State of California The Resources Agency

Memorandum

To : Whom It May Concern Date : April 3, 1986

From : Department of Fish and Game

Subject : "Lead and Zinc Levels in the Eggshells of California Condors"

Here is a copy of subject final report of Rob Ramey's study, which was funded by our Department, WFVZ, and NWF.

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Attachment

LEAD AND ZINC LEVELS IN THE EGGSHELLS OF CALIFORNIA CONDORS

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Introduction

California condors (Gymnogyps californianus) are one of the most critically threatened species of birds in the world today. Only 5 individuals are known to exist in the wild and 21 in captivity. Identifying and eliminating sources of condor mortality in the wild are crucial to the long term survival of the species.

The first evidence that condors may suffer from the effects of lead contamination came from blood samples taken from a condor live trapped in 1982 and bone samples from a dead condor collected in 1976. Both birds were found to have lead concentrations considered to be above background levels (Wiemeyer et al. 1983). In 1984 a subadult condor was found dead of lead toxicosis, resulting from the ingestion of a lead bullet fragment (Anderson 1984). In 1986 a breeding adult female condor also died of acute lead contamination.

Acute lead contamination, through the ingestion of bullet fragments or shotgun pellets, was reported as the cause of death in a captive Andean condor (Vultur gryphus) (Locke et al. 1969) and two captive King vultures (Sarcorhampus papa) (Decker et al. 1979). Although acute lead contamination through the ingestion of lead bullet fragments has been implicated in the deaths of these birds and two California condors, chronic lead contamination of other California condors could not be ruled out. This investigation was designed to test whether chronic or acute lead contamination has been the source of condor morbidity and mortality.

Lead concentrates in bone of birds following contamination. This is a result of competition between lead and calcium during active bone metabolism, such as during eggshell formation (Finley and Dieter 1978). Female birds contaminated with lead prior to egg laying may be expected to have elevated levels of lead in their medullary bone and eggshells (Finley and Dieter 1978, Haegele et al. 1974, Grandjean 1980). Knowing the concentration in the eggshell of a female condor therefore may be useful in determining if it has been exposed to lead contamination.

Methods

Eggshell fragments from California condors were collected from wild nests or obtained from zoos following the hatching of captive incubated eggs. Eggshell fragments collected from wild nests since 1982 were obtained by sifting the nest substrate by hand or through a fine fiberglass screen. Fragments were then placed in acid washed vials provided by Patuxent Wildlife Research Center. Samples collected prior to 1982 were not stored in chemically clean containers. Following collection, all samples were curated and stored at the Western Foundation of Vertebrate Zoology in Los Angeles, California.

Throughout the laboratory phase of this investigation eggshell fragments were handled only with polyethylene gloves or acid washed Nalgene polypropelene forceps and stored in acid washed polyethylene containers.

Eggshell fragments with membranes still attached were soaked in deionized distilled water for 30 minutes and the membranes were removed with polypropylene forceps. Samples were then transfered to polyethylene vials, covered with deionized distilled water and placed in a sonicator for two minutes. The water from each container was then poured off, the container refilled, and the process repeated. Persistent dirt particles were gently removed with polypropylene forceps.

Followng cleaning, all samples were dried in a vacuum oven for 1 hour at 100°C. Immediately upon removal from the oven, large fragments were broken into pieces weighing <0.05 grams each and each sample was weighed to the nearest 0.0001 gram. Samples were then digested in 1 ml of concentrated Ultrex suprapure nitric acid in chemically clean, acid washed test tubes. Foaming was minimized by digesting one fragment at a time. The total weight of each sample was between 0.5 and 0.1 grams.

Following digestion, the test tubes were placed on a heat block at 80°C for 1 hour and vortexed at 15 minute intervals to remove all undigested material from the test tube walls. The samples were then dried at 105°C, cooled and reconstituted in 1-2 ml of 0.2% nitric acid (deionized distilled water and Ultrex nitric acid). Each test tube was vortexed to insure complete digestion and covered with parafilm until analyses were made.

Prior to injection in the spectrophotometer, a 50 µl aliquot of each sample was diluted in 2 parts of an ammonimn phosphate $(NH_4)_2HPO_4$ solution. Ammonium phosphate was used as a matrix modifier to stabilize the analyte during atomization (Slavin et al., 1983). Stabilizing the analyte delays atomization, allowing a closer approach to isothermal conditions in the graphite furnace. This procedure maximizes analyte recovery. Dilution of the sample also reduced background readings to between 0.4 and 0.7 absorbance-seconds.

All lead determinations were made by flameless atomic absorption spectrophotometry using a Perkin Elmer model 3030 atomic absorption spectrophotometer equiped with an HGA400 graphite furnace, deterium background corrector and graphics display. Integrated absorbance and baseline offset correction were used in signal processing.

Pyrolytic coated graphite tubes and L'vov platforms were used in the graphite furnace. The use of L'vov platforms along with a matrix modifier allows atomization of the analyte element to occur in a stabilized temperature environment, maximizing accuracy (Paschal et al. 1985).

The following furnace conditions were found to optimize lead recovery from samples and were used for all condor eggshell analyses:

110°C dry

600°C char (gas mini flow during char)

1800°C atomization

2500°C burnoff

20°C cooling

After calibration with 0, 5, 10 and 20 µg/dl standards, multiple lead determinations were made for each sample until repeatable results were obtained. This was a necessary quality control procedure in the absence of an auto sampler. The volume of each injection was 15 µl.

Due to the buildup of calcium oxides on the graphite tube walls and contact rings, the graphite furnace was disassembled and cleaned after 3-4 eggshell samples were analyzed. The spectrophotometer was then recalibrated before further analyses were made.

Reagent blanks were carried through the analytical procedure and the following values obtained: ammonium phosphate - none detected; 0.2% nitric acid - none detected; deionized distilled water <3ppb. Recovery of lead from spiked samples and by the method of addition averaged 84.7% for all analytical runs (range 79 - 95.4%). All lead values reported in this paper are uncorrected values.

All zinc determinations were made via flame atomic absorption spectrophotometry.

Results and Discussion

Lead concentrations in California condor eggshells (n=25) ranged from none detected (<0.05 μg/g) to 5.79 μg/g (Table 1). Except for two extreme values (4.93 and 5.79 μg/g) all lead values were less than 0.41 μg/g. Although the two elevated values could be due to lead contamination of the female prior to egg laying, they may also be explained by post laying contamination of the eggshells in the nest site. All samples, except the two in question were collected from nest sites within three years of laying (Table 2).

It is possible that post laying contamination of S-14 and S-156 may have resulted from lead being absorbed into the eggshell through soil moisture. Contamination by lead absorbed from soil will not occur as dirt on the surface or pores of the shell and cannot be removed by cleaning (Ericson et al. 1979). Determinations of soil lead concentrations at S-14 and S-156 could be used to test the soil moisture hypothesis. Due to the uncertain source of lead in the eggshells collected from these sites, these values have been excluded from the data analysis.

Based on comparisons with other species reported in the literature, the lead concentrations found in California condor eggshells appear to be at or below background levels (Table 3).

The lead concentrations in eggshell produced by 3 individual condors did not show any apparent trends within or between breeding seasons (Table 4). A significant difference however, was found between eggs laid in the 1960's and the late 1970's-1980's ($t = 2.82$, $p<0.02$, $df=20$) (Figure 1). Although lead concentrations in condor eggshells have increased in recent years, they are still below background levels reported for other species. These data tend to refute the hypothesis that condors buildup toxic levels of lead through chronic low level contamination. The data however, are consistent with the hypothesis that acute contamination, presumably through the ingestion of bullet fragments, is a source of condor morbidity and mortality. Female condors that die of acute lead contamination do not lay eggs and therefore are absent from the record.

Zinc concentrations in condor eggshells (n=23) ranged from 0.79μg/g to 11.84μg/g. No significant correlation was found between levels of lead and zinc in condor eggshells (Figure 2). A significant increase was found between eggs laid in the 1960's and late 1970's-1980's ($t = 3.29$, $p<0.01$, $df=20$) suggesting an increase in background contamination (Figure 3). Three of the eggshells sampled were elevated above the rest of the distribution and may represent episodes of acute contamination (Figure 5). Background levels, and the modes of uptake, transport and deposition of zinc in avian tissues is not well documented so these results should be interpreted with caution.

Acknowledgements

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Table 1 - Lead and zinc concentrations in condor eggshells.

Sample No.	Location	Name	Year	lead (µg/g)	zinc (µg/g)
S-15	High Mtn.	-	1968	0.06	1.93
S-24	Red Rock	Pismo	1984	0.29	7.72
S-34	Red Rock	-	Late 1970's	0.40	2.02
S-41a	April Cyn.		1966	0.09	1.56
S-41b	April Cyn.	-	1967	0.00	1.83
S-44	Pine Cyn.	-	1922	0.10	1.52
S-49	Trough Cyn.	Sespe	1983	0.08	3.59
S-111	Mono Narrows	Ojai	1984	0.16	3.89
S-112	Sulfur Creek	Squapuni	1984	0.27	11.84
S-135	West Big Pine	-	1985	0.13	2.98
S-145	Madulce	Yosemite	1984	0.39	3.15
S-146	Don Victor	Kaweah	1985	0.05	4.9
S-158	Starvation Gr.	Sequoia	1984	0.06	4.06
S-162	Dough Flat	-	1967	0.13	2.75
S-251	Sycamore Cyn.	-	1967	0.00	0.79
S-313	Indian	Almiyi	1983	0.33	1.47
S-342	Red Rock	Piru	1984	0.10	2.81
S-353	Red Rock	Sisquoc	1983	0.26	6.97
S-355	Red Rock	-	1982	0.27	2.55
S-356	April Cyn.	Tecuya	1983	0.22	3.84
S-357	Red Rock	Inaja	1984	0.07	3.50
S-363	Agua Blanca	Anapa	1984	0.16	2.30
S-364	Pine Mtn.	-	1984	0.28	1.97

Table 2 - Data for outliers deleted from analysis.

Sample No.	Location	Lead Concentration	Time in nest before collection
S-14	Eaton Canyon	5.79 µg/g	75 years
S-156	Piru Gorge	4.93 µg/g	16 years

Table 3 - Lead levels in eggshells of avian species.

Source	Species	Egg source	n	Mean (µg/g)	Median (µg/g)	Range (µg/g)
Grandjean (1980)	Kestrels	wild nests 1875-1953	12	-	1.73	1.27-3.38
		wild nests 1972-1974	7	-	1.52	1.02-2.19
Haegele (1974)	Mallards	captive	15	0.52	-	-
Sigurslid (1984)	Night herons	wild nests	135	4.78	-	1.0-40.1
Pattee (1984)	Kestrels	captive	-	1.2	-	0.0-2.9
this study	Condors	wild nests	23	0.17	-	<0.05-0.4

Table 4 - Lead and zinc concentrations in eggshells produced by individual female condors.

Location	Sample No.	Date Laid	Name	Lead (µg/g)	Zinc (µg/g)
Red Rock	S-34	late 1970's	-	0.40	2.02
	S-355	1982	-	0.27	2.55
	S-353	Feb. 1983	Sisquoc	0.26	6.97
	S-49	March 1983	Sespe	0.08	3.59
	S-342	Feb. 1984	Piru	0.10	2.81
	S-357	March 1984	Inaja	0.07	3.50
	S-24	April 1984	Pismo	0.29	7.72
Santa Barbara	S-313	March 1983	Almiyi	0.33	1.47
	S-145	March 1984	Yosemite	0.39	3.15
	S-111	April 1984	Ojai	0.16	3.89
	S-146	1985	Kaweah	0.05	4.90
Agua Blanca	S-356	April 1983	Tecuya	0.22	3.84
	S-363	March 1984	Anapa	0.16	2.30
	S-112	April 1984	Squapuni	0.27	11.84

Figure 1 - Plot of lead levels in condor eggshells vs year.

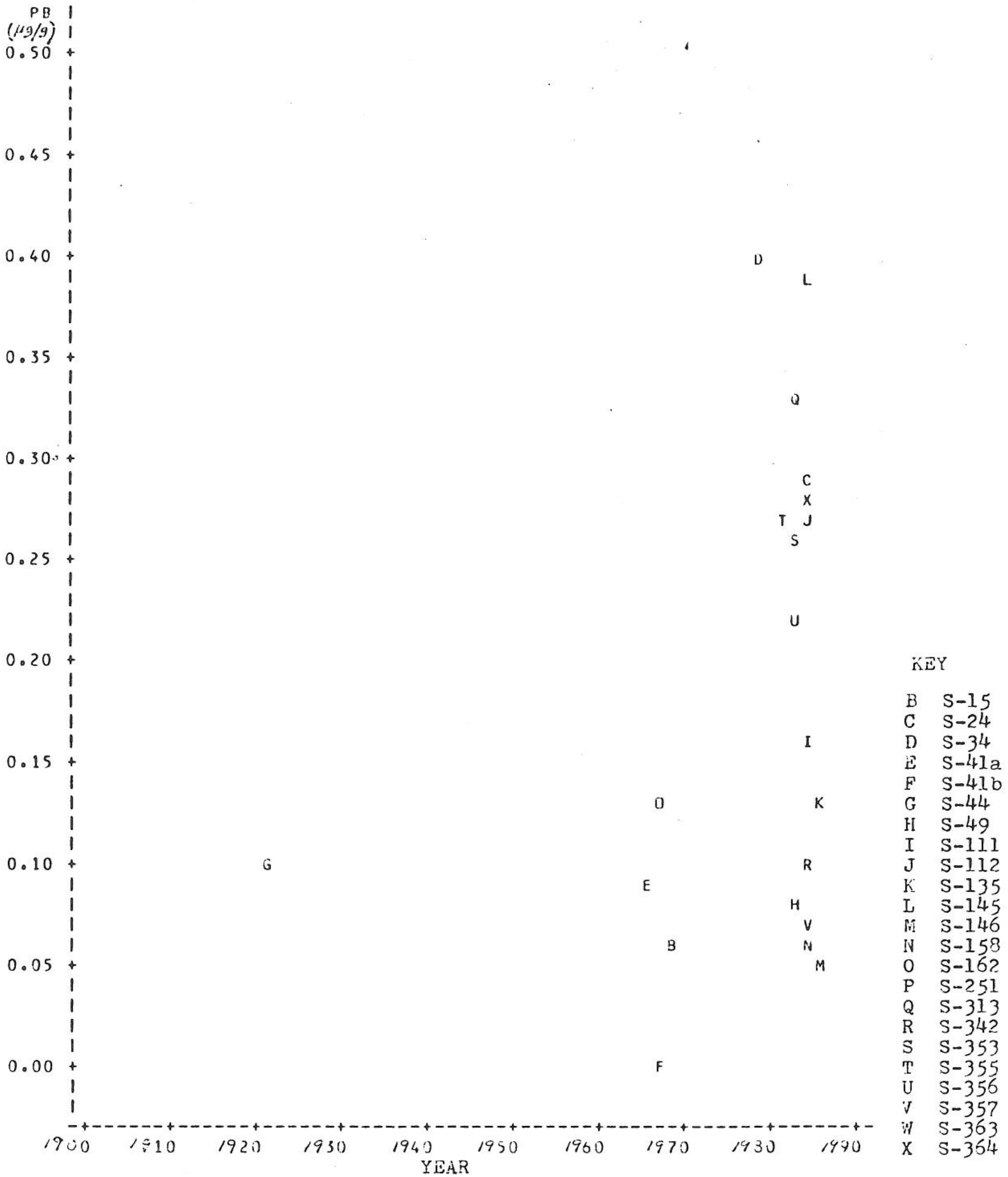

Figure 2 - Plot of lead vs zinc levels in individual samples.

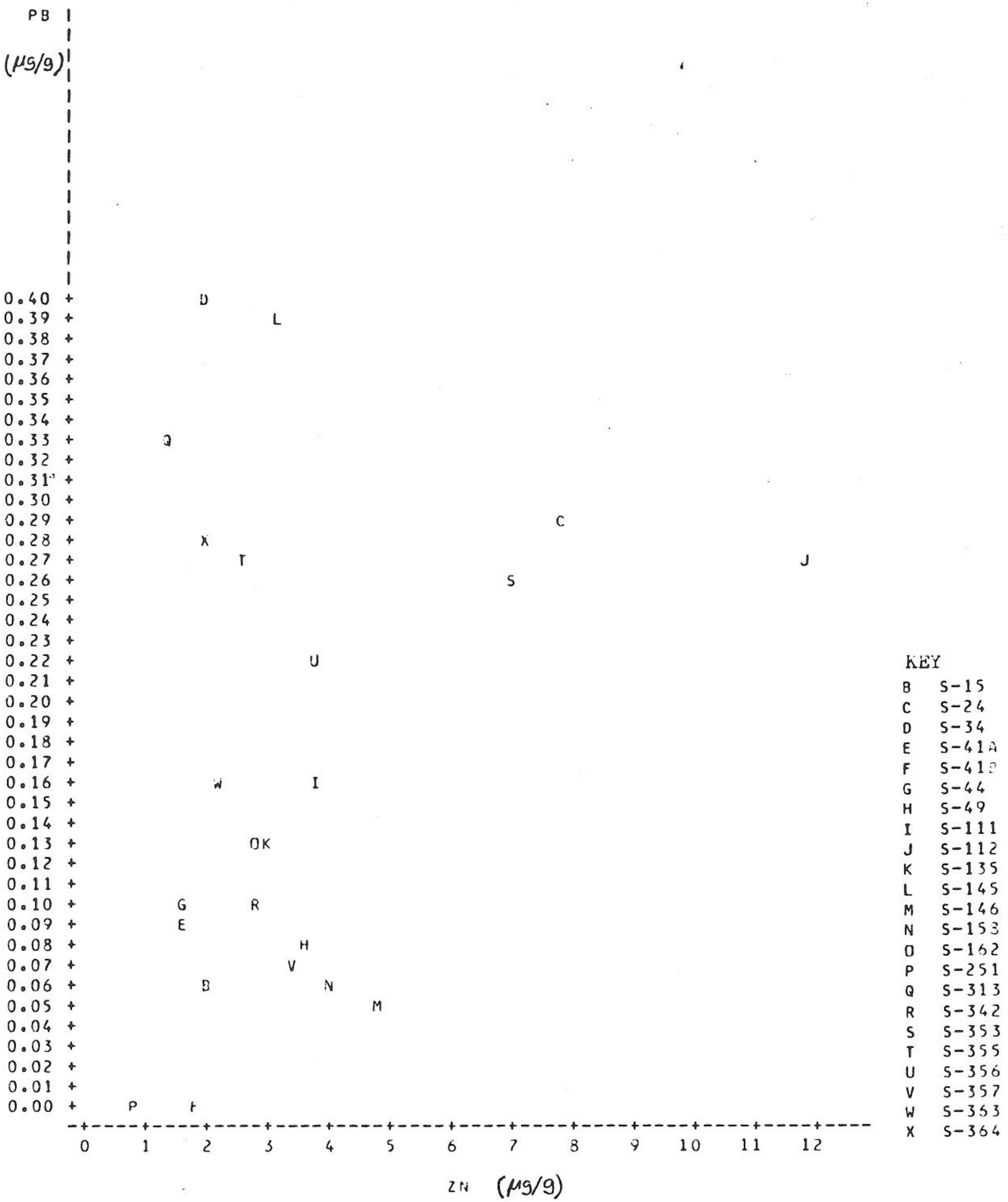

Figure 3 - Plot of zinc levels in condor eggshells vs year.

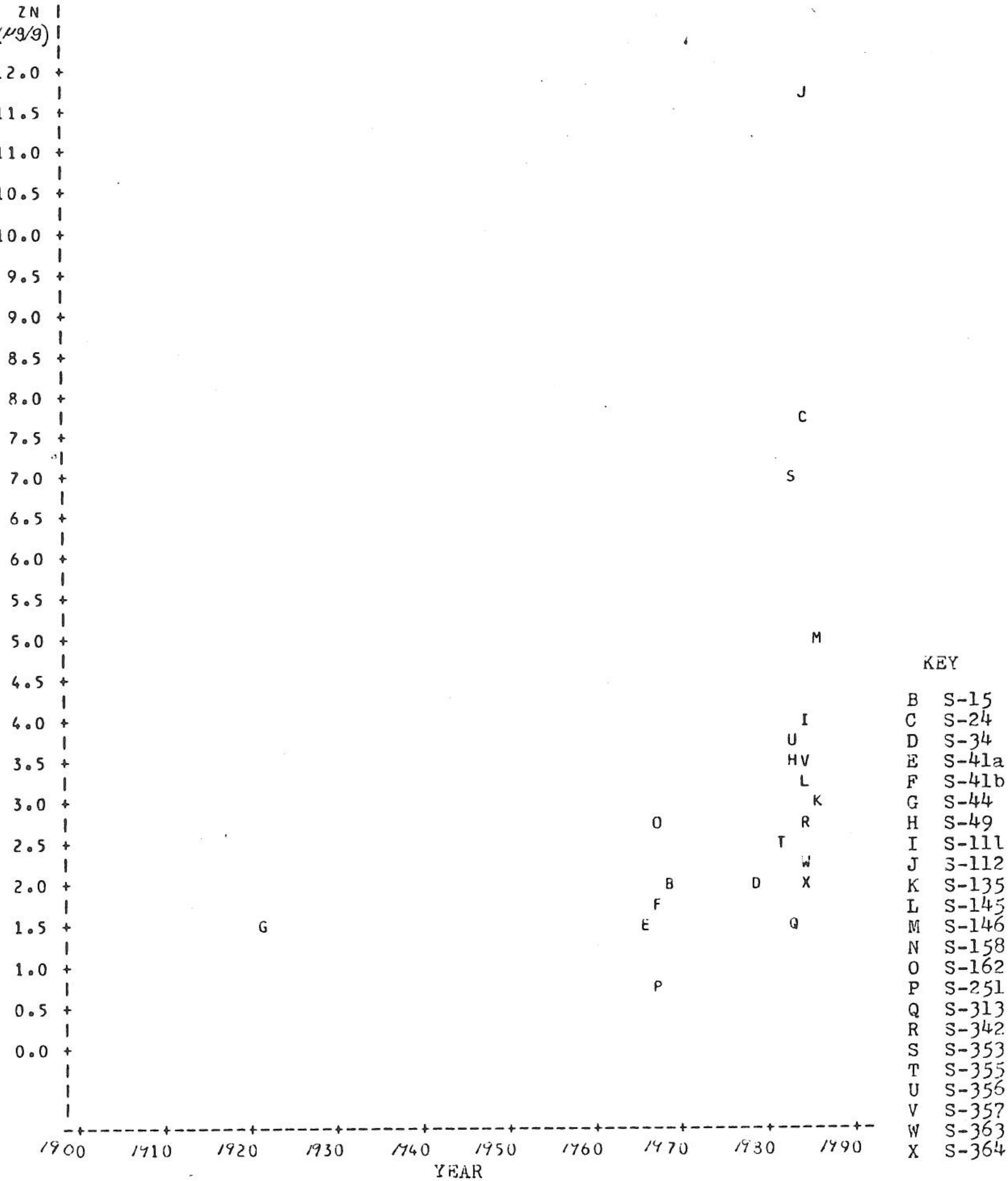

Figure 4 - Frequency bar chart of
condor lead levels.

```
MIDPOINT
   PB                                   FREQ
 (ppm)
          |
  0.00    |**********                      2
          |
  0.05    |********************            4
          |
  0.10    |********************            4
          |
  0.15    |********************            4
          |
  0.20    |*****                           1
          |
  0.25    |***************                 3
          |
  0.30    |**********                      2
          |
  0.35    |*****                           1
          |
  0.40    |**********                      2
          |
  0.45    |                                0
          |
  0.50    |                                0
          |
          -----+----+----+----+
               1    2    3    4

              FREQUENCY
```

Figure 5 - Frequency bar chart of condor zinc levels.

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MIDPOINT
 ZN                                                                  FREQ
(ppm)
        |
  0     |                                                              0
        |
  1     |**********                                                    2
        |
  2     |***********************************                           7
        |
  3     |******************************                                6
        |
  4     |********************                                          4
        |
  5     |*****                                                         1
        |
  6     |                                                              0
        |
  7     |*****                                                         1
        |
  8     |*****                                                         1
        |
  9     |                                                              0
        |
 10     |                                                              0
        |
 11     |                                                              0
        |
 12     |*****                                                         1
        |
        -----+----+----+----+----+----+----+
             1    2    3    4    5    6    7

                       FREQUENCY
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