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COMPARATIVE ASPECTS OF ECOTOXICITY BETWEEN DIFFERENT ENVIRONMENTAL COMPARTMENTS AS EVIDENCED BY SPECIFIC BIOMARKERS

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Abstract

Biomarkers can be defined in ecotoxicological meanings as the body" s biological response to the action or the impact of a toxic chemical that leads to a deviation from normal. Under this definition are analyzed only at an individual level those considered relevant and specific biological responses in ecotoxicological point of view. In this study, trace the development of ALAD (delta-aminolevulinic acid dehydratase), specific biomarker of lead in fish and ruminant mammals to be correlated with the concentrations of lead in the environment. State environmental health of the biotic and abiotic thus indirectly can thus be monitored and may show clear histotoxicologic and citotoxicologic alterations type generated by the chemicals. Comparison of data from monitoring biomarkers and potentially toxic chemicals from abiotic environmental compartments (water, air, soil) can make a major contribution to the establishment of tolerable levels of concentrations of these toxic substances. On the other hand, emphasizing the functional levels of biomarkers help to clarify the etiopathogenic mechanisms of many ecotoxicological processes, risk to human and the environment health, thereby establishing the relationship between cause and effect.

Keywords: biomarker, metals, lead, enzymes, ecotoxicological, spectrometry.

1. INTRODUCTION

The main reason is the knowledge of biomarkers of human health problems and also the biotic environment caused by metals, usually with high atomic weight such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As), with negative implications, but also from low atomic mass, the best known example is aluminum (Al). Decontamination for toxic metals is different from organic toxins: because toxic metals are elements, they cannot be destroyed. Toxic metals may be made insoluble or collected, possibly by the aid of chelating agents. Toxic metals can bioaccumulate in the body and in the food chain. Therefore, a common characteristic of toxic metals is the chronic nature of their toxicity. This is particularly notable with radioactive heavy metals such as thorium, which imitates calcium to the point of being incorporated into human bone, although similar health implications are found in lead or mercury poisoning. The exceptions to this are barium and aluminum, which can be removed efficiently by the kidneys.

Ecotoxicological chemicals risk create problems as a result of relatively recent highly accelerated industrial and economic development, being less severe in developed countries where more stringent measures have been introduced in recent decades. These chemicals persist in the environment because they have natural origin and are biodegradable.

Exposure to metals is the occurrence of adverse consequence of chronic (Akesson et al.2006, Chen et al.2007).

Target populations of these studies were changed to bioindicator species monitoring. It is necessary however, biological studies to be correlated with the presence of these substances in environmental compartments (water, air, soil).

In the case of human fetuses are studied as they are at high risk from exposure to metals, can be observed combined effects of these metals. Important to investigate is the degree of bioaccumulation of chemical elements in various bodies mentioned above, placental transfer of these metals and their relationships with maternal and fetal circulation.

The specificity of the biomarkers is an important criterion in ecotoxicological investigations. And non-specific biomarkers in the division are necessary because the effects of bioindicator organisms by specific biomarkers (biological responses) are safe and effective means of specifying the toxic chemicals involved in metabolism.

Identifying specific biomarkers is possible in kingdoms, plant and animal.

Thus, seleno-or fluorosensibile plants, plant selenoproteins. They are excellent biomarkers for

stress, and excess fluoride causes fluorocitrat bioaccumulation of a specific biomarker of fluoride poisoning.

In animals and humans, are very useful as biomarkers of enzymatic parameters, such ALAD inhibition has been studied since 30 years ago as a means of detecting human exposure to environmental lead and became a standard bioassay for this reason, frequently used in investigations of wild world.

The test is very specific for lead and other metals are 10 times less active because of inhibition. At this, ALAD inhibition is rapid, but the effect is poorly expressed ALAD returning to normal only after four months. Delta-aminolevulinic acid dehydratase (ALAD) is an enzyme that in humans is encoded by the *ALAD* gene (1).

The ALAD enzyme is composed of 8 identical subunits and catalyzes the condensation of 2 molecules of delta-aminolevulinate to form porphobilinogen (a precursor of heme, cytochromes and other hemoproteins).

ALAD catalyzes the second step in the porphyrin and heme biosynthetic pathway; zinc is essential for enzymatic activity.

ALAD enzymatic activity is inhibited by lead and a defect in the ALAD structural gene can cause increased sensitivity to lead poisoning and acute hepatic porphyria. Alternatively spliced transcript variants encoding different isoforms have been identified.

The neurobehavioral effects of Pb, especially in children, have also been well studied (Canfield et al., 2003; Lanphear et al., 2005).

2. MATERIAL AND METHODS

The work plan includes three types of methods to highlight the influence of lead in the environment. Thus, we can mention:

A. Determination of ALAD specific lead biomarker by PCR;

B. Determination of phenotypic indicators of bioindicators species under metalotoxical influence, fish and mammals ruminant;

C. Spectrometric investigations of lead in the abiotic environment.

Comparison of data obtained and their correlation gives precise indications on the influence of metals. We know that found to have elevated free erythrocyte protoporphyrin (FEP) levels.

Additional collaborations have been established to study ALAD genotypes by PCR and MspI cleavage analysis in populations in which a chronic measure of lead exposure is available. H Hu, D Bellinger (Harvard), H Needham (Pittsburgh): chronic measure, dentine of deciduous teeth. B Fowler, E Silbergeld (University of Maryland): acute measure, blood lead; chronic measure, bone lead. DR Chettle (McMaster), L Cerhardsson, S Skerfving (Lund), V Englyst (Boliden Metall AB): acute measure, blood lead history; chronic measure, bone lead. A Todd and P Landrigan (Mount Sinai, New York): acute measure, blood lead; chronic measure, bone lead.

PCR ALAD genotyping by PCR is carried out as previously described (32).

The PCR is commonly carried out in a reaction volume of 10–200 μl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler.

The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below).

Atomic absorption spectrometry (AAS) is a spectrometric method which allows a selective determination of metals in environmental samples (water, air, soil) to highlight relevant elements eco, lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As). AAS techniques work, that standard operating procedures, quality assurance of analytical data have a number of specific elements.

Analytical methods are differentiated by type of sample origin, reflects the conditioning / pre-treatment of the sample to be analyzed by AAS and applied to the production, depending on the type of sample.

Lead is the metal which was analyzed by atomic absorption spectrometry (AAS) with graphite furnace according to ISO 8288:2001.

Atomic absorption spectrometer radiation source with hollow cathode lamp and a deuterium lamp for correction, with a monochromator, equipped with a source of atomization furnace with heating and longitudinal sound detector associated with an electronic amplifier and measuring equipment.

Complementary methods of study

I. Morphological indicators calculated by the method described by Bagenal and Tesche (1978) Conditioning factor (CF): [weight (g) / lungime3 (cm) 3] × 100) and Slooff et al. (1983) for the liver somatic index (LSI): [liver weight (g) / body weight (g)] × 100) - chosen for tissue sampling as liver and muscles (7).

II. Chemical metabolic stress indicators:

a. ***glycogen content***- to measure glycogen content, mix 1 g of muscle in 4 mL of 0.33 M perchloric acid and subjected to hydrolysis with amiloglucosidase, a method described by Keppler

and Decker (1974). Glucose formed is determined by the Sigma enzymatic kit (Cat. no. 716 251), using hexokinase and glucose-6-phosphate dehydrogenase.
Content is extrapolated from glucose into glycogen formed by acid hydrolysis.
The tissues selected for analysis were the liver and white muscle (the bulk of the fish body).
At each sampling time, muscle samples from each specimen were analysed for glycogen and lactate contents.
At the same time, liver samples from each specimen were analysed for glutathione S-transferase (GST) activity.
All biochemical response analyses were performed in triplicate.
b. lactate content - to measure the content of the lactate, mix 1 g of muscle in 4 mL of 0.33 M perchloric acid.
After adding 8 ml distilled water, adjust the pH to 10-11 with 2 M KOH. Transerată solution is a 20 mL flask and filled to the mark.
Clarified solution is analyzed for lactate with a Boehringer Mannheim test kit (Cat. no. 10,139,084 0359), using lactate dehydrogenase - the method described by Gutmann and Wahlefeld (1974).

Table 1. Limit values of toxic metals

Limit values	U.M.	Quality grades I	II	III	IV	V
Metal Items Ecotoxicity						
Lead	µg /l	Fond	5	10	25	>25
Arsenic	µg /l	Fond	1	2	5	>5
Mercury	µg /l	Fond	0,1	0,2	0,5	>0,5
Cadmium	µg /l	Fond	5	10	25	>25

3. RESULTS AND DISCUSSIONS

The present study investigated bioaccumulation markers and biochemical responses in fish and ruminant mamals under different environmental conditions.
Species, season, diet, location, life stage and age all have a major impact on both nutrient and contaminant levels in fish.
Contaminants present in fish are derived predominantly from their diet, and levels of bioaccumulative contaminants are higher in fish that appear high up in the food chain.

Table 2. Fish weight and length measured at each sampling time.

	Weight (g)		Length (cm)	
	April	**July**	**April**	**July**
Site 1	656±57	576±93	37±2	38±3
Site 2	678±122	609±134	38±3	35±3
Site 3	487±66	443±67	36±1.5	33±2
Site 4	265±59	368±40	36±1	43±1

Values are expressed as mean±standard deviation of 8 fish.
The overall results from both chemical analysis and biochemical assays of the tissues represented the environment in which the bioindicators animals were raised. Bioindicators of the health of individual fish included a general condition factor (CF), the liver somatic index (LSI) and a biomarker of contaminant exposure, glutathione S-transferase (GST) activity (Hamed et al., 2003). Glycogen and lactate contents of the muscle, metabolic indices of stress that are sensitive to a wide variety of stressors, including contamination level, were also assessed. &Aminolevulinate dehydratase (ALAD) is the second enzyme in the heme biosynthesis pathway. ALAD is a zinc metalloenzyme, and its inhibition by lead substitution for zinc is one of the most sensitive indicators of blood-lead accumulation, a measure of recent lead exposure. Stoichiometry calculations indicate that a significant portion of blood lead is stored in ALAD.

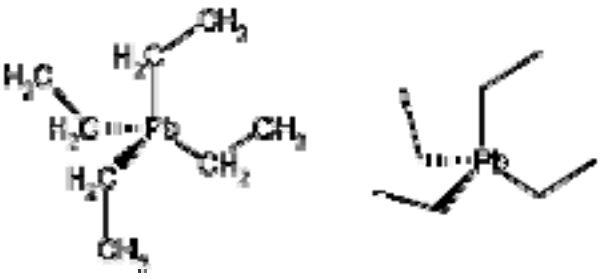


Figure 1. Addition of Pb to the chemical formula acid hydrolase aminonolevulinic

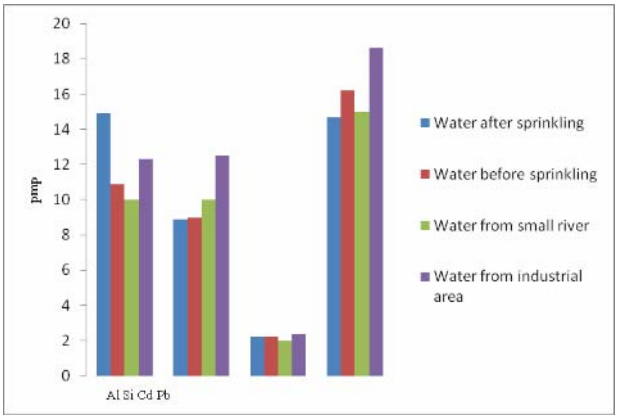
Human ALAD exhibits a charge polymorphism, with about 20% of Caucasians expressing the rarer ALAD2 allele. Human ALAD1 and ALAD2 cDNAs and the 16-kb ALAD gene have been cloned and sequenced. A simple polymerase chain reaction test has been established and validated for determining ALAD genotypes. Two population

studies have indicated that lead-exposed individuals with the ALAD allele have blood-lead levels about 10 pg/dl greater than similarly exposed individuals carrying only the ALAD1 allele. Ongoing work is directed toward determining the biochemistry underlying the allele-specific accumulation of blood lead and toward determining the contribution of human ALAD genotype to lead accumulation in other tissues in transgenic mouse models and to

Table 3. Water and soil samples from different periods subject to chemical stress

Item No.	Metals	water after spraying	water before spraying	creek water	industrial area water.	soil treatment station	industrial area soil
		ppm	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**
1	Al	14.9	10.9	10.0	12.3	62.30	71.1
2	Si	8.9	9.0	10.0	12.5	317.00	361.38
3	Ca	1964.4	2538.	2700.0	3213.0	1200.0	1368.0
4	Fe	836.6	872.0	800.0	888.0	4670.	5510.6
5	Cd	2.2	2.2	2.0	2.34	1.2	1.4
6	Pb	14.7	16.2	15.0	18.6	75.0	9.3

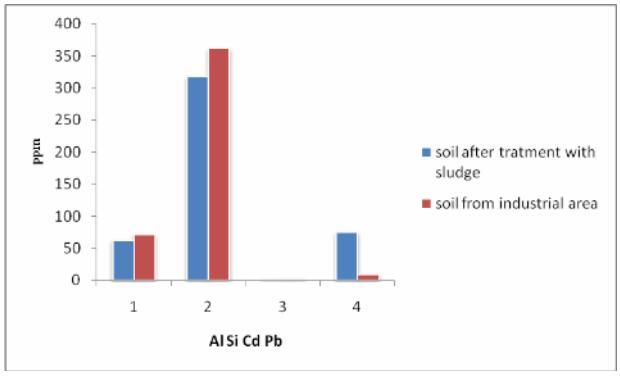
final lead deposition in bone in both mouse and man. - Environ Health Perspect 102(Suppl 3):215-219 (1994).



Al (blue), Si (red), Cd (green), Pb (violet)

Figure 2. Presence of toxic metals in different water samples

We noted some differences in samples of water content of some toxic metals properties. Aluminum is present in large amounts after taking herbal and industrial area. Arsenic, cadmium and lead have a peak far beyond what other evidence showing the involvement of human factor enrichment of these chemicals.



Al (1), Si (2), Cd (3), Pb (4)

Figure 3. Presence of toxic metals in different soil samples

The soil samples we noted significant differences in the amount of lead that occurs higher in the treated soils which shows the risk of exposure to wastewater use for agricultural activities.

4. CONCLUSIONS

- This study demonstrates that environmental factors, including them here and those generated by human activity a crucial role in maintaining environmental health;
- Metal bioacumulation is influenced by artificial chemical processing of industry;
- extending it has taken a human activity has contributed to global cycling of metals, phenomena that can be described as rich antropogenetic factors (RAF);
- Human activity is responsible for most of the global movement of cadmium, lead, mercury. Information obtained in the study of mutations suffered by abiotic components (air, water, soil) and biotic show deviations from normal state in which living organisms;
- Determination of pollution agents becomes more and more important in the protection of environment and health, taking into account the cumulative effects of some metallic ions.
- We know that metals are nonbiodegradable and cannot be broken down into less harmful components. Detoxification by organism consists of hiding active metal ions within a protein such as metallothionein (binding covalently to sulfur) or depositing then in an insoluble form in intracellular granules for long times storage or excretion in the feces.
- Nonessential metals such as lead or mercury or cadmium can be toxic above certain levels, may also affect organism by inducing deficiencies of

essential elements through completion at active sites of certain molecules, and these can play an significant role in the global cycles of metals described by anthropogenic enrichment factor (AEF).

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