

## RESEARCH ARTICLE

# Toxic metals and autism

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**Abstract:** Toxic metals (MT) are the major pollution to the land (37.3% in Europe), which are produced mainly by industrial activities, entering a far part of our life cycle, through the skin, breathing, ingestion of foods and water. Especially children in the first three years of life are biologically more vulnerable. The spreading epidemic of Autism and developmental disorders, that has been recorded in industrialized countries during the last 20 years is a sensitive thermometer of the pollution. Tests were carried out on 20 MT in a group of 73 subjects diagnosed with autism, the mean age of 8 years old. All 73 patients were over threshold for at least one metal. Seven metals (mercury, lead, antimony, tin, arsenic, thallium, cesium) had values over 50% (thirty-seven patients). The 26% (nineteen patients) exceeded the threshold for ten or more metals. Gadolinium 1521.21 exceeds threshold times. Mercury (x 379.95), tin (x 118), lead (x 108): presenting the coefficients more elevated. The average values exceed the threshold a lot: Mercury (x 28.14), Lead (x 10.56), Tin (x 9.98). With age increases the threshold exceeded for a lower number of metals. The analysis shows that gender has no significant difference, possibly results from the reduced number of females (18%). A future comparison research remains. We will explore the detoxification capacity difference between two genders affected by autism.

**Keywords:** Toxic metals, Autism

Consistent scientific research in the past thirty years shows progressive environmental toxicity signals,<sup>[1-3]</sup> which affect and threaten life on the planet, such as the decrease in the number and variety of plant and animal species and the spread of chronic degenerative diseases in a globalized society. Over 40 years, autism has risen from an incidence of 1 in 2,500 (1975) to 1 in 68, while learning and developmental disorders affecting 1 in 6 children (Center for Disease Control: Surveillance Summaries / April 1, 2016/65 (3); 1-23). The level of toxicity that characterizes every aspect of our daily lives systematically exceeds the analytical limits of the most sophisticated technologies available to us for five basic reasons:

1) The astronomical number of pollutants produced by humans and released to the environment: more than 126 million new substances, organic and inorganic, produced by the industry since 1957; currently, average 12,000

new substances are added each day (www.CAS Registry; home page).

2) The incomplete certification relating to health risks.<sup>[4]</sup>

3) The inability to evaluate the detoxifying power of the individual, against specific toxins.<sup>[5]</sup>

4) The inability to assess over time the biological action summation exerted by different toxic substances in the individual.<sup>[6,7]</sup>

5) The lack of a practice dedicated to the detoxification of metals by the Public Health. *AAA/BBB*

Environmental pollution, food toxicity and drug addiction are largely characterized by the presence of heavy metals (MP).<sup>[8,9]</sup> The Heavy Metals have higher atomic numbers than iron, a density greater than 5 g/cm<sup>3</sup>, and are a common cause of pollution and toxicity in biological organisms.<sup>[10]</sup> The formation of salts behave as cations. They tend to form complex bonds and show great affinity for the sulfides in different oxidation states depending on the pH, they bind proteins in the blood, and then distributed to the different compartments depending on their properties. MP essential at very low doses become toxic at high doses: chromium, manganese, iron, cobalt, copper and zinc.<sup>[11]</sup> Metals toxic anyway (MT) include: beryllium, aluminum, antimony, thallium, tin, uranium, tungsten, nickel, arsenic, cadmium, mercury, lead, uranium, gadolinium.<sup>[12]</sup> Sources Man-made include: electrical installations (Hg, As, Tl),

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vehicles (Cd), diesel engines (Ni), metallurgical industries and foundries (Pb, As, Cu, Al, Co, Zn, Fe), agriculture (Cu, As, Al, Zn), of waste disposal systems (Hg, Cd, Pb, as) paints (Hg, Cd, Pb), insecticides, rodenticides, fungicides (Hg, as, Cd, Ti), cookware (Al, Cu), wood protection products (As, Cu), disinfectants (Cu, Hg, Ag), vaccines (Hg, Al), dental materials (Hg, Al, Au, Ag, Ni), contrast medium for the resonance nuclear magnetic (Gd).<sup>[13]</sup> Since 2003, in EU countries it is applied a protocol for monitoring air emissions of cadmium, lead and mercury and a detailed report of the situation was published in 2007 by a task force of experts selected by European governments, which describes the technologies as available to control the emission of these metals, pointing to plans, strategies and policies to curb emissions within agreed limits. The emissions resulting from industrial activities, traffic of vehicles and from energy production. Between 1990 and 2003, emissions to air in Europe decreased on average by 50%. However, this significant reduction is not able to counteract the continuous and progressive accumulation in soils, where the MT constitute the main pollutant (37.3%).<sup>[14]</sup>

The toxicological research has recently demonstrated the extreme danger of chronic low-dose exposure, because the MT will slowly accumulate in our tissues, which do not participate in any biological functional activity and their action is to block the many complex active enzyme, with cumulative effect and mutual reinforcement over time.<sup>[15]</sup> In particular, the MT inhibit enzymes and molecules which provide antioxidant activity, by blocking the sulfhydryl groups (glutathione, metallothioneins, cysteine, alpha-lipoic acid) with a consequent increase of oxidative stress, alter the ionic homeostasis (calcium, sodium, potassium), mitochondrial function, in a selective manner by interfering with the Krebs cycle and with the methylation-Sulfoxidation circuit.<sup>[16]</sup> It should be noted that by the latter circuit it depends on the synthesis of creatine (for energy charging processes), neuromediators, melatonin, the blockade of histamine (allergic reactions), the modulation of DNA (protein synthesis), synthesis myelin (nerve impulse conduction) and finally the synthesis of cysteine and glutathione (antioxidant and detoxifying activity). In other words, the MT enter our metabolism and profoundly alter, so the formulation of security lapses is always purely conventional.<sup>[17,18]</sup> In the brain, liver, kidney, immune system, bones, lungs, MT create localized inflammatory reactions and generalized reactions with toxic genesis and / or immune, predisposing us to a long series of disorders and diseases.<sup>[19,20]</sup>

The absorption of MT at the gastrointestinal level and in lung level varies depending on the host condition, the

composition (inorganic or organic) and the state of valence (elementary or ionic) of the metal.<sup>[21]</sup> The blood is the primary means of transport of the metals according kinetics dependent on: diffusibility, the form of bonding, speed of processing and bio-availability of intracellular ligands.<sup>[22]</sup> The main streets of metal excretion are renal and biliary-intestinal. Minimally elimination can happen for salivation, sweating, exhalation, breast, skin exfoliation and loss of hair and nails.<sup>[23,24]</sup> Some organs (bone, brain, liver and kidney) sequester certain metals in relatively high concentration and chronically for years.

The absorption of labor origin takes place mainly through the respiratory route; the digestive tract is secondary, and generally linked to poor hygiene measures or accidents.<sup>[25]</sup> The percutaneous absorption has been demonstrated for the hexavalent chromium and certain organo-metallic compounds, such as tetraethyl lead and methylmercury.<sup>[26]</sup> The distribution of metals in the organism can occur in free form or bound to plasma proteins, such as albumin, transferrin, metallothionein, ceruloplasmin, to reach the critical concentration, at which there are early toxic effects.<sup>[27]</sup> Each metal is deposited in a specific way in different organs and tissues based on selective affinity. In general, the metals do not undergo metabolic processes, except that the organometallic forms, such as alkyl lead and the organo-mercurials.

Autism occurs between the first and the third year, children in 90% of cases are free of genetic diseases, but frequent polymorphisms (SNPs) that affect oxidative stress (GSTT1, GSTM1, SOD 2), and the detoxifying capacity (CYP2D6, CYP2C9, CYP2C19, NAT 2).<sup>[28,29]</sup> Clinically, this syndrome multifactorial and multisystemic, appears with a slowing of cognitive behavioral development (isolation, indifference, loss of contact with peers and family members) regression of the word, self behaviors and hetero aggressive and a long line of organic disorders, affecting intestinal function, immune and energy metabolism.<sup>[30]</sup> Since 1995 it is stated in the international arena the multidisciplinary approach for the prevention and treatment of developmental disorders (Defeat Autism Now, DAN!), Which made it possible to investigate the molecular biological and clinical aspects of this syndrome and to start integrated programs and personalized therapy and rehabilitation.<sup>[31,32]</sup> The research and clinical practice that have resulted in multidisciplinary autistic child represented a "successful model", which promoted new diagnostic and therapeutic acquisitions to numerous systemic multi adult chronic diseases.

## 1 Materials and methods

From 2010 to 2016, 73 patients, mainly from central and northern Italy, with a diagnosis of autism, including 13 females (18%) and 60 males (82%), the average age of 8 years (2 to 23 years old), were subjected to a test for the analysis of toxic metals. The test involves the administration of an oral powder DMSA (meso-2-3- dimercaptosuccinic acid) <30 mg/kg of body weight, in a single intake, and in the collection of urine of the next six hours, the end of which is extracted to be as the urine sample, which is analyzed in the laboratory. The dosage of toxic metals is read in micrograms/gram of creatinine. The beds metals include lead (Pb), mercury (Hg), aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), bismuth (Bi), cadmium (Cd), cesium (Cs) gadolinium (Gd), gallium (Ga), nickel (Ni), niobium (Nb), platinum (Pt), rubidium (Rb), tellurium (Tl), thorium (Th), tin (Sn), tungsten (W), uranium (U).

The DMSA is a water soluble molecule, such chelating action, with its two sulfhydryl groups (SH) manifests a great affinity for mercury and lead, cadmium, arsenic, tin, thallium, antimony, uranium and nickel, the lower the affinity with aluminum. It increases the excretion of potassium and chromium. For oral absorption, approximately 20% and the highest values are found in the circulation after 2 to 4 hours, excretion occurs mainly via the urine. In 1991, due to its low toxicity, DMSA has been approved by the FDA (Food and Drug Administration, USA) for the treatment of pediatric poisoning Lead from 1 year old.

## 2 Results

The results are presented through a series of tables and charts (Table 1,2,3,4,5,6; Figure 1,2,3,4).

1. Number of metal for patients: All 73 patients are suprathreshold for at least one metal; Mercury (95.89%) and lead (94.52%) are suprathreshold for the higher number of patients; Seven metals: mercury, lead, antimony, tin, arsenic, thallium, cesium suprathreshold values present in over 50% of patients (37 patients); Three metals are suprathreshold in over 80% of patients: Hg (95.89%); Pb (94.52%); Sb 83.56%); The average theoretical value for the metal suprathreshold patients is equal to 31% (23 patients); Thorium and niobium involving only one patient; No detectable: thorium (98.63%), bismuth (97.26%), niobium (91.78%).

2. Number of patient metals: Nineteen patients (26%) below the threshold for 10 or more metals; The patient 71 exceeds the threshold for 14 metals; The patient 46 exceeds the threshold for only two metals (Hg and U); The theoretical mean value is 38.3%, equivalent to 8 metals

above for patient threshold.

3. Maximum Value / Value Threshold: Gadolinium exceeds the threshold of 1521.21 times; Hg (x379.95), Sn (x118), Pb (x108); Thorium exceeds the threshold of 1.67 times and has the lowest coefficient; Excluding the Gadolinium, the average of the maximum values exceeds the 42% threshold.

4. Medium / Threshold value: The average value of Gadolinium is 254.98 times higher than the threshold; The highest average values: Hg (x28.14), Pb (x10.56), Sn (x9.98); The average theoretical value for the 19 metals is 4 times higher than the threshold; The lowest coefficient is observed for the Cadmium, which is lower than the threshold.

It is a statistical model which is estimated to relate the number of metals that exceed the threshold with the age of patients. It appears to be an inverse relationship between the two variables, ie with increasing age the threshold is exceeded for a lower number of metals. This statistical model (Poisson model) is having a very good fit to the data (P value = 0.9038). The coefficient for the years made variable = 0.2157, P value = 0.031.

The analysis in relation to the gender did not produce any meaningful result, possibly also in relation to the small number of females.

The correlations between pairs of metals have a double interest: environmental and metabolic.

The Table 1 shows the associations between metals, which were statistically more significant.

**Table 1.** The associations between metals

Associated metals	r value <sup>1</sup>	P value
Bi Th	r=0.6922	p=0.0000**
Rb Tl	r=0.6503	p=0.0000**
Rb Cs	r=0.5588	p=0.0000**
Cs Tl	r=0.5554	p=0.0000**
Sb Sn	r=0.4812	p=0.0000**
Al Ba	r=0.4697	p=0.0000**
Pb Tl	r=0.4138	p=0.0003**
Sb Cd	r=0.3866	p=0.0007**
Hg Sn	r=0.3806	p=0.0009**
Cd Sn	r=0.3783	p=0.0010**

Note:\*\*P<0.001 \*P<0.05

The Spearman r value also allows to work on very low numerosity, as in the case of the correlation between Bi and Th, with very few detectable observations. The corre-

lations between the seven metals that are suprathreshold in over 50% of patients, include in descending order for metal couple:

- Pb con: Tl, As, Hg, Cs.
- Hg con: Sn, Al, As.
- Sb con: Sn, Cd, As, Ni.
- As con: Cs, Cd, Sn, Tl, Ni.
- Cs con: Tl.

The pervasive developmental disorder is a heterogeneous group of neurodevelopmental conditions that are manifested at an early age, characterized by difficulties in social interaction and communication and the presence of restricted and repetitive behaviors and interests. These disorders debut in the early years of life, but they involve the whole mental development of the individual. The terminology used by the new diagnostic classification of the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-S) (American Psychiatric Association, 2013) refers to a dimensional view of disease that appears at the ideal moment to represent the continuity between autism and other disorders included in the same category. According to this perspective, the symptoms of Autism Spectrum Disorder would represent the final extreme, with pathological significance, of a continuum of difficulties which may also be highlighted within the general population.

The most recent interpretations attribute a fundamental role to autism in neurophysiological and genetic factors. This interpretive approach has been gradually replacing the initial psychodynamic and environmental hypotheses, mainly incurred by the authors to psychodynamic.

Compared to the etiology of autism, the theory commonly accepted today is that genetic and environmental elements act in the early stages of child development, during pregnancy, or during the first years of life. The current hypothesis is that autism are involved no fewer than three and no more fifteen to twenty genes. Each of these acts as a risk factor, which is due to the onset of the disease, unless there are other risk factors (genetic or environmental, favoring expression of genes "sick"). According to this theory, in the general population there exist many normal subjects, carriers of genes predisposing to autism, who have not developed Autism, because there have been no other "precipitating factors".

The first evidence of a genetic basis of autism comes from the observation that identical twins have a much higher chance (from 36% to 90% according to various studies) of disease recurrence compared to dizygotic twins. Except for a minority of cases (less than 10%), in which autism was associated with chromosomal abnormalities or diseases in Mendelian transmis-

sion, for the remaining 90% of cases the most likely pattern of inheritance is not what monogenic, but a multi-locus model, where multiple genes contribute to predisposition to the disorder. The complexity of the picture, adding the influence of environmental factors. Among the biochemical factors which appear to be mainly involved in the genesis of autism are the dopaminergic, serotonergic and noradrenergic; glial protein and ganglioside; brain metabolism (Ciaccio et al., Conference A.G.S.A.S.2003). The alteration of these systems leads to an alteration of neuromodulators, which leads to an alteration of neurotransmission. Among the neuromodulators involved is dopamine, serotonin, the gaba, glutamate, glycine. The dopamine system is invoked in having a role in autism because functions such as perception, attention, etc., which are regulated by dopamine, are compromised in individuals with autism; Furthermore, because dopamine agonists, such as amphetamines, aggravate the symptoms; Finally, because neuroleptic drugs with a competitive mechanism on specific receptors for dopamine, causing an improvement in the symptomatology. Instead, Lambiase (2004) states that a deficiency of serotonin in the frontal lobes may be related to impulsivity, and obsessive-compulsive symptoms may be related to a deficiency of serotonin in the basal ganglia.

On the relationship between serotonin and autism it is based on the theory of De Long (1999). This theory assumes two types of autism: 1. The first is characterized by an early brain damage, usually of the temporal lobes, predominantly of the side, which prevents the achievement of the basic semantic structures of language, social skills and intentional activities organized. These children can not build a structure of meaning and are usually low-functioning. In this case of autism with bilateral temporal sclerosis post epileptic, those of herpes simplex encephalitis, infantile spasms, tuberous sclerosis with thunderstorms tubers, cases of congenital rubella, etc.

The second group is not associated with brain damage. This would be the most common idiopathic form and not accompanied by neurological signs or alterations of any kind to the instrumental investigations. Usually, it has a family-based, or genetic roots. Initially, it begins with phenomena of regression in the second year of life, there is some language development and cognitive activities with some normal functions islands, while the affective symptoms are prominent. The prognosis would be better than the first group.

Other studies have been conducted in neurobiology and showed the existence of deficits at the level of the limbic system, cerebellum, hippocampus, amygdala, frontal lobe and cerebellum.

Recent studies have focused on the role of mirror neurons. Between the 80s and 90s Welsh and Rizzolatti discovered the existence of mirror neurons in monkeys. These neurons are activated in the moment in which the individual performs an action or sees someone else perform an action allowing the understanding of the action or the "ability to recognize that an individual is performing an action, this action to differentiate from another similar to it, and to use this information in order to act in such a way appropriate" (Welsh, 1996, p.606). In 1995 Fogassi, Welsh et al., we demonstrated the presence in humans. These neurons are involved not only in the moment in which an individual observes an action implemented by another person but also when observing the expression of emotion.

Again the data are controversial. The research that questions the report is conducted by a group of researchers at New York University (2010) who published a paper in *Neuron* in 2010. In the paper they have refuted the hypothesis of a link with autism-mirror neurons demonstrating an experiment that autistic people do not show differences compared to the control group.

Today we can say that the causes of autism, despite the proliferation of various theories about them, are unknown. I think it's anachronistic reference to the work of Wakefield because it is well established that there is no correlation between the trivalent vaccine and autism. In fact in the journal *Journal of the American Medical Association (JAMA)*: April 21, 2015-313 (15) 1534-40, the MMR (measles, rubella, anti, anti mumps) was not found associated with an increased risk of the disorder autism spectrum, after a study of 95,000 children with autistic siblings more.

The conclusion is therefore to be found in the epidemiology of autism spectrum disorders when it can be stated that: the pathologies autism spectrum represent an example of multifactorial disease, ie a situation of disease that when it comes to producing a genetic predisposition meets with noxious stimuli which may be of chemical nature, a physical nature, of immunologic origin or other origin, for example infectious.

Figure 2 shows: Seven metals are suprathreshold in 20% of patients: Ni (17.81); Ga (8.22); Cd (5.48); Gd (4.11); Bi (2.74); Nb 1.37); Th (1.37); Fourteen metals are suprathreshold in 50% of patients: Cs (53.42); Rb (46.58); W (43.84); At (35.62); Pt (30.14); U (26.03); Ba (23.29); Ni (17.81); Ga (8.22); Cd (5.48); Gd (4.11); Bi (2.74); Nb 1.37); Th (1.37); Seven metals are suprathreshold in over 50% of patients: Hg (95.89); Pb (94.52); Sb 83.56); Sn 79.45; As 57.53; Tl 54.79; Cs 53.42); Three metals are suprathreshold in over 80% of patients: Hg (95.89); Pb (94.52); Sb 83.56).

**Table 2.** Percentage of patients above the threshold for metal

Metal	Patients above threshold
Pb	94.52%
Hg	95.89%
Al	35.62%
Sb	83.56%
As	57.53%
Ba	23.29%
Bi	2.74%
Cd	5.48%
Cs	53.42%
Gd	4.11%
Ga	8.22%
Ni	17.81%
Nb	1.37%
Pt	30.14%
Rb	46.58%
Tl	54.79%
Th	1.37%
Sn	79.45%
W	43.84%
U	26.03%

### 3 Conclusion

MT is the major pollutant to the environment we live, which are the result of industrial and market activities. MT affect every aspect of our daily lives, as a permanent tax on health, starting from conception. Thermometer sensitive for this condition is the child population, with particular regard to the first three years, in relation to the fragility, complexity, dynamism, that characterize this stage of development. MT and developmental disorders are two antibiological sensitive marker of the quality of the environment in which we live. In Italy and Europe health care is long overdue on both fronts. The MT is treated from a few dozen "alternative" doctors in private practice and autistic children under the care of a Neuropsychiatry, pronouncing the verdict incurable and simultaneously denies the multidisciplinary dialogue with the biological person-centered medicine. The current trend in the epidemic of autism (1 child in 50) and developmental disorders (1 in 6 children) is an absolute alarm to our species, when the statistical confirmation projection that over 30 years the autism affect half of the child population. Toxic Metals source ia-

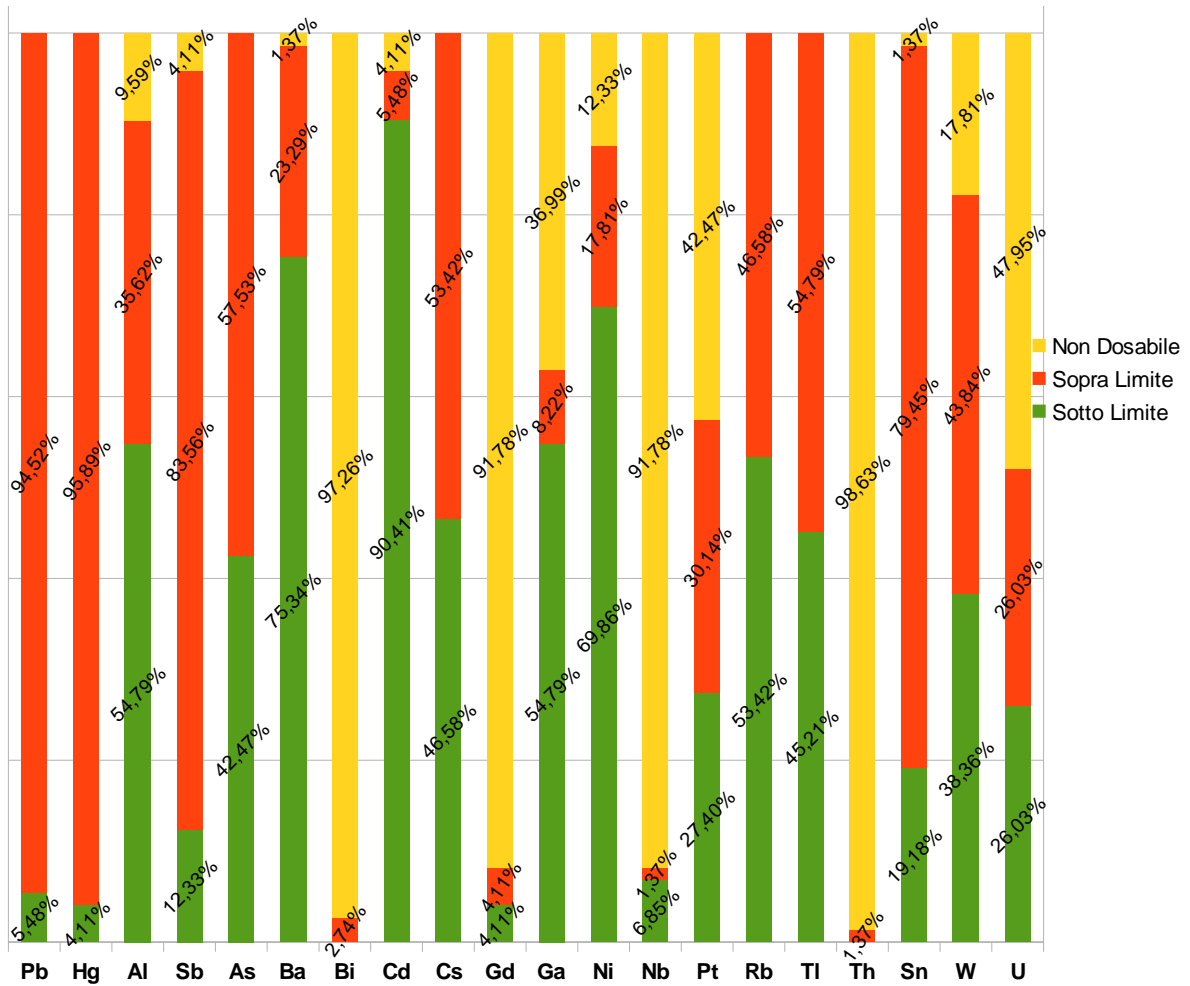


Figure 1. Records for each metal, the percentage of patients

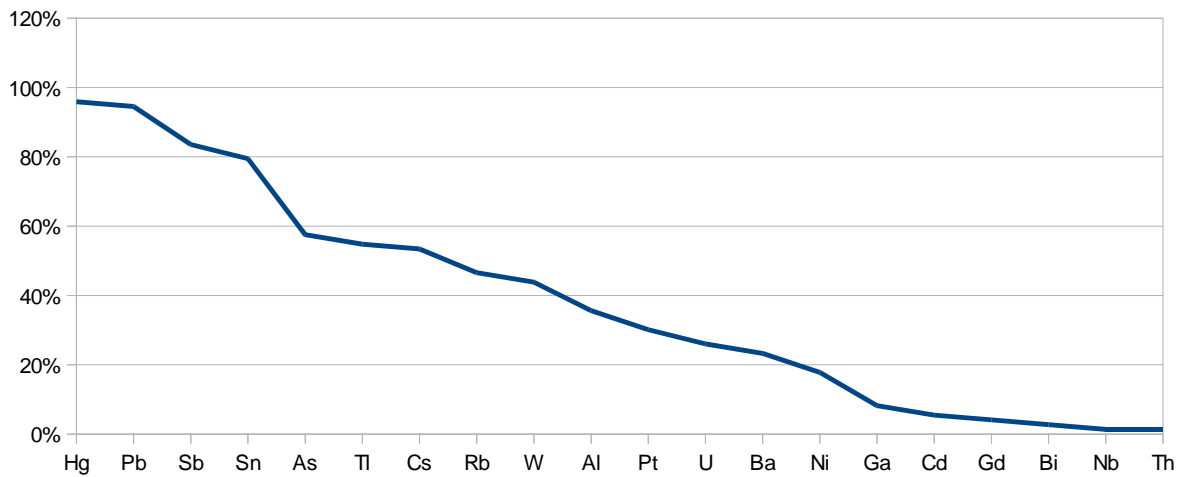


Figure 2. Percentage of patients suprathreshold for each metal

trogenic include vaccines (ethyl mercury and aluminum) and dental amalgams (mercury, silver and tin), affecting

a large percentage of the population over age 35 and are properly removed in a narrow minority of cases from a



**Table 3.** Percentage of metals above for patient threshold

Case	Suprathreshold metals	Case	Suprathreshold metals
Patient 1	30%	Patient 38	55%
Patient 2	40%	Patient 39	30%
Patient 3	15%	Patient 40	55%
Patient 4	45%	Patient 41	55%
Patient 5	35%	Patient 42	40%
Patient 6	45%	Patient 43	45%
Patient 7	55%	Patient 44	20%
Patient 8	30%	Patient 45	35%
Patient 9	35%	Patient 46	10%
Patient 10	50%	Patient 47	45%
Patient 11	35%	Patient 48	45%
Patient 12	20%	Patient 49	45%
Patient 13	40%	Patient 50	15%
Patient 14	30%	Patient 51	30%
Patient 15	50%	Patient 52	35%
Patient 16	55%	Patient 53	25%
Patient 17	25%	Patient 54	20%
Patient 18	35%	Patient 55	45%
Patient 19	40%	Patient 56	60%
Patient 20	30%	Patient 57	55%
Patient 21	25%	Patient 58	35%
Patient 22	50%	Patient 59	35%
Patient 23	50%	Patient 60	50%
Patient 24	60%	Patient 61	30%
Patient 25	25%	Patient 62	20%
Patient 26	50%	Patient 63	40%
Patient 27	25%	Patient 64	45%
Patient 28	40%	Patient 65	25%
Patient 29	30%	Patient 66	45%
Patient 30	50%	Patient 67	45%
Patient 31	30%	Patient 68	50%
Patient 32	40%	Patient 69	30%
Patient 33	60%	Patient 70	30%
Patient 34	25%	Patient 71	70%
Patient 35	35%	Patient 72	45%
Patient 36	30%	Patient 73	50%
Patient 37	20%		

Note: All patients appear to be above the threshold for at least a metal; 19 patients (26%) exceeded the threshold for 10 or more metals; The patient 71 exceeds the threshold for 14 metals; The patient 46 exceeds the threshold for only two metals (Hg and U); The theoretical mean value is 38.3%, equivalent to 8 metals above for patient threshold.

few dozen dentists. The gadolinium as a contrast agent for Magnetic Resonance Imaging, is deposited in tissues and its removal requires a custom chelation therapy protocol, which is systematically ignored by the Neurology and Neuroradiology and that no public institute now offers.

In conclusion, toxic metals and autism promote a new medical approach strategy. Not the diagnosis, but the

**Table 4.** The maximum value and times exceeds the threshold

Metal	Max value	Times ( the max value exceeds threshold)
Pb	151.200	108.000
Hg	832.080	379.950
Al	629.200	28.220
Sb	6.814	45.730
As	700.000	14.000
Ba	57.900	8.640
Bi	9.620	4.220
Cd	3.810	5.950
Cs	44.800	4.270
Gd	28.903	1521.210
Ga	0.081	2.890
Ni	16.740	4.310
Nb	0.303	3.610
Pt	0.684	20.730
Rb	11.013	4.870
Tl	3.776	12.670
Th	6.991	1.670
Sn	240.730	118.000
W	3.304	15.660
U	0.167	6.420

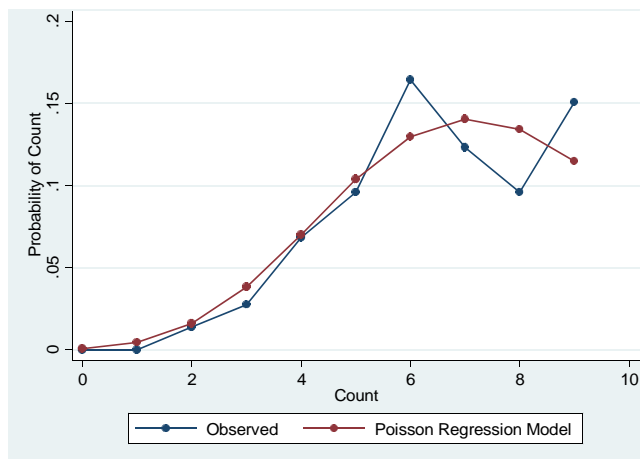
Note: The gadolinium exceeds the threshold of 1521.21 times; Excluding gadolinium, the average value exceeds the 42% threshold; Hg (x379.95), Sn (x118); Pb (x108): these are the metals with the highest coefficients; The thorium exceeds the threshold of 1.67 times and has the lowest coefficient; Below the corresponding graph, in which we have excluded the gadolinium, because its insertion, with the exceptionality of the result, flattens the reliefs, as significant, the other metals.

**Table 5.** The relationship between metals and threshold

Metal	Average value	Times (the average value exceeds threshold)
Pb	14.786	10.56
Hg	61.624	28.14
Al	37.597	1.69
Sb	0.601	4.03
As	88.565	1.77
Ba	5.946	0.89
Bi	7.860	3.45
Cd	0.306	0.48
Cs	11.317	1.08
Gd	4.845	254.98
Ga	0.016	0.58
Ni	3.108	0.80
Nb	0.092	1.10
Pt	0.070	2.11
Rb	2.619	1.16
Tl	0.487	1.63
Th	6.991	1.67
Sn	20.353	9.98
W	0.396	1.88
U	0.044	1.68

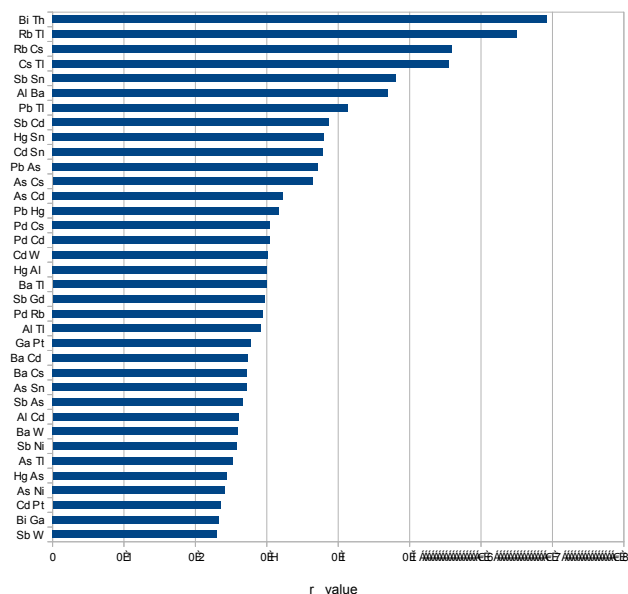
Note: The average value of gadolinium is 254.98 times higher than the threshold; The highest average values: Hg (x28.14); Pb (x10.56); Sn (x9.98); The average theoretical value for the 19 metals is 4 times higher than the threshold; The lowest coefficient is observed for cadmium, which is lower than the threshold.

patient is at the center of the investigation, as the only real evidence, that science and conscience are called to



**Figure 3.** Poisson regression model

Note: Spearman test



**Figure 4.** Correlation between metals

Note: 1. The statistical model is estimated to evaluate the number of metals that exceed the threshold with the age of patients. It appears to be an inverse relationship between the two variables. This statistical model (Poisson model) has a very good fit to the data (P value = 0.9038); 2. Coefficient for years is 0.2157; P value = 0.031; 3. The analysis in relation to the gender did not produce any meaningful result, possibly also in relation to the small number of females.

investigate, according to an authentic approach multidisciplinary.

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**Table 6.** Correlations between pairs of metals

Associated metals	r value <sup>1</sup>	P value
Pb Hg	r= 0.3169	p= 0.0063**
Pb As	r= 0.3719	p= 0.0012**
Pb Cd	r= 0.3042	p= 0.0089**
Pb Cs	r= 0.3042	p= 0.0089**
Pb Tl	r= 0.4138	p= 0.0003**
Hg Al	r= 0.3001	p= 0.0099**
Hg As	r= 0.2435	p= 0.0379*
Hg Sn	r= 0.3806	p= 0.0009**
Al Ba	r= 0.4697	p= 0.0000**
Al Cd	r= 0.2605	p= 0.0260*
Al Tl	r= 0.2923	p= 0.0121*
Sb As	r= 0.2662	p= 0.0228*
Sb Cd	r= 0.3866	p= 0.0007**
Sb Gd	r=-0.2968	p= 0.0108*
Sb Ni	r= 0.2576	p= 0.0278*
Sb Sn	r= 0.4812	p= 0.0000**
Sb W	r= 0.2307	p= 0.0497*
As Cd	r= 0.3223	p= 0.0054**
As Cs	r= 0.3639	p= 0.0016**
As Ni	r= 0.2412	p= 0.0398*
As Tl	r= 0.2522	p= 0.0314*
As Sn	r= 0.2725	p= 0.0197*
Ba Cd	r= 0.2738	p= 0.0191*
Ba Cs	r= 0.2725	p= 0.0197*
Ba Tl	r= 0.2995	p= 0.0101*
Ba W	r= 0.2593	p= 0.0268*
Bi Ga	r= 0.2329	p= 0.0474*
Bi Th	r= 0.6922	p= 0.0000**
Cd Pt	r= 0.2351	p= 0.0452*
Cd Sn	r= 0.3783	p= 0.0010**
Cd W	r= 0.3015	p= 0.0095**
Pd Rb	r= 0.2949	p= 0.0113*
Cs Tl	r= 0.5554	p= 0.0000**
Ga Pt	r= 0.2774	p= 0.0175*
Rb Cs	r= 0.5588	p= 0.0000**
Rb Tl	r= 0.6503	p= 0.0000**

Note: 1. Spearman test; 2. \*\*P<0.001 \*P<0.05

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