In vivo Experiments of Natural Products Protection of Antagonistic Effects of Lead on Iron

MARIOARA NICULA[1], NICOLAE PACALA[1], LAVINIA STEF[1], IOAN PET[1], DOREL DRONCA[1]*, ADRIANA GHERBON[2], MIRELA AHMADI[3]*

[1]Banat's University of Agriculture Sciences and Veterinary Medicine King Michael I of Romania from Timisoara, Faculty of Animal Sciences and Biotechnology, 119 Calea Aradului, 300645, Timisoara, Romania

[2]University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Medicine, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[3]Banat's University of Agriculture Sciences and Veterinary Medicine King Michael I of Romania from Timisoara, Faculty of Veterinary Medicine, 119 Calea Aradului, 300645, Timisoara, Romania

Living organisms take nutrients from the environment, and together with them, substances with toxic potential – such as heavy metals. Lead is one common metal pollutant especially in aquatic environment, from where the fish can be intoxicated very easily. Bioavailability, distribution, toxic action, synergistic and antagonistic effects are characteristics which can alter the fish health. Our experimental study followed the effects of lead overload in water on iron distribution, in different tissues sample Carassius gibelio Bloch fish. We performed the experiment in four different fish groups: control C; lead – Pb (administration of lead in water 0.075mg/mL of water, as $Pb(NO_3)_2$ x ½ H_2O); lead (the same dose) and 2% of freeze-dry garlic incorporated into fishes' food – Pb+garlic; lead (the same dose) and 2% chlorella incorporated into fishes' food – Pb+chlorella, for 21 consecutive days. The iron concentration was analysed with AAS (Atomic Absorption Spectroscopy) from gills, muscle, skin (and scales), intestine, liver, heart, brain, ovary, testicles, and kidney. The obtained data presented a significantly decrease of iron content in all tested tissue samples that demonstrated, alteration of iron homeostasis, explained by a strong antagonistic effect of lead on iron. Our experiment showed that biologic active principles from garlic and chlorella act like natural protectors, and potentiate the iron deficiency even in the case of lead overload in aquatic environment, for fish.

Keywords: fish, lead, iron, garlic, chlorella

Ecosystems consists in complex community of living organisms that needs balance nutrients and energy, and tolerate low or moderate pollutants. Because the ecosystems components interact each other, some of them can act like protective organisms for others-by biodegradation the pollutants [1-4]. Heavy metals are major pollutants for terrestrial and aquatic environments and cannot be biodegraded, causing the pollution of water, soil and/or air [5].

According to US Environmental Protection Agency (1999) it was established *The Water Quality Criteria* by assessing a model for biotic ligand for some metals found as pollutants in aquatic systems (Cu, Ag, Pb, Cd). The *biotic ligand model* presents the bioavailability of copper, silver, lead and cadmium in aquatic ecosystems, referring to a comprehensive evaluation of total metal content, metal complexation and possible interactions of the site of action (named biotic ligand). Therefore, in aquatic organisms the mortality occurs when the concentration of the metal bound to some biotic ligands exceeds a certain concentration limit (US Environmental Protection Agency, 1999; Health and Ecological Criteria Division – US EPA, 2002). The Environmental Quality Standards and The Specific Quality Standards established the recommended some guide values for lead concentration and are presented in table 1 (Lead EQS dossier 2011).

Chelation (pulling heavy metals from cells with synthetic or natural agents) is the standard treatment for heavy metal toxicity [6,7]. Organic or inorganic compounds acting as chelating agents are chemicals which can bind metal ions and form complex ring-like structure named in chemistry *chelates*. Beside of advantages of synthetic chelating agents such as DMPS (2,3-dimercaptopropane 1-sulfonate), DMSA (2,3-dimercaptosuccinic acid), EDTA (ethylenediamine-tetraacetic acid), due to their strong ability to capture and removing poisonous metals from the body, there are also some disadvantages to using them, such as: incomplete removal of toxic metals, high-energy requirements for living organism, production of some other toxic potential compounds [8,9]. But natural chelators from some vegetal sources, if used properly have proved to be highly effective at removing heavy metals from the body.

Generally, in water, heavy metals are considered toxic when their concentration exceeds 5g/cm^3 (5g/mL). Maximum contaminated level for most prevalent heavy metals summarized by Babel and Kurnianwan in 2003 – cited by Barakat [9, 10] are presented in table 2.

Heavy metals presented in table 2 are considered systemic toxicants, being classified - according to U.S. Environmental Protection Agency (U.S. EPA); International Agency for Research on Cancer (IARC) and National Health and Nutrition Examination surveys (NHANES) - as human carcinogens, being correlated with dose, chemical species, exposure route, age, gender, genetics, nutritional status, income and degree or urbanization [9, 11].

Lead can also be present in drinking water especially in the countries where the drinking water pipes are made of lead. Various factors influence the reactivity between lead and chemical compounds from water, such as water *p*H, temperature, time, and others [10].

Fish are also organisms which can be contaminated with heavy metals due to water pollution and trophic chain, but the toxic effect is correlated also with genetic characteristics [12]. Hence, the mechanism action of

* email: ddronca@animalsci-tm.ro; mirelaahmadi@gmail.com

Table 1
ENVIRONMENTAL QUALITY STANDARD AND SPECIFIC QUALITY STANDARD FOR LEAD

Specification	Ecosystem aquatic protection	Unit measure	Value
Environmental Quality Standards (EQS)	*Freshwater* Proposed AA-EQS[a*] Proposed MAC-EQS[b*]	[μg / L]	1.2 14.25
	Marine water Proposed AA-EQS Proposed MAC-EQS	[μg / L]	1.3 14.25
Specific Quality Standard (QS)	*Freshwater* Pelagic community Benthic community	[μg / L] [mg / kg]	EQS Covered 131 (based on total Pb)
	Marine water Pelagic community Benthic community	[μg / L] [mg / kg_{dw}][c*]	1.3 123
	Mammalian predators (considered as secondary toxicity)	[mg / $kg_{biota\ ww}$][d*] [μg / L]	3.6 2.3
	Human health due to translocation chain (consumption of fresh aquatic products)	[μg / $kg_{biota\ ww}$]	fish muscle 200 crustaceans 500 molluscs 1000 cephalopods (without viscera) 1000
	Human health due to water consumption	[μg / L]	10

Note: [a*] Annual Average-Environmental Quality Standards (AA-EQS); [b*] Maximum Annual Concentration-Environmental Quality Standards (MAC-EQS); [c*] dw – dry weight; [d*] biota ww – biota wet weight.

Table 2
MAXIMUM CONTAMINATED LEVEL (MCL) STANDARDS FOR SOME HAZARDOUS HEAVY METALS

Heavy metal	MCL (mg/L)	Toxicity
Arsenic (As)	0.05	Dermatological disorders (skin damage), vascular and circulatory disorders, visceral cancer
Cadmium (Cd)	0.01	Renal disorders, carcinogen for humans
Chromium (Cr)	0.05	Headaches, nausea, vomiting, diarrheic, carcinogenic, allergic dermatitis
Copper (Cu)	0.25	Gastrointestinal distress in short term exposure; Liver (including Wilson diseases) and kidney damage in long term exposure, insomnia
Lead (Pb)	0.006	Children: fetal brain disorders, delay physical and mental development, deficits in attention and learning abilities; Adults: renal, circulatory and nervous system disorders, high blood pressure
Mercury (Hg)	0.00003	Rheumatoid arthritis, renal, circulatory and nervous system disorders
Nickel (Ni)	0.20	Dermatological disorders, nausea, coughing, chronic asthma, carcinogen for humans
Zinc (Zn)	0.80	Lethargy, depression, increasing thirst, neurological disorders

heavy metals could have various explanations. One important mechanism involves the high affinity of heavy metals for proteins, and more exactly amino acids and –SH groups, because these proteins act as enzyme poisons. In water chemical form of heavy metals is also very important, because complex organic and inorganic compounds are less toxic then ionic metals and simple inorganic compounds [13].

Most of heavy metals are accumulated in fish tissues, negatively affecting the vital processes, reproduction capacities, immune system, inducing severe pathological changes. Based on the results of different experimental researches, fishes can be used such bio-indicators of water pollution, and can play important role in pollution monitoring the heavy metals contamination [14].

Experimental part

Our research experiment was conducted on *Carassius gibelio Bloch* fish, and for lead contamination we used $Pb(NO_3)_2$ x ½ H_2O (S.C. Corozin S.R.L.). To counteract the toxic effect of lead we add into fish's water freeze-dry garlic and powder chlorella (Medica Laboratories) – natural products with demonstrated detoxifying properties, used to evaluate the lead antagonistic effect of other metals.

We selected the fish by gravimetrical measurements (22-25g / fish) and we used a 60L aquarium for each group from experiment with included 30 fishes / group. The aquariums were optimum aerated and the fish were acclimated for two weeks before the experiments just to eliminate any suspicion of using fish with poor or pathological health status. The fish were feed twice a day with fish granulated feed, and they had 12 h of light followed of 12 h of darkness. Water quality was checked using a portable dissolved oxygen meter HI9145 Hanna for dissolved oxygen; Termatest kit for *p*H, NO^-_2, NO^-_3, and water hardness; and thermometer for water temperature. The lead was administrated as 0.075mg/mL of water (as $Pb(NO_3)_2 \times 1/2\ H_2O$) and the circulation was assured by a compressor which circulated the water into aquarium. The natural products as protection of lead contamination for fish were used as 2% of freeze-dry garlic and 2% chlorella incorporated into fishes' feed. After 21 days we add into the aquarium water clove oil for fishes euthanasia, and we sampled gills, muscle, skin (and scales), intestine, liver, heart, brain, ovary, testicles, and kidney.

In the present research paper we analysed only the iron distribution in fish tissues, noted with C-tissues from control experimental group (without any administration or protection); with Pb - tissues from experimental group with lead administration (without any protection); Pb+chlorella -tissues from experimental group with lead administration and chlorella protection; and Pb+garlic – tissues from experimental group with lead administration and freeze-dry garlic protection.

The results for iron concentrations were analytical analysed using atomic absorption spectrometry (AAS) and the data were statistical analysed using ANOVA program.

Results and discussions

Heavy metal contamination in fish did not present any external modification, but there were some synergic and antagonistic effects on metals from different fish tissues. Taking in account this fact, our experiment tested the influence of lead toxicity on iron presented in fish tissues, as an mineral responsible for vital functions (detoxify- liver, kidney; respiration - gills; reproduction - ovary, testicles; digestion- liver, intestine; circulation- heart; nervous and sensitive system -brain and skin). Iron concentration from different fish tissues after lead and natural products administration are presented in figure 1.

Lead influenced the iron concentration in all tested samples. Our experiment demonstrated that lead administration severely affected the iron distribution in liver (liver Fe decreases with 81.73% after Pb administration compared to control), gills (Fe decreases with 75.16% after Pb administration compared to control), intestine (Fe decreases with 74.12% after Pb administration compared to control), ovary (Fe decreases with 73.91 % after Pb administration compared to control), and brain (Fe decreases with 71.75% after Pb administration compared to control). The least affected by lead intoxication was the heart tissue – Fe decreases only with 27.71% after Pb administration compared to control group. Absorption process for iron is most active in small intestine, but liver is the detoxifying organ, and gills are the place where the oxygen has to be taken over by haemoglobin – the iron-protein from blood [6, 15, 16, 17]. Also, in most of the cases, administration of chlorella or garlic protects the iron from organs, being less effective for muscle iron. Table 3 presents the statistical signification for analytical data obtained after AAS analysis of tissue samples of *Carassius gibelio Bloch* fishes after lead administration.

Iron is very important in respiratory process and gills play a very important role in oxygen transport. Lead intoxication reduces the iron from gill tissue, but chlorella acts like a good protector of iron content, while the garlic seems to be a very good natural protector for iron distribution in gills. In muscle tissues chlorella and garlic act similar in lead contamination of fishes, having almost the same protection effect on iron concentration (Fe

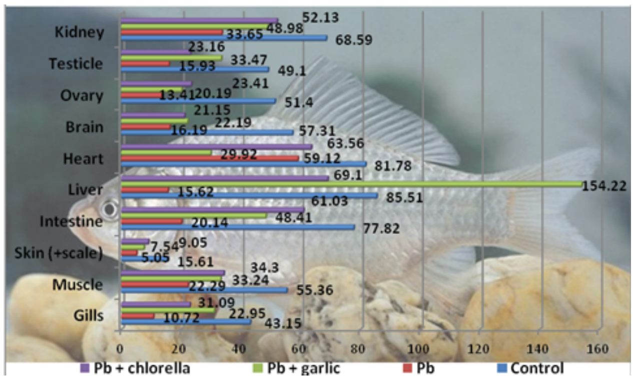

Fig. 1. Influence of lead contamination in water on iron from fish tissues

concentration decrease with 38.04% after chlorella administration and with 39.96% after garlic administration). Skin and scales do not contain too much iron, but in the case of Pb contamination, the Fe concentration is diminished almost to half (51.70% with garlic protection, and 42.02% with chlorella protection), this could be explain also due to the direct contact of contaminated water with the skin and scales.

Iron absorption is made mainly in small intestine, but some metal contaminant can have synergistic or antagonistic effect. Accordingly, lead is an antagonistic metal for iron, diminishing the iron concentration in intestine with 74.12%, but administration of chlorella decreases the iron only with 21.58%, while garlic administration decreases the iron concentration with 37.79%. Liver acts strangely in our experiment because Pb overload severely decreases the Fe concentration (with 81.73%), chlorella administration decreases the iron only with 19.19%, while the garlic administration high significance increases the iron with 80.35% compare to control. This fact could be explained by liver detoxing function, because this organ concentrates on the excess of metals in order to excrete them and adjust the metals homeostasis. Iron-overload in liver could be the result of cellular damage or iron-mediated hepatic injury and alteration of divalent metal transporter 1 [18].

For heart, iron only chlorella administration reduces the lead effect on iron concentration, while garlic accelerates the loss of iron in heart muscle. In cardiac cells, for metabolic normal purposes, most of the iron as bounded of proteins as iron-associated-proteins, being released after transferrin endo-cytolysis, and regulated by uptake/storage mechanism in order to keep the iron homeostasis. This complex process could be disturbed in potential toxic scenarios, when transferrin saturation can exceed 70% with formation of labile iron which enter to circulation arriving to different types of cells through resident channels or transporters, generating various reactive oxygen species, inducing oxidative stress damages. These scenarios could be avoided if the patients receive adequate therapy that include iron-chelation agents, which remove labile and excess iron, protection the heart function against heart failure [19].

Brain is affected by metals' overload because most of these metals are cumulated in brain tissues, with alteration of cerebral function mostly in young patients with cognitive deficits. Thus, lead exposure and toxicity involve neurodegenerative diseases, particularly Alzheimer and Parkinson diseases [20]. Our experimental study reveals antagonistic effect of lead on iron in brain. Iron concentration decreases with 71.75% after lead overload, but chlorella and garlic show little protection of iron content (because Fe decreases with 63.10% after chlorella

Tissue	*Number*	*Sum*	*Average*	*Variance*	*SD*
Fe (mg x kg^{-1} wet weight)					
Gills	4	107.91	26.97	186.32	11.82
Muscle	4	145.19	36.29	190.97	11.967
Skin and scales	4	37.25	9.31	20.34	3.90
Intestine	4	207.40	51.85	592.02	21.07
Liver	4	324.45	81.11	3265.87	49.49
Heart	4	234.38	58.59	461.57	18.60
Brain	4	116.84	29.21	357.79	16.38
Ovary	4	108.41	27.10	279.75	14.48
Testicle	4	121.66	30.41	206.97	12.45
Kidney	4	203.35	50.83	205.22	12.40
Group					
Control ©	10	585.63	58.56	441.30	19.92
Pb	10	212.12	21.21	234.50	20.29
Pb + freeze-dry garlic	10	429.25	42.92	1682.73	21.44
Pb + chlorella	10	379.84	37.98	460.65	13.82
Source of variance		*p*			
Between tissues		$p<0.001$			
Between treatments		$p<0.01$			

Table 3
FISH TISSUE IRON CONCENTRATION – STATISTICAL SIGNIFICATION OF LEAD TOXICITY

protection and 61.28% after garlic administration). Similar data were recorded also for ovary and testicle samples, with a slightly better protection of garlic compared to chlorella in case of iron in testicle.

Excess of metals are regulated through kidney, being excreted in urine. Kidney tissue is very well vascularised and iron has a very important role in renal function [21, 22]. Our experiment showed that lead overload acts antagonistic with iron in kidney, reducing the concentration with 50.94%. But, in fish administration of chlorella and garlic as protective natural products decrease the iron load only with 24% (chlorella) and 28.59% (garlic), acting as a potent agent of renal iron load.

Our experimental study demonstrated that lead contamination in water acts as an inhibitor of iron absorption and metabolism in fish. This complex process could be explained by iron bonding to high affinity proteins, such as blood ferritin and transferrin. Decreases of iron in blood and different tissues negatively influence the hem and haemoglobin biosynthesis, needed for erythrocytes, leading to anemia (anaemia), and serious oxygen deficiency that modify the metabolic processes with alteration of energy and nutrients needs.

Conclusions

Our experiment study reveals important data regarding the lead overload and iron tissue distribution in aquatic organisms - respectively in fish. Obtaining data presents critical antagonistic effect of lead on iron in analysed sample of gills, muscle, skin and scales, intestine, liver, heart, brain, ovary, testicle, and kidney.

Lead decreases significantly the iron concentration in all fish tested samples, acting as an antagonistic metal for iron homeostasis. Administration of chlorella and freeze-dry garlic potent the iron deficiency due to lead contamination for all samples, with the exception of liver where garlic highly increases the iron content in hepatic tissue, and decreases iron content in heart tissue.

Water contamination with lead affects the iron distribution in fish tissues negatively, but active bioactive principles from garlic or chlorella can diminish the antagonistic effect, being a good protector or iron homeostasis.

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