cu, Pb 137Cs and other radioisotopes	ICP-MS Radioisotope methods	Gamberg et al.-2005 [5]
Salmon	Fatty tissue	Pb, Cd	AAS, ICP-MS	Jamil et al. (2023) [2]
Deer Odocoileus virginianus	Blood Antlers Hooves	Hg and Pb Pb, Hg, Mg, Ca Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	ICP/AAS ICP CP-OES	Miller et al. (1989) [6] Miller et al. (1985) [7] Archie et al. (2017) [8] Reynolds et al. (2018) [9]
European bison Bison bonasus	Hooves	Cd, Pb, Zn	AAS	Skibniewska et al. (2015) [10]
Honeybee Apis mellifera	Honey	neonicotinoids	GC-MS	Mitchell et al. (2017) [3]
Monarch butterfly Danaus Plexippus	Wings	Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn	ICP-OES	Reynolds et al. (2018) [9]
Asian lady beetle Harmonia axyridis	Whole body	Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn	ICP-OES	Reynolds et al. (2018) [9]
Marmorated stink bug Halyomorpha halys	Whole body	K, Al, Fe, Mn, V, Cr, Cu, F, Li,	ICP-OES	Cason, et al. 2020 [11b]
Mussel Mytilus eduli	Mussel body only	dioxin, organic compounds	GC-MS	NOAA Mussel Watch Program Mar. Environ.Res., 65 (2008), pp. 101-127
Lichen Parnaria sphinctrina	Whole organism	organic compounds, DDT	GC-MS ICP-MS	Glavich & Gleiser -2008 [12]
Exotic moth orchid Phalaenopsis amabilis	Petals	Al, Cr, Fe, Mn, Cu, K, Au, Ag, Pb, Sn, Ni	ICP-OES	Cason and Murphy -2021 [11a]

Technique abbreviations for methods: AAS (Atomic Absorption Spectroscopy), AES (Atomic Emission Spectroscopy), GC-MS (Gas Chromatography/Mass Spectrometry), ICP-MS (Inductively Coupled Plasma/Mass Spectrometry), ICP- OES (Inductively Coupled Plasma Optical Emission Spectroscopy), MS-mass spectrometry. DDT is dichloro-diphenyl-trichloroethane. Applications used in sentinel studies include environmental monitoring (Mitchell et al., 2017) [3], public health assessment, risk assessment, ecosystem health monitoring, military detection of gases and nerve agents (Brankowitz, 1987) [13], and historical use (Burrell and Seibert, 1914) [14].

**Table 1:** Representative sentinel species analyzed (1985-2023), adapted from Ritter (2017) [1].

## Environmental Monitoring

Abdullahi et al. demonstrated that sentinel species such as *Daphnia* can act in dual roles as diagnostic tools for chemical pollution and remediation agents.

## Public Health Assessment

Reif in 2011 discovered that animals may be sensitive indicators of environmental hazards and provide an early warning system for public health intervention. A classic example of an animal sentinel system is the well-known canary in the coal mine [14]. Canaries are sensitive to the effects of poisonous gases, particularly carbon monoxide, and were routinely taken into the mines to warn of dangers. Its inclination to sing much of the time, coupled with its brightly colored plumage offered both “audio and visual” cues to the miners. If the canary stopped singing and/or fell from its

perch, this was the signal for the miners to don their respirators or evacuate. Many miners owe their existence and livelihood to this historic animal sentinel.

## Risk Assessment

In 2017, Neo et al. demonstrated the use of animals as a surveillance tool for monitoring environmental health hazards, human health hazards and bioterrorism. Sentinel species data can be utilized in human health hazard and risk assessments, evaluating the mechanisms of chemical effects. Though it is unlikely that sentinel species data will be used as the sole determinative factor in evaluating human health concerns, such data can be useful as for additional weight of evidence in a risk assessment, for providing early warning of situations requiring further study, or for monitoring the course of remedial activities.

## Ecosystem Health Monitoring

Because sentinel species can be studied in their natural environments, the biologic effects of suspected toxic substances in nature can be evaluated while the animals, insects, fish, or plants remain in their natural habitat, such as a field, farm, body of water, or human home. Certain species and substances, such as beeswax, birds, and insects can indicate long-term health impacts from chemical exposure or the presence of pollutants in ecosystems.

## Military Detection of Nerve Gases and Agents

Birds, horses, cats, guinea pigs, rats, mice, and rabbits were employed as sentinels for chemical agent exposure during World War I (WWI) and WWII. Until 1969, rabbits were placed in small cages in railcars during the transportation of nerve gases. Sudden animal mortality would warn of gas release [13].

## Historical Use

In addition to the canary in the coal mine historically used to detect the presence of deadly carbon monoxide in coal mines, chickens have been employed to complement the M22 ion-mobility spectrometer, which was used to tag nerve and blister agents [15]. They were meant to act as a backup to false alarms the automated detectors were notorious for.

## Biological Sensors

Biological sensors that serve as sentinels can be animals, insects, flora, or microscopic systems.

## Technological Sensors

Technological sensors are devices capable of detecting, measuring, and transmitting data about physical, chemical, or biological parameters. These types of sensors play a fundamental role in automation, data collection, environmental monitoring, and industrial control systems. Technological sensors may play an important role in the future for remote monitoring of sentinel species in ecosystems. Key milestones in sensor technology include the development of infrared sensors for remote temperature measurement, capacitive touch sensors, and biosensors for health monitoring and medical diagnostics. Today, sensors are not only limited to industrial and scientific applications but have become integral to daily life, found in everything from household appliances, pet and baby monitors, to advanced space exploration rovers. IoT sensor technology allows sensors to communicate with other devices and systems, creating a fully integrated ecosystem that can respond to changing conditions and automate processes. Artificial Intelligence (AI) is another emerging trend that is changing the sensor industry. Each of these trends is currently under active research and implementation, contributing to a future where sensors are more intelligent, sustainable, and integrated into our daily lives and are all amendable to apply to the study of

sentinel species to discern the effects of chemicals and pollutants on their environments.

- **AI Integration:** Sensors with AI capabilities will enhance real-time data processing and decision-making.
- **IoT Connectivity:** Real-time remote monitoring and control, applicable to ecosystems monitoring.
- **Nanotechnology:** Further miniaturization for advanced applications embedded in sentinel species.
- **Self-Powered Sensors:** Energy harvesting technologies will enable standalone sensor systems.

## Case Studies

Some examples of successful applications in chemical and environmental monitoring using sentinel species in our research program are detailed using four sentinel species in nature: deer, exotic moth orchids, Asian lady beetles, and Monarch butterflies.

## Materials and Methods

All chemicals were obtained from Sigma-Aldrich Chemical Co. and all ICP -OES standard samples were used as received from Inorganic Ventures, Inc. Mineral free nitric acid ( $\text{HNO}_3$ ) was purchased from Sigma-Aldrich Chemical Co. Ultra-high purity (UHP) argon gas used for plasma generation and auto-sampler was obtained from Air Gas, Inc. Deionized water was used to make samples.

## Elemental Analysis of Samples

Determination of Al, B, Cu, Zn, Co, Mg, Mn, Fe, Cr, Cd, Ni, Pb, and Zn content was made directly on each final solution using an inductively coupled plasma optical emission spectrometer (Varian VISTA ICP-OES) and associated auto-sampler. Standard solutions of each sample were prepared according to Varian manufacturer procedures for ICP-OES. Known standards (IV-26) containing metal ions were purchased from Inorganic Ventures, Inc. at various concentrations, including 1000 mg/L and 5000 mg/L. Standards were volumetrically diluted to 500 ppb, 1000 ppb, 2000 ppb, and 5000 ppb for use as calibration standards. All metals/elements standard solutions selected for analysis passed the calibration test of the ICP-OES software within  $\pm 5.0\%$ . A calibration blank was composed of the same solvent as was used in the analysis of samples and standards (matrix-matched). All samples and standards were analyzed in triplicate in the same run and under the exact same instrumental settings. Prior to loading each measured sample into the auto-sampler of the ICP-OES, each sample was filtered through a syringe filter into a polycarbonate sample tube. The analysis of each sample via ICP-OES was completed without error signals. All calibration curves exhibited  $r^2$  values greater than 0.9990. The sample preparation and analysis methods used in this research duplicate those of other researchers [16,17] in which

percent recovery tests were conducted to certify the ICP-OES protocol used in this paper.

### Deer Hoof Sample Preparation

The deer hooves were removed from each male deer just after killing and were immediately frozen at 4°C after assigning a sample number to each hoof. For analysis, each hoof was cut 1.0 cm above the keratin hoof to remove non-hoof material, dried, and weighed. The mean mass of each deer sample (2 hooves per sample) was 65.03 g, with samples ranging from 57.05-71.43 g. When ready for analysis, the trimmed hooves were allowed to defrost and were dissolved completely in concentrated nitric acid and kept covered in a hood overnight to prevent excessive foaming, while digesting. After filtering each sample solution through Whatman-Grade 4 qualitative filter paper, exactly 1.0 mL of each sample was removed from the hooves-nitric acid mixture and was diluted volumetrically to 100 mL with 7% nitric acid. A total of 34 deer hooves from ten counties in Alabama were analyzed.

### Moth Orchid, Asian Lady Beetle, and Monarch Butterfly Wing Sample Preparation

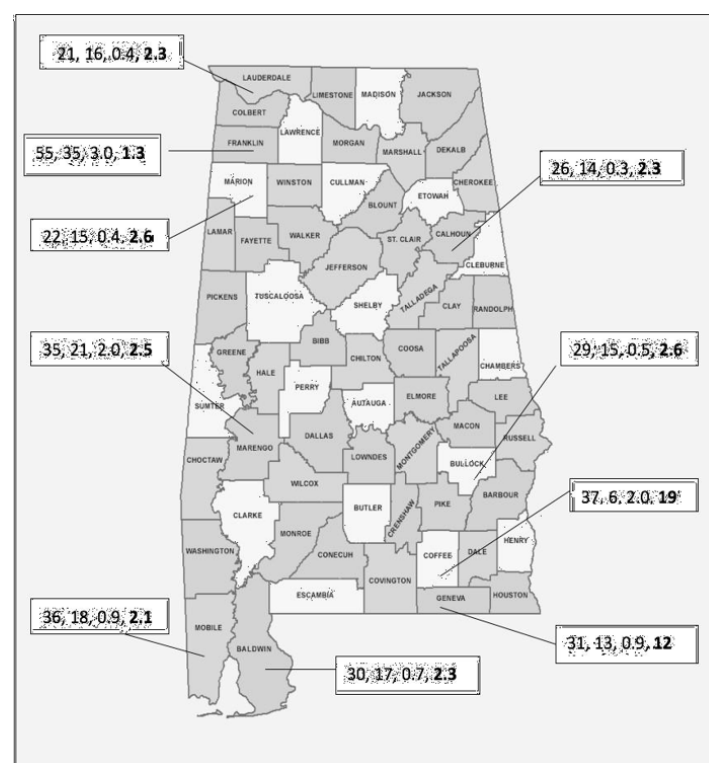
Orchid petal, Asian lady beetles, and Monarch butterfly wings samples were separately weighed and dissolved completely in measured amounts of concentrated nitric acid and digested. After filtering each sample solution through Whatman-Grade 4 qualitative filter paper, exactly 1.0 mL of each sample was diluted volumetrically to 100 mL with 7% nitric acid (aqueous) and analyzed via ICP-OES.

## Results and Discussion

### Analysis of Deer Hooves

Previous research demonstrated the feasibility of using ICP -OES to analyze metal content in deer hooves from selected counties in Alabama [18]. A more extended study and expanded number of samples provided results seen in Figure 1. Deer hooves from ten counties in Alabama (Baldwin, Bullock, Calhoun, Coffee, Franklin, Geneva, Lauderdale, Marengo, Marion, and Mobile) were analyzed for nine different metals. The overall metal content from 34 hooves (17 samples) killed in ten counties showed the following mean metal concentration ranking consistent with that of our previous studies [19].  $Zn > Fe > Cu > Ni > Mn > Co > Pb > Cr > Cd$ . Samples from locations where Zn deer mineral blocks were visibly in use (Deer #1, #2, #3) showed high Zn content, some six times higher than the lowest level from the other samples. Deer hooves obtained from an iron mining spoils lake (Number Six Lake) in

Franklin County, Alabama showed the highest concentration of Fe, Ni, and Pb of all samples from ten counties in this study, some 2-10 times higher than the levels on the same metals found in deer hooves from the other nine counties (See Figure 2). Deer killed in counties that were high in agricultural production of peanuts, potatoes, soybeans, cotton, and corn (Geneva, Marengo, Baldwin, Mobile, and Franklin) showed high concentration in Mn, prevalent in plants and peanuts. The health of deer was gauged by the normal range of mean concentration of all deer hoof samples: Cu (21 ppm), Fe (33 ppm), and Zn (48 ppm) seen in our study. A ratio of Zn:Cu concentration of approximately 2:1 ensures healthy keratin production needed for antler and hoof growth and health [20]. These ratios also are like Zn:Cu concentrations found in European bison hooves [10] and cattle hooves [16]. Figure 1 depicts the mean Zn:Cu ratios detected in deer hooves from ten Alabama counties.



**Figure 1:** Mean iron, nickel, and lead content (Fe, Ni, Pb in ppm) and mean **Zn:Cu** ratio (in boldface) of deer hooves from ten counties in Alabama. A ratio of Zn:Cu concentration of approximately 2:1 ensures healthy keratin production needed for antler and hoof growth and health [20].





**Figure 2:** Sixteen known variants of the multicolored Asian lady beetle.

Deer hooves from seven of the ten counties showed healthy Zn:Cu ratios in the range of 2.0-2.6:1, while three of the counties had deer hoof samples that exhibited unhealthy Zn:Cu ratios. The deer hooves analyzed from a mining spoils/lake region in Franklin County had comparatively higher Cu and Zn present (Zn:Cu= 1.3), reflective of the water quality in the associated mining spoils lake. The deer hooves isolated from deer killed in Coffee and Geneva counties indicated unhealthy Zn:Cu ratios (19:1 and 12:1, respectively), due to the high use of Zn mineral supplemental feeding stations located in Geneva and Coffee counties, where the deer were killed. Mean Lead (Pb) and Cadmium (Cd) levels detected in deer hooves were at low levels (less than 1.5 ppm), indicating no contamination via bioaccumulation of deer due to nutrition or environmental effects. Deer were identified by county according to the kill site location. We assumed deer kill site locations were associated with the respective county based on a comprehensive deer GPS study of 101 mature whitetail deer bucks and their travel behavior during the “rut.” [21]. The rut is the one- to-three-month breeding season for deer. Foley and coworkers spent five years tracking and tagging

(via global-positioning devices) whitetail mature bucks in south Texas. They found that 90% of the “collared” deer displayed two types of travel: 60% displayed “periodic search” travel behavior, moving from 0-2.75 km from their starting position during the rut, while 30% showed “resident search” pattern travel behavior, moving up to 5.50 km from their starting position during the rut. Only 10% of their sample displayed “nomadic behavior,” roaming erratically up to 11 km from their starting location. All of our deer hooves were obtained from male deer.

#### Analysis of Exotic Moth Orchids

The color of the orchid petals in the four species of *Phalaenopsis* orchids studied herein was related to the metal content detected in the orchid petals (See Table 2). Dissection and analysis of individual parts of the orchid flower revealed differentiation of elements based on the structures. Further research is in progress to study a wider variety of species of *Phalaenopsis* orchids using our established ICP-OES analytical techniques for orchid elemental analyses.

Orchid Color	Ag	Al	As	B	Cd	Co	Cr	Cu	Fe	K	Mn	Mo
Deep purple	10.58	10.19	nd	14.16	9.14	8.37	8.36	10.01	9.492	nd	nd	15.11
Lavender	7.658	8.134	nd	15.15	7.852	7.327	9.155	9.983	9.966	7.644	nd	nd
Yellow with deep purple	3.041	3.07	4.663	10.69	nd	nd	nd	nd	7.978	3.961	4.179	nd
White with purple center	nd	nd	0.288	9.373	nd	nd	nd	11.22	0.435	1.715	2.137	nd

**Table 2:** Elemental Analysis of Phalaenopsis orchid **petals** analyzed by ICP-OES in ppm (parts per million), corrected for dilution. Pb was detected only in deep purple sample at the level of 8.557 ppm. Not detected=nd.

### Analysis of Asian Lady Beetles

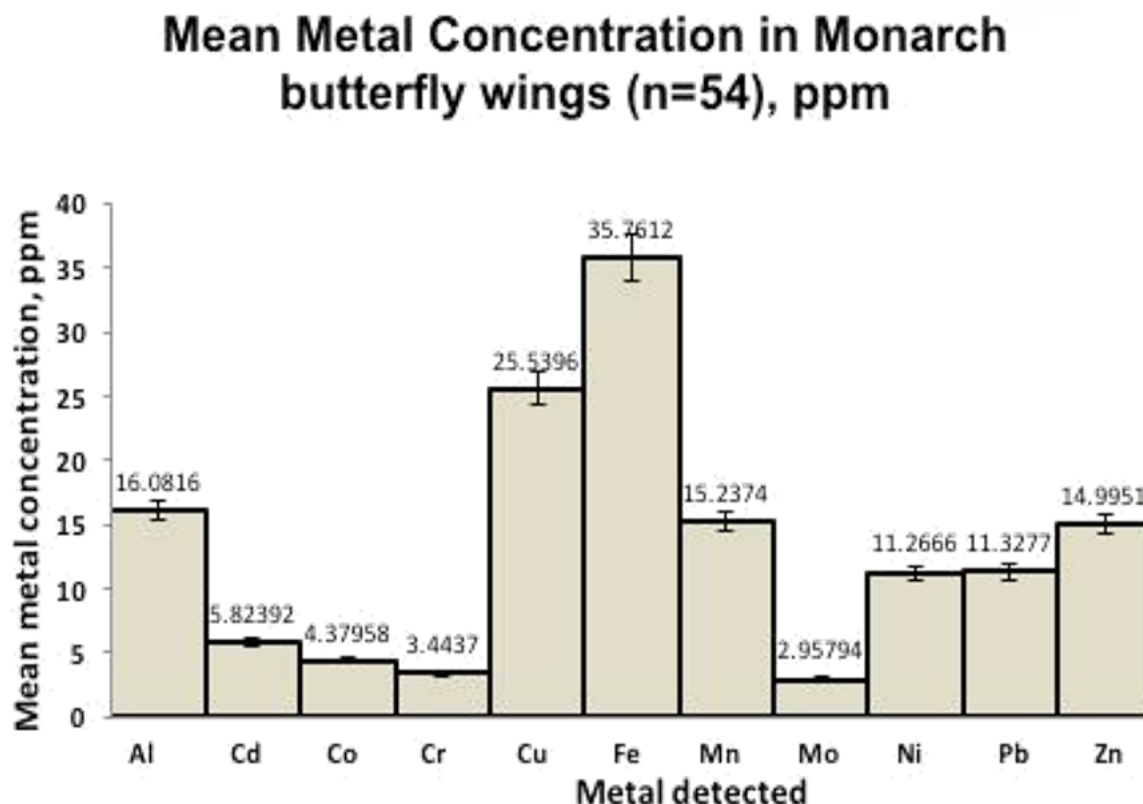
Results from the ICP-OES analysis of three variants of multicolored Asian lady beetles collected from an iron mining spoils lake in Franklin County, Alabama showed comparative mean metal concentrations of 398 beetle samples to be Ni>Fe>Cu>Zn>Al>Mo>Pb>Co>Cr>Cd. However, as seen in Table 3, these results are affected by the extremely high Ni content detected in one variant (Beetle #13, See Figure 2 below), the multicolored Asian lady beetle that was orange with no spots. In addition, the enzyme that catalyzes the reaction that places a methyl and/or a methoxy group on the pyrazine molecule is a nickel-containing enzyme [22]. Detection of comparatively high levels of Ni in this variant of multicolored Asian lady beetles may be an indicator of the different role of this variant in the multicolored Asian lady beetle population. This lady beetle with no spots was more difficult to find, as it was always present in lower numbers in groups of lady beetles discovered. For five separate samples of 23, 23, 24, 23 and 16 beetles of this kind (orange, no spots), the Ni concentration detected was over 100 ppm for each sample, with a mean of 150 ppm for all Beetle #13 samples (See Table 3).

Sample (n=)	Al, ppb	Cd, ppb	Co, ppb	Cr, ppb	Cu, ppb	Fe, ppb	Mo, ppb	Ni, ppb	Pb, ppb	Zn, ppb
Beetle #3 (n=141)	104.91	1.899	5.7414	4.3873	663.13	186.29	27.191	18.185	35.119	38.1217
Beetle #10 (n=138)	38.079	1.8211	8.4094	5.3756	20.125	56.116	67.879	6.3401	23.819	140.56
<b>Beetle #13 (n=109)</b>	<b>68.463</b>	<b>1.6427</b>	<b>6.9251</b>	<b>7.4107</b>	<b>23.749</b>	<b>188.87</b>	<b>53.983</b>	<b>1503.5</b>	<b>58.176</b>	<b>158.19</b>
Mean, ppb	70.484	1.7876	7.0253	5.7245	235.67	143.75	49.684	509.34	39.038	112.29
Mean, ppb dilution corrected	7.0484	0.1788	0.7025	0.5724	23.567	14.375	4.9684	50.934	3.9038	11.229

**Table 3:** Mean metal content of three multicolored Asian lady beetle variants: #3: red with black spots, #10 orange with black spots, and #13 orange (with no spots) determined by ICP-OES. See Figure 2 for detail of each variant.

### Analysis of Monarch Butterfly Wings

Results from metal content in 54 Monarch butterflies (27 sets of 2 wings per sample) showed high concentrations of Fe and Cu, some 12 times higher than other metal concentrations detected. Every sample analyzed showed elevated Fe and Cu concentrations compared to the other metals detected (See Figure 3). The order of concentration levels detected in our Monarch butterfly wings was found to be: Fe>Cu>Al>Mn>Zn>Pb>Ni>Cd>Co>Cr>Mo. Monarch butterflies can obtain these metals from drinking water which may be contaminated with mining spill-tainted water in Mexico. A toxic spill in August 2014 at the Grupo Mexico copper mine in Sonora, Mexico spewed 10 million gallons of mining waste, including copper sulfate, into the Sonora and Bacanuchi rivers. Hundreds of miles of waterways in Mexico were contaminated with metals from mining waste. The Monarch butterfly population has decreased by over 80 percent throughout the past few decades and estimates placed 145 million butterflies at the wintering grounds in 2017 compared to 1 billion in 1990 [23]. Our results show high levels of metals in Monarch butterflies collected in Mexico from 2014-2017, which may indicate risks to the health of the species.



**Figure 3:** Mean metal concentration (ppm) in Monarch butterfly wings (n=54 butterflies) from Mexico, collected during 2014-2017. Error bars reflect +/- 5% of measured value, based upon 5% error in each metal concentration calibration curve (ICP- OES).

### Conclusions from Case Studies

Our sentinel case studies research demonstrates that ICP-OES analyses of fresh deer hooves, exotic moth orchids, multicolored Asian lady beetles, and Monarch butterflies reflect comparative levels of metals and indicating similar nutrition and health of these sentinel species and in some cases, indicating elevated levels of elements produced by environmental effects (concentration of metals in water and the environment). These sentinel species in our study reflect comparatively high Fe levels in deer hooves from iron mining lake areas, healthy Zn:Cu ratios in deer hooves from 70% of counties studied, and high Fe and Cu content in Monarch butterflies due to mining spills in Mexico affecting wintering sites of the butterflies. In addition, Ni-containing enzymes that catalyze the O-methylation of pyrazine [22] may be responsible for increased Ni content in the orange, non-spotted multicolored Asian lady beetle that has been found to secrete the most methoxypyrazine as an odorant when compared to other variants of these Asian lady beetles [24]. This odorant has been found to spoil grapes in wineries where the beetle has not been eradicated. The results from this research can be used to gauge the health, nutrition, and environment of these sentinel species. Elemental

analysis of the petals of four different variants of Phalaenopsis orchids demonstrated higher metal concentration (Co,Cr, and Fe) for the more highly colored orchid petals. Finally, the use of ICP-OES coupled with standard analytical methods, provides an engaging method to involve undergraduate research teams in broad biomonitoring projects with a common goal of understanding the chemistry and biochemistry of sentinel species in nature.

### Challenges and Limitations in the Detection of Chemicals and Pollutants Using Sentinel Systems

Challenges and Limitations in the Detection of Chemicals and Pollutants Using Sentinel Systems in Nature Challenges for future researchers in the detection of chemicals and pollutants using sentinel systems in nature include sensitivity of instrumentation used for detection, low concentration of analytes in sentinels, improper use of standards and dilutions, data interpretation and analysis. In some cases, solubility of samples may be a limiting problem. In addition, regulatory considerations, state and federal laws for monitoring species must be followed. Ethical considerations should also be considered, so that no undue harm is done to the environment or species [25-35].



## Future Directions

The future directions in sentinel chemistry and biochemistry research include international collaboration on research projects, inclusion of the effects of increasing temperatures on sentinel species and species migration, study of loss of habitats of sentinel species due to increasing sea levels, and rapid growth in technology and the use of AI to augment and model and explore future research projects.

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