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Geochemical and Biological Enrichments with Toxic Metals; Anthropogenic Effects

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Abstract

Historical and scientific data suggest that neurotoxins may have affected human health and consequently the history of Renaissance Europe. During the Anthropocene, the epoch we now live in, huge quantities of neurotoxins such as mercury, lead and manganese are added to the biosphere. Here we searched for proxies in biological materials such as plants and animal products for anthropogenic enrichment with such toxins. To put our results into geological perspective we also searched for such toxins in ancient materials dated to pre-human occupation of the earth. We examined the metal content in putative proxies for enrichment in fossil plants from the early Paleocene (~64 million years old) in New Mexico and from present-day New Mexico and compared this to similar proxies from Peru, a country with a rich mining history, which continues to this day, where neurotoxins ravaged pre-Hispanic settlers and affects present-day miners. We found proxies for metals in the plants and other biological materials. Sixty four million year old plant samples found in New Mexico contained more neurotoxins such as mercury, lead and manganese than samples from present-day New Mexico. Contemporaneous samples from Lima, Peru, had even more neurotoxins than the pre-human samples. Despite such geochemical and human enrichments the stability of systems over geological times was not affected by neurotoxin additions to the biosphere. Though intoxications have been well-documented in historical personages and in contemporaneous epidemics of poisoning, our findings imply that the addition of neurotoxins to the biosphere over geological time periods has, as yet, no discernible influence on human health.

Keywords: Neurotoxins; Human health; Proxies plants biologic materials; Peru mining; New Mexico plant fossils; Statistical modeling; Tipping points; Neurotoxins renaissance Europe; Epidemics of poisoning

Introduction

The Anthropocene, the man-made world we now live in, is thought to have started ~14,000 years ago; others posit that it began with the industrial revolution from ~1760 AD and continues to this day. This epoch is characterized by ever increasing levels of neuro-toxic metals such as arsenic (As), mercury (Hg), manganese (Mn), and lead (Pb), in the biosphere. But geochemical enrichment evidenced by soil content of such toxins was already detectable ~ 64 million years ago (MYR), before the advent of humans on earth, in fossil plants. Here we show that in highly polluted locations such as modern Lima, Peru, the neurotoxins Hg, Mn, and Pb increased significantly when compared to millennia and decades ago. We attribute this to human activities during the Anthropocene resulting in biological enrichment. We posited that current environmental pollution by humans may have affected health in the recent past and continues to do so in present times.

Materials and Methods

Sample collections

We collected samples from sites in New Mexico, USA and various locations in Peru. The samples were analyzed in the laboratories of the Earth and Planetary Sciences at the University of New Mexico. The New Mexico Health Enhancement and Marathon Clinics (NMHEMC) Research Foundation's IRB committee gave approval for this study (NMHEMC 16-2014). The geological samples were donated by the New Mexico Museum of Natural History and Science, Albuquerque NM, USA. Samples were also received for analyses from various sites in Peru including Lima. All authors declare no conflict of interest.

New Mexico: In 1980 Barbara Standhardt of the University of Arizona discovered a site named "Black Toe" after the distinctive black clay layer immediately below the fossil level. This site was revisited in 2014 (GPS coordinates are: UTM zone 13, 2041704E, 4012066NAD 83). The locality is in the Paleocene Nacimiento Formation. The geological age is: Early Paleocene (Puercan) about 64.5 million year old. It is in the *Bisti/De-Na-Zin* National Conservation Lands, NM, USA.

Surface rock: 6" deep and 18" deep rock (Figure 1). Sheep jaw and teeth found nearby at the site visited were also collected for analysis. Plants growing in the same area (Figure 2) and a piece of petrified wood ~120 MYR found in the same location were also included in our analyses.

Other collection sites

Albuquerque NM: Cottonwood leaves, top soil, chicken feathers, pigeon feathers, llama wool from local animals. Sandia Crest trails: leaves, decade old deer jaw with teeth, deer bones. Sites publicly owned. Siberia: Mammoth bone 24,000 years old; from the Utah mammoth site. Sites publicly owned; Peru: Teeth from the coastal region (Ilo Peru) dated to 1500 year ago.

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Figure 1: Black Toe Early Paleocene (Puercan) ~64 MYR. This is the formation where the fossil plants were found in 1980 (the black round toe-like structures, red arrows) and the site was revisited in 2014. A sample of petrified wood ~120 MYR was also found lying on the surface and analyzed in this study.

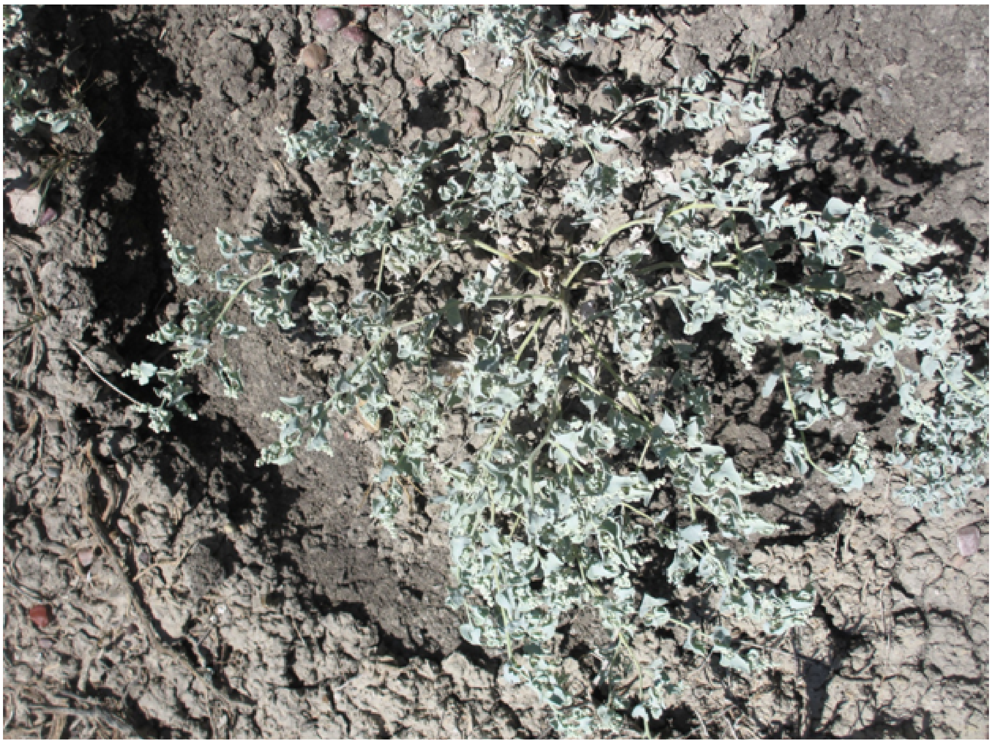

Figure 2: Saltbush. This plant was found growing at the edge of our 2014 excavation at Black Toe. The roots; stem and leaves were analyzed separately; but gave comparable results in metal content.

Lima, Peru: Contemporaneous pigeon feathers, chicken feathers. Janca Punta Cerro Pasco (4900 masl. (masl=meters above sea level) wool; Huachón, Pasco (4900 masl) wool, Chicrin, Pasco (4900 masl) wool. We compared the metal contents of ancient, millennia old, recent (decade old) and contemporaneous samples from Peru and New Mexico USA.

Sites and permissions

New Mexico Museum of Natural History and Science 1801 Mountain Road NW. Albuquerque, New Mexico 87104 USA 35.1107° N, 106.6100° W permit #841-2808-2014c; # of specimens 23 fossil plants. One living salt bush. Specimens are publicly accessible. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens collected by Spencer Lucas.

Bisti/De-Na-Zin NM, USA UTM zone 13, 2041704E, 4012066NAD 83 Specimen destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens collected by Otto Appenzeller.

NMHEMC Research Foundation Albuquerque NM 87102 35.1107° N, 106.6100° W permit #1989-66-2014; 20 cotton-wood leaves; 250 Gr. top soil: 25 chicken feathers 33 pigeon feathers; 15 Grams llama wool; one deer jaw with 5 teeth in situ. Specimens destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected from the streets by Otto Appenzeller.

Universidad Peruana Cayetano Heredia, Lima, Peru. 12.0433° S, 77.0283° W Permit #196687-16-2014. 10 pigeon feathers; 8 chicken feathers Specimens destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected Guido Lombardi.

South Dakota Mammoth site 43.4317° N, 103.4742° W permit #1566-0113-2013 One spinous process of a mammoth. Specimen destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimen collected by Dr. Larry Agenbroad.

Twenty six teeth from the Museum at Ilo Peru. 17.6459° S, 71.3453° W permit #100--2003. Samples destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens collected by Dr. Sonia Guillen.

Janca Punta, Peru -9.5655500 (latitude in decimal degrees), -76.5653500 (longitude in decimal degrees). Permit #16--20013. Samples destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected Guido Lombardi.

Cerro Pasco, Peru 10.6864° S, 76.2625° W permit#01-2013. Samples destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected Guido Lombardi.

Huachón, Pasco, Peru 10°33′57″S 75°56′30″W permit #60-2013. Samples destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected Guido Lombardi.

Chicrin, Pasco, Peru -10.48272, -76.51023 permit #02-2013. Samples destroyed by analyses. All necessary permits were obtained for the described study, which complied with all relevant regulations. Sites publicly owned. The site is on public lands and no permits or permission was required to collect the sample from this site. Specimens were collected Guido Lombardi.

Peruvian samples were provided By Dr. Guido Lombardi, Peru and Dr. Sonia Guillen, Peru. The mammoth specimen was provided by Dr. Larry Agenbroad Legacy Fund for Research, Hot Springs, South Dakota, USA.

Sample preparation

Samples were dried at 65°C for at least two hours and crushed manually using a pestle and mortar. The samples were then pulverized using a SPEX mixer mill with carbide vessel and mixing balls. About 1.000 to 2.000 grams of the sample were weighed and digested using a mixture of 3 ml concentrated nitric acid (HNO_3) and 2 ml concentrated hydrochloric acid (HCl). Samples were digested using DigiPrep heating block equipped with temperature controller at 95°C for about two hours. After digestion was completed, samples were filtered (0.45 micron) and brought to final volume "25 ml".

CP-OES analysis

Digested samples were transferred into 15 ml glass test tubes and were setup on the Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP-OES) (PerkinElmer, Optima 5300DV ICP-OES; and for Hg FIMS) autosampler. The system was optimized using mercury optical alignment and manganese (Mn) view touch alignment.

FIMS mercury analysis

Digested samples were diluted two times using three percent hydrochloric acid (HCl). Samples were transferred into 15 ml glass test tubes and were setup on the Flow Injection Mercury System (FIMS) autosampler. The system was optimized and calibrated for mercury (Hg) using a blank and three calibration standards that were diluted sequentially in order to achieve a linear calibration curve. A set of quality control check samples (Initial Calibration Blank Verification "ICBV", Initial Calibration Verification "ICV", and Continuing Calibration Verification ("CCV") were measured to verify and validate calibration and data quality. Samples were analyzed; data were reduced, verified, validated, and reported in mg/Kg unit of measurement.

Statistical analyses

We used the 64 million year old plant material as our standard for geochemical enrichment and compared everything else to this standard to determine biological (anthropogenic) enrichment. We used Hg content of our materials as a measure of biological enrichment. We compared the feathers and wool Hg results from Peru as a standard for anthropogenic enrichment. To insure that plants and other contemporaneous material were good proxies for soil Hg content we compared plant and soil samples from Albuquerque with the results from *Bisti/De-Na-Zin* National Conservation Lands NM. Comparisons are done using Analysis of Variance (ANOVA); and post hoc pairwise comparisons are done by Fisher's least significance difference method.

Approximate entropy (ApEen)

We used approximate entropy (ApEn) to determine the stability of the system derived from our analyses of the metal content of the samples.

Results

We found proxies for metal content in the soil in the plants and other biological materials collected from specific sites examined in this study (Figures 3-5).

Evidence for anthropogenic enrichments with neurotoxic metals such as Hg, Mn, Pb, was found in Lima, Peru (Figures 6 and 7).

Approximate entropy was not statistically significantly different when comparing geochemical with anthropogenic enrichments (P=0.42) (Figure 8).

We also modeled the concentrations of Hg, Pb and As all significant neurotoxins with huge increases in levels in the biosphere during the Anthropocene (Figure 9).

Discussion

Our data and conclusions are derived from regional materials. However, pollution is a global issue; we believe, therefore, that similar studies from other sites might eventually lead to better global assessments of anthropogenic threats to human health.

Geochemical enrichments, as shown by increased concentrations of metals in the soil have been well documented [1]. However, biological enrichment, which includes anthropogenic enrichment, with increased concentrations of toxic metals in the biosphere and in

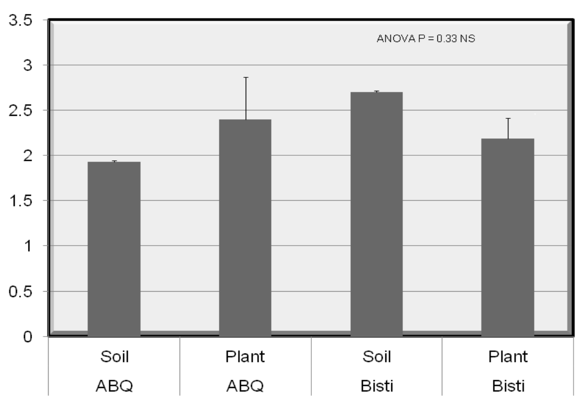

Figure 3: Mercury content (on y-axis in units of log_{10}- mg/kg) in soil and plant materials from Albuquerque and *Bisti/De-Na-Zin* National Conservation Lands both in NM. There were no significant differences between the samples supporting the validity of our assumption that in these sites plants can be used as proxies for metal content.

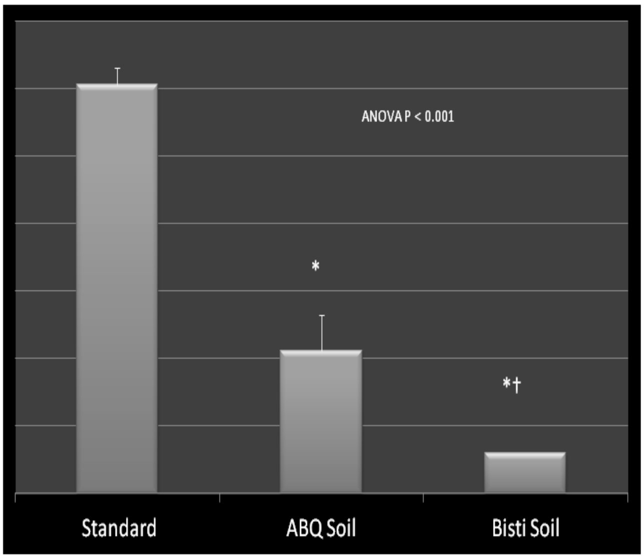

Figure 4: The 64 MYR old plants (standard) had significantly more Hg content (on y-axis in units of log_{10}- mg/kg) than present day plant material growing in Albuquerque NM soil (*). The levels were also significantly higher than in ~120 MYR old petrified woods found in the same location as the standard (†). This finding implies that 64 million years ago some geochemical event (s) lead to the Hg enrichment at this site in New Mexico.

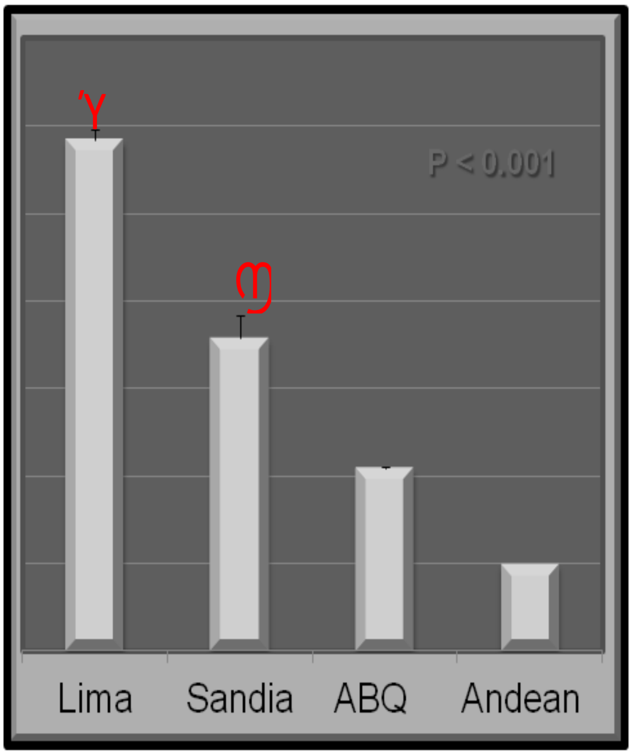

Figure 5: Mercury content (on y-axis in units of log_{10}- mg/kg) of biological materials. Samples collected in Lima, Peru, compared to material collected in Albuquerque New Mexico and in the high Andes of Peru (*) are significantly higher than in other sites. The mercury content of material collected in New Mexico (Sandia) is significantly higher than in material collected in Albuquerque (†), which in turn, is significantly higher than in Andean material (‡). The Hg content of biological materials from Lima, Peru reflects anthropogenic enrichment due to contemporaneous pollution in this location.

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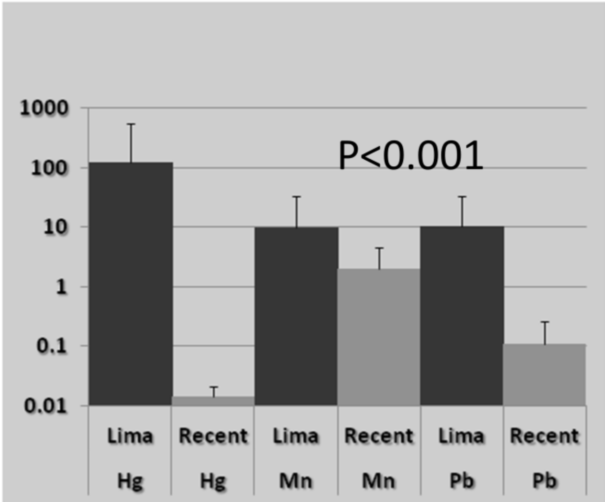

Figure 6: The neurotoxic metal content (on y-axis in units of mg/kg; ANOVA) in Lima, Peru. Metal content is significantly higher in Lima than in other locations (Recent).

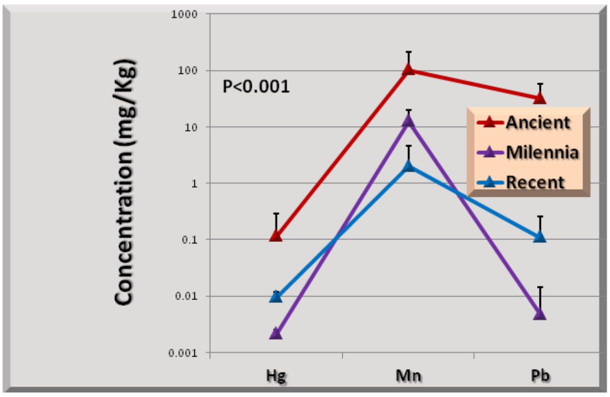

Figure 7: Significantly higher levels of neurotoxins were present in pre-human geochemically enriched biological materials (ancient). We found higher levels of these metals in Lima, Peru than in millennia old and recent material (main effect, P<0.001).

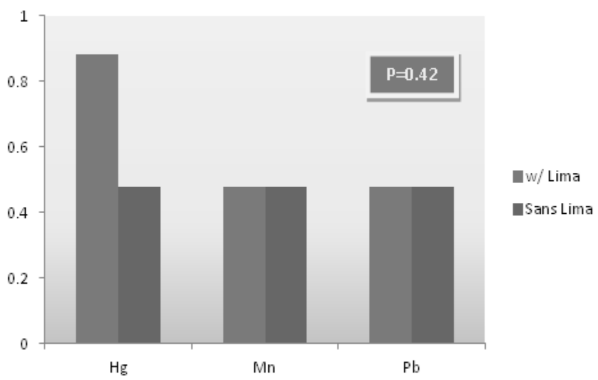

Figure 8: Approximate entropy (ApEn) on y-axis. The proportion (0-1) of repetitive patterns of metal levels derived from neurotoxins in Lima compared to other sites did not reach statistical significance. (Also see: Supplemental on line material (S1) Nevertheless, the highest levels of Hg were found in Lima when compared to other sites (Sans Lima).

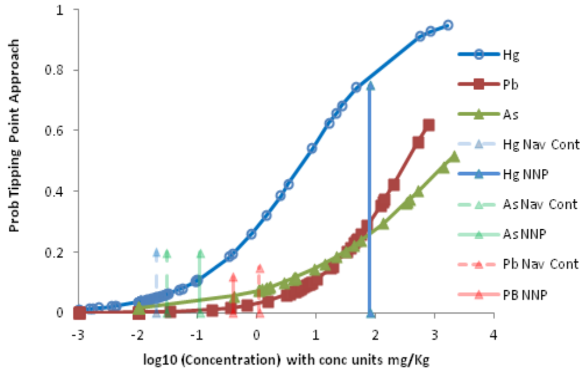

Figure 9: The concentrations of Hg, Pb, and As approaching the probability of reaching the biological "tipping points". The concentrations of Hg (blue arrow), Pb (red arrow), and as (green arrow) for Navajo Control (dashed line) and Navajo Neuropathy (NNP) patients (solid line). The biological "tipping point" is defined here as Hg concentration >11 mg/Kg or Pb concentration >200 mg/Kg of tissue based on the accumulation of Hg or of Pb that lead to death due to metal intoxication. Only Hg had reached the tipping point level on the Navajo reservation in patients with Navajo neuropathy but not in Navajo controls.

biologic tissues has not been sufficiently studied. Here we show that such enrichments can be deduced from the metal content of fossil plants and contemporaneous biological materials.

The Anthropocene

The *Anthropocene* is the epoch we now live in. This epoch has been altered by human activities. Mercury is a neurotoxin with especially devastating effects on the nervous system. Recent work shows that the emissions of Hg to the biosphere peaked in 1870 but that further additions of Hg occurred in 1970 and although declining until about 2000, the levels of Hg are increasing again [1,2]. We focused here on the analyses of Hg and other neurotoxins such as Mn, As and Pb. We examined sites in New Mexico, as examples of less polluted environments and in Peru, more polluted because of extensive mining activities in the past and more recently further addition of Hg by artisanal mining in some parts of Peru. Our results support our contention that Lima is a site of considerable anthropogenic enrichment with neurotoxins and imply that high levels of neurotoxins can occur independently of anthropogenic enrichment but the contribution to neurotoxic levels during the Anthropocene is higher than it ever was before [3,4].

Reports that plants growing in soil containing gold also contain gold in their leaves [5] supports our contentions that plants are proxies for metal content of the soil in which they grow. Other biologic sources such as pigeon feathers and wool accumulate neurotoxins through metabolic activity of the animals, for example in Lima, Peru, as shown here. These results implied that proxies for soil content of neurotoxins could be found in fossil plants; a hypothesis confirmed here in ~64 million year (MYR) old material from the Early Paleocene, Nacimiento Formation in National Conservation Lands near Farmington NM. USA. Thus signatures of enrichments over geologic time scales (geochemical enrichment), before the advent of humans on earth, could be derived from fossil material. Conversely, biologic materials in Lima characterized anthropogenic enrichment due to current human activities.

Evidence from human materials such as bone for lead content has provided estimates of natural lead levels too. These were reported to be some 600-fold lower than what is considered the maximum safe lead level for contemporary children [6]. During the Renaissance bioaccumulation of neurotoxins (Hg and Pb) caused intoxications and death in humans, mostly confined to the nobility who could afford to drink wine (full of Pb contaminants in the form of sapa, (lead oxide) added as a sweetener) rather than water which, at that time, was heavily contaminated. Similarly, this affluent group could indulge in pharmaceuticals which contained, almost universally at that time, large quantities of Hg [7]. These examples of bioaccumulation-induced diseases underscore the importance of metal content of the biosphere.

Tipping points

Over geologic timescales earth's climate has remained stable but sudden transitions from such stability occurred often leading to enrichments with neurotoxic metals. These transitions have been attributed to critical tipping points when the prevailing climate unexpectedly changes. The best predictor of such transitions appears to be a slowing of fluctuations in the oscillations of the system starting before the climate transition actually occurs, thus providing warning of impending change [8]. Analogues of such sudden transitions (tipping points) occur also in biologic systems where stability or homeostasis is maintained by the autonomic nervous system (ANS) [9]. The timescales in biology are much shorter than in geology but they are sufficient to warn of impending clinical deterioration or death [10]. Our analyses are confined to New Mexico and Peruvian geologic and anthropogenic enrichments however, we also define biological tipping point in terms of the accumulation of Hg or of Pb known to cause death; this definition applied to our samples yields the probability of biological tipping point as function of the various metal concentrations using logistic regression. We found that tipping points have not, as yet, reached levels that were historically known to have caused death in humans during the Renaissance but did reach the tipping point in patients with Navajo neuropathy.

Approximate entropy (ApEn)

Recovery from perturbations of stable systems such as geochemical and anthropogenic enrichments becomes slower when approaching so called "tipping points". Such slowing foreshadows catastrophic collapse in a variety of settings [11]. Repetitive patterns of metal levels render systems more predictable whereas small numbers of repetitions in patterns makes them less predictable (more unstable), more repetitions have a small ApEn; those with fewer repetitions, therefore more chaotic patterns, have higher ApEn and better stability [11].

To statistically validate the evidence presented and to estimate the stability of the geochemical and anthropogenic enrichments we used approximate entropy (ApEn). This analysis quantified the regularities in the fluctuations of metal content [11-15] such as in geological and biological samples. We confined our study to the effects of the neurotoxins Hg, Pb and Mn. Unexpectedly, we found no significant differences in the robustness of the equilibrium of the systems (no slower recovery after changes in metal content) in samples dating to ~64 MYR with high neurotoxic metal content and those obtained from present-day Lima with even higher Hg content. This implies that the stability of the systems over geologic times has not been significantly affected, even though Hg levels in Lima were higher than at any other time-scale examined in this study. Furthermore the ApEn of Manganese and Pb were the same further implying that Hg is an appropriate proxy for anthropogenic enrichment (Figure 9).

Finally, we estimated the levels of Hg, Pb and As that could significantly affect the stability of the system. This suggests that tipping point-levels of such neurotoxins in the biosphere will be reachable in the not too distant future (Figure 9). Details of the statistical analyses are given in Supplemental on line information (Supplementary Information S1).

Conclusions

Biological materials such as plants, feathers and wool are good proxies for soil content of metals. Statistical analysis of metal content of soil and biologic materials compared over appropriate sites and epochs can distinguish geochemical and anthropogenic enrichments. This conclusion cannot be generalized to all locations but is indicative only of the sites examined.

Sixty four MYR ago, before the advent of humans on earth, there were significantly more neurotoxins in certain locations in New Mexico (geochemical enrichment) but the contents were lower ~120 MYR ago and are still low today. There are significantly more neurotoxins in Lima (anthropogenic enrichment) when compared statistically to the recent past (millennia ago) in other sites in Peru.

The stability of geological and biological systems as expressed by neurotoxin content that define geochemical and anthropogenic enrichments has not changed over geological times. The significance of these findings will emerge after the passage of more time and especially after examination of additional sites in various parts of the globe where such enrichments may be relevant to biology and to predicting the tipping points.

Study limitations

1. The results apply to the sites examined.

2. The evidence is limited to the periods analyzed.

3. Plants and other biological material absorb metals at different rates dependent on precipitations and other climatic conditions, our analyses are restricted to the materials used, time periods examined and locations studied and cannot be generalized to other living matter, locations or epochs.

4. Statistical analyzes of bio- geological data do not imply causations but suggest correlations only.

Acknowledgements

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References

1. Castillo PR, Rigby SJ, Solidum RU (2007) Origin of high field strength element enrichment in volcanic arcs: Geochemical evidence from the Sulu Arc, southern Philippines. Lithos 97: 271-288.
2. Horowitz N, Jacob DJ, Amos HM, Streets DG, Sunderland EM (2014) Historical Mercury Releases from Commercial Products: Global Environmental Implications. Environmental Science and Technology 48: 10242-10250.
3. Colin A, Cookea L, Prentiss H, Balcomb H, Biesterc A, et al. (2009) Over three millennia of mercury pollution in the Peruvian Andes. PNAS 106: 8830-8834.
4. Krabbenhoft DP, Elsie MS (2014) Global change and mercury. Science 341: 1457-1458.
5. Lintern M, Anand R, Ryan C, Paterson D (2013) Natural gold particles in Eucalyptus leaves and their relevance to exploration for buried gold deposits. Nature Communications 4: 1-8.
6. Flegal RA, Smith DR (1992) Lead Levels in Preindustrial Humans. N Engl J Med 326: 1293-1294.

7. Rasmussen KL, Boldsen JL, Kristensen HK, Skytte L, Hansen KL, et al. (2008) Mercury levels in Danish Medieval human bones. Journal of Archaeological Science 35: 2295-2306.

8. Dakos V, Scheffer M, Van Nes EH, Brovkin V, Petoukhov V, et al. (2008) Slowing down as an early warning signal for abrupt climate change. PNAS 105: 14308-14312.

9. Appenzeller O, Oribe E (1997) The Autonomic Nervous system. An Introduction to Basic and Clinical Concepts. 5th edn. USA, p: 910.

10. Scheffer M, Bascompte J, Brock W, Carpenter SR (2009) Early-warning signals for critical transitions. Nature 461: 53-59.

11. Pincus SM, Gladstone IM, Ehrenkranz RA (1991) A regularity statistic for medical data analysis. J Clin Mon 7: 335-345.

12. Richman JS, Moorman JR (2000) Physiological time-series analysis using Approximate entropy and sample entropy. Am J Physiol Heart Circ Physiol 278: 2039-2049.

13. Carpenter SR (2011) Early warnings of regime shifts: a whole-ecosystem experiment. Science 332: 1079-1082.

14. Qualls CR, Appenzeller O (2015) Modeling metabolism and disease in bioarcheology. BioMed Research International.

15. Pincus SM, Goldberger AL (1994) Physiological Time Series Analysis – What does regularity quantify? Am J Physiol 266: 1643-1656.