Journal of Elementology ISSN 1644-2296

Fiłon J., Ustymowicz-Farbiszewska J., Karczewski J., Żendzian-Piotrowska M. 2017. *Analysis of trace element content in hair of autistic children.* J. Elem., 22(4): 1285-1293. DOI: 10.5601/jelem.2016.21.4.1355

ORIGINAL PAPER

ANALYSIS OF TRACE ELEMENT CONTENT IN HAIR OF AUTISTIC CHILDREN*

Joanna Fiłon[1], Jolanta Ustymowicz- Farbiszewska[1], Jan Karczewski[2], Małgorzata Żendzian-Piotrowska[1]

**[1]Department of Hygiene, Epidemiology and Ergonomics
Medical University of Bialystok, Poland
[2]Department of Public Health
State Higher School in Biała Podlaska, Poland**

Abstract

Determination of the pathogenesis and treatment of autism spectrum disorders is one of the major challenges for modern scientists. The main pathogenic factors implicated in these disorders are epigenetic changes caused by environmental factors, which include toxic metals (Cd, Pb, Hg, Mn, Al and As). Also, the nervous system may be affected by the deficiency of micro- and macroelements (e.g. Zn and Mg). The aim of the work was to analyze the content of particular trace elements in the hair of autistic children. The study material comprised samples of hair collected from both autistic children (study group) and children chosen randomly from general population (control group). The concentrations of Mg, Zn, Cd and Mn were measured by electron scanning microscopy on a Hitachi TM-3000 apparatus, and by X-ray EDS microanalysis. The content of trace elements in hair, mutual interactions between them and their impact on the development of autism in children were evaluated. The results were statistically analyzed with the use of Statistica PL 12.5 software, including the Mann-Whitney U test, Wilcoxon matched-pair test and R-Spearman correlation. According to the study, children diagnosed with autism suffer from both Zn deficiency and toxic metal burden (Cd and Mn). The changes observed in Mg level were statistically insignificant. These abnormalities, as an environmental factor, may play a pivotal role in the pathogenesis of this disease.

Keywords: cadmium, zinc, manganese, magnesium, children, autism, hair.

Joanna Fiłon, PhD, Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Mickiewicza 2c, 15-089 Białystok, Poland, e-mail: joanna@umb.edu.pl
* The study was supported by the Medical University of Bialystok (Project No. ANZ-0604-153--02624/15).

INTRODUCTION

Autism develops in 62 out of 10 000 children and is a syndrome of neurotic disorders characterized by poor non-verbal communication and social interaction as well as the presence of restrictive and repetitive types of behavior. Autism related disorders are genetic, yet both lifestyle and environmental aspects are also mentioned among a number of risk factors. Epigenetic changes in gene expression caused by environmental factors are considered as key ones in the pathogenesis of many diseases including autism (Yasuda et al. 2011, 2013, Priya, Geetha 2011, Tabatadze et al. 2015).

Compared to adults, children tend to be more exposed to environmental toxins as a consequence of their behaviour, in addition to which they have higher absorption rates, lower detoxification abilities and xenobiotic elimination capability. Metabolic pathways in fetuses and infants are immature. In their bodies, particular toxic substances may transform into active forms. Also, poorly developed mechanisms of renal excretion in small children combined with a concurrent underdevelopment of biotransformative mechanisms cause high sensitivity to poisoning with both medications and substances present in the environment. Many studies have confirmed the relationship between heavy metal toxicity and neurobehavioral disorders (Lonsdale et al. 2002, Adams, Romdalvik 2004, Al-Ayadhi 2005, Grandjean, Landrigan 2006, Priya, Geetha 2011, Blaurock-Busch et al. 2012, Yasuda et al. 2013).

A developing brain is highly sensitive to chemical agents. In prenatal and early postnatal periods, this organ is in the phase of fast growth and even a slight exposure to toxic environmental factors may cause further impairment of cognitive functions. There is a list of 201 industrial chemical agents showing neurotoxic effects on humans. They may cause autism as well as other disorders such as a deficit of concentration, mental retardation and cerebral palsy (Walsh et al. 2001, Serajee et al. 2004, Fido, Al-Saad 2005, Priya, Geetha 2011, Yasuda et al. 2011, 2013).

The aim of the work was to analyze the content of particular trace elements in the hair of autistic children.

MATERIAL AND METHODS

The material consisted of hair samples taken from children from urban areas. The research group consisted of 30 children with autism (25 boys and 5 girls) aged 2 to 8 years (mean age of 5.250 ± 1.586 years) attending randomly selected therapeutic kindergartens in Bialystok. The control group consisted of 30 formally healthy children (25 boys and 5 girls, children with chronic diseases and any illness symptoms were excluded) aged 2 to 8 years (mean age of 5.094 ± 1.510 years), randomly selected from the general population of Bialystok and the surrounding area. In both study and control groups, hair samples were collected from six different areas of the head. Each hair sample of a mass of 0.2 - 0.5 g consisted of 3-cm-long parts (from the scalp). Concentrations of Mg, Zn, Cd and Mn were tested by electron scanning microscopy on a Hitachi TM -3000 apparatus with X-ray EDS microanalysis. The accuracy of this method was verified on certified reference material GBW 09101 Human Hair (China).

An assessment of the content of the elements studied in hair and their impact on the development of autism in children was performed and the correlations between the concentrations of the trace elements in hair were evaluated.

The results for each child were averaged and subjected to statistical analysis using Statistica PL 12.5 for Windows (Copyright© StatSoft, Inc. 1984-2014) software. The analysis included calculations of mean, standard deviation, minimum and maximum and medians as well as the values of 10^{th} and 90^{th} percentile of the elements in hair. Prior to analysis, the evaluation of normal distribution was carried out with the Shapiro-Wilk test and Kołmogorow-Smirnow test. Next, the homogeneity of variance with the use of F test and Levene's test was examined. The results of the analysis of the content of elements in hair were submitted to the Mann-Whitney U test and Wilcoxon matched-pair test. Also, the correlations between the studied variables were evaluated with the Spearman rank correlation coefficient (R). The statistical significance was considered $p \leq 0.050$. The Bioethics Committee of Medical University of Bialystok issued approval number: R-I-002/18/2015.

RESULTS AND DISCUSSION

Magnesium plays a role in the normal function of the brain and nervous system. This element decreases cell activity in the nervous system and shows a calming effect. The symptoms of Mg deficiency include: anxiety, depression, increased susceptibility to stress, hyperactivity, irritability, nervousness, aggression, psychomotor hyperactivity, concentration disorders and memory disorders (Starobrat-Hermelin, Kozielec 1997, Mousain-Bose et al. 2004, Priya, Geetha, 2011, Yasuda et al. 2011, 2013, Tabatadze et al. 2015).

A low level of Mg may cause a decreased ability and function of the ATP/ ADP system and impede the processes of homocysteine methylation (characteristic in autistic children). A high concentration of homocysteine, which plays a crucial role in the development of neurodegenerative disorders, causes a decrease in genome transcription and plasticity of synapses by the impaired synthesis of neurotransmitters such as: dopamine, noradrenaline and serotonin. According to research, as well as showing a positive impact on persons suffering from autism and ADHD, a diet high in Mg improves concentration and reduces aggressive behaviors in children (Starobrat-Hermelin, Kozielec 1997, Mousain-Bose et al. 2004, Priya, Geetha, 2011, Konikowska et al. 2012).

The mean concentration of Mg in the hair of the studied children was 29.31 ± 10.79 mg kg^{-1}, thus being lower than the mean content of this element in the control group (35.67 ± 18.52 mg kg^{-1}), statistically insignificant differences – Figure 1.

Apart from the Mg deficiency, a number of autistic children also show the deficiency of Zn and Ca. Moreover, a relationship between the deficiency of Zn and Mg has been observed. Every fourth child suffers from the deficiency of both elements concurrently (Yasuda et al. 2011, 2013, Tabatadze et al. 2015).

Although Zn may be an environmental pollutant as well as the cause of sideroblastic anemia in cases of high exposure, it is an essential trace element for human beings. Zn is vital in the synthesis of nucleic acids, cell replication as well as tissue growth and repair, especially in infants. Therefore, Zn deficiency leads to: taste disorders, poor wound healing, immunity disorders, delayed growth and neurodegenerative disorders. Meat and fish proteins are the main sources of this element (Yasuda et al. 2011, Konikowska et al. 2012, Gogia, Sachdev 2012, Yasuda et al. 2013, Gonzalez-Reimers et al. 2016). Also, Zn deficiency results in high absorption of toxic metals such as Cd and Pb, and enhances the formation of their deposits in the body. Deficiency of Zn and Mg combined with an increased burden of toxic metals may play the main epigenetic role in the pathogenesis of diseases from the autism spectrum (Akhondzadeh et al. 2004, Yasuda et al. 2011, Gogia, Sachdev 2012, Yasuda et al. 2013, Tabatadze et al. 2015).

The mean Zn content in the hair of children without neurological disorders was 149.66 ± 42.56 mg kg^{-1} and was statistically higher than the mean content of this element in the hair of autistic children (99.93 ± 67.50 mg kg^{-1}) – Figure 1.

Cd is a xenobiotic of high toxicity. It has neurotoxic, hepatotoxic, mutagenic, carcinogenic and teratogenic effects (it is included by the IARC – The International Agency for Research on Cancer into the group 1 of agents of a confirmed carcinogenic effect on humans). Moreover, Cd impairs metabolism of Ca, Mg, Fe, Zn, Se and Cu, which causes morphological and functional changes in particular organs. Cd is absorbed into a human body mainly through the digestive tract and respiratory system. As regards Cd in food in the digestive tract, it is absorbed in approximately 6% in adults and up to 60% in children (Al-Ayadhi 2005, ATSDR 2012, Sanders et al. 2012, Yasuda et al. 2013, Tabatadze et al. 2015).

A three-fold higher mean Cd content in the hair of autistic children compared to the mean Cd content in the control group's hair was observed (0.224 ± 0.114 mg kg^{-1} and 0.063 ± 0.028 mg kg^{-1} respectively) (statistically insignificant differences) – Figure 1.

Mn is a microelement vital for life functions. As a component of active

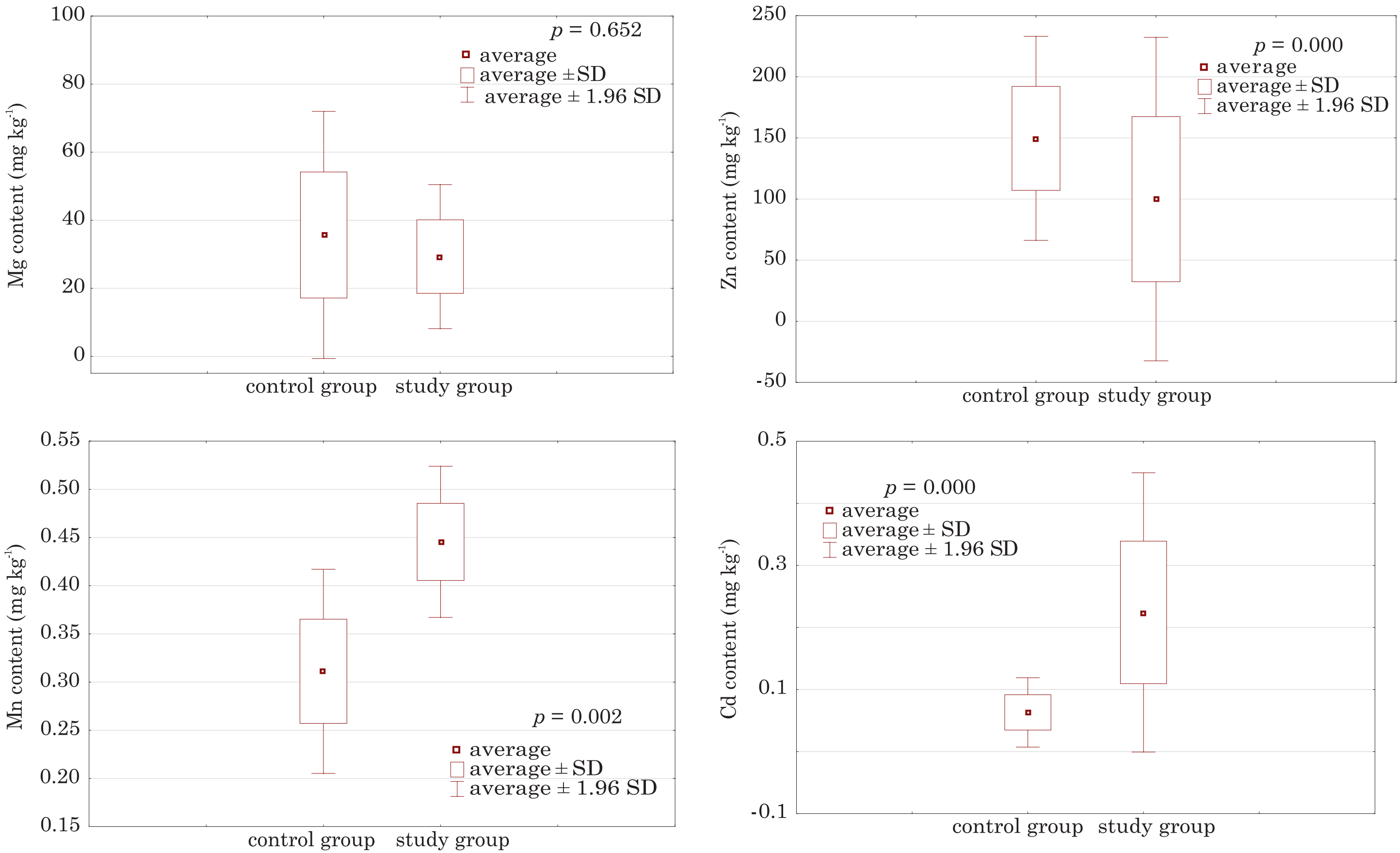

Fig.1. Relationships between the content of the elements in the hair of the study group and control group (mg kg^{-1})

enzymes, and it participates in the metabolism of amino acids, proteins and fats. However, its excess may have a toxic effect on nerve tissue. According to previous studies, exposure to Mn dust at workplace and environment correlates with neurological issues in children and adults. Chronic exposure to Mn compounds leads to the development of Parkinson's syndrome. Its major symptoms include neurological disorders mostly related to the damage to extrapyramidal system (limb tremor, cogwheel phenomen, hypersalivation), pyramidal system (myoclonus, pathologic Babinski reflex) as well as bulbar lesions (swallowing disorders, dysarthria). These changes are accompanied by psychiatric symptoms called 'Mangan madness', which include increased psychomotor activity, impulsiveness, emotional lability and increased talkativeness. Psychiatric symptoms may be indicative of the damage to the areas located in the frontal lobe (apart from the pyramidal lobe). Also, Mn deteriorates the absorption of particular microelements in the digestive track, Ca, Zn, Cu, K (Takeda 2003, Al-Ayadhi 2005, Zawadzki et al. 2008, Sanders et al. 2012, Tabatadze et al. 2015).

According to own research, a statistically higher (Figure 1) mean Mn content in the studied children compared to the control group was observed (0.446 ± 0.219 mg kg^{-1} and 0.311 ± 0.296 mg kg^{-1} respectively).

Exposure in modern times is a complex problem. Interactions may occur between individual elements. Toxic metals affect trace element absorption, and the interaction between essential elements and toxic metals affects threshold values and toxicity effects. The toxic metals may interact metabolically with nutritionally essential metals. The interaction between Cd and Zn is related to the competition for binding of metallothionein. It has been observed that the increased supply of zinc in diet reduce the absorption of Cd. Oversupply of Mn reduces the absorption of Zn, Ca and Mg, also Mg deficiency increases the absorption of Zn and Cd. (Brzóska, Moniuszko-Jakoniuk 2001, Al-Ayadhi 2005, Sanders et al. 2012, Blaurock-Busch et al. 2012, Tabatadze et al. 2015).

In own research, statistically insignificant weak positive correlations between Mg and Mn (r = 0.222) as well as Cd and Mn (r = 0.146) and negative ones between Mg and Zn (r = -0.292) were observed in the hair of the studied children. The correlation analysis between analogous elements in healthy children revealed reverse values, i.e. weak negative correlations between Mg and Mn content (r = - 0.159) and Cd and Mn (r = - 0.114) as well as positive ones between Mg and Zn (r = 0.108) – Table 1.

The correlation coefficients (from r = - 0.271 to r = 0.248) also indicated a distinct *albeit* low correlation between the amounts of the studied elements in children from both study and control groups (Table 1). Statistically insignificant negative correlations were observed between the Mg, Zn and Mn content and positive ones appeared between the Cd level in the hair of study group and control group.

Furthermore, a statistically significant relationship between the level of

Table 1

Correlation between the content of the elements in the hair of the study group and control group

Group		Study group				Control group			
		Mg	Zn	Cd	Mn	Mg	Zn	Cd	Mn
Study group	Mg	-	- 0.292	0.032	0.222	- 0.271			
	Zn	- 0.292	-	0.005	- 0.093		- 0.020		
	Cd	0.032	0.005	-	0.146			0.248	
	Mn	0.222	- 0.093	0.146	-				- 0.016
Control group	Mg	- 0.271				-	0.108	0.159	- 0.159
	Zn		- 0.020			0.108	-	0.027	- 0.078
	Cd			0.248		0.159	0.027	-	- 0.114
	Mn				- 0.016	- 0.159	- 0.078	- 0.114	-

the studied elements in the hair of children from the studied and control group was found.

The present results are supported by the study of YASUDA et al. (2011, 2013) who detected Zn deficiency in 29.70% of children. The mean concentration of this element in the studied group was 129.0 ppm. Mg deficiency was observed in 17.60% of children while 2.000% of children were diagnosed with the deficiency of other elements, including Mn or Cu. The mean Mg concentration in the study group was 39.50 ppm. The quoted authors also showed an increased Cd concentration in 8.500% of autistic children. The mean Cd concentration was 782.0 ppm. Approximate content of trace elements in the hair was measured by authors from Arabic countries (AL-AYADHI 2005, BLAUROCK-BUSCH 2011), India (PRIYA, GEETHA 2011), Egypt (ELSHESHTAWY et al. 2011) and the USA (VAGHRI 2008, 2011, TABATADZE et al. 2015).

Many authors also observed that Mg and Zn levels were significantly decreased ($p < 0.001$) in autistic children when compared to controls. This indicates that children with autism are prone to deficiency of these elements (AL-AYADHI 2005, PRIYA, GEETHA 2011, BLAUROCK-BUSCH 2011, 2012, YASUDA et al. 2011, 2013, TABATADZE et al. 2015). FABER et al. (2009) confirm that the shortage of Zn is very common among children with autism.

Our study supports the historic evidence that heavy metals play a role in the development of autism. LONSDALE et al. (2002), AL-AYADHI (2005) and BLAUROCK-BUSCH (2011, 2012) also observed significantly higher levels of toxic heavy metals (e.g. Cd) in the hair of children with autistic spectrum disorders as compared to healthy children.

CONCLUSIONS

1. A statistically significantly higher Cd and Mn content and a decreased Zn content in the hair of autistic children compared to the control group were observed.

2. The decrease in the Mg content in the autistic children's hair compared to the control group was statistically insignificant.

3. Abnormal concentrations of the studied elements may be indicative of a pathophysiological role of heavy metals and trace elements in the occurrence of autism.

REFERENCES

Adams J.B., Romdalvik J. 2004 *Arizona State University: Autism Baby Hair Study*. http://www.bridges4kids.org/articles/8-04/AZ7-04.html

Akhondzadeh S., Mohammadi M.R., Khademi M. 2004. *Zinc sulfate as an adjunct to methylphenidate for the treatment of attention deficit hyperactivity disorder in children: A double blind and randomised trial.* BMC Psychiatry, 4: 9-14.

Al-Ayadhi L.Y. 2005. *Heavy metals and trace elements in hair samples of autistic children in central Saudi Arabia.* Neurosciences, 10(3): 213-218.

ATSDR (Agency for Toxic Substances and Disease Registry). 2012. *Draft Toxicological Profile for Cadmium. Atlanta, Georgia: US Department of Health and Human Services.* Atlanta, GA, USA.

Blaurock-Busch E., Amin O.R., Rabah T. 2011. *Heavy metals and trace elements in hair and urine of a sample of Arab children with autistic spectrum disorder.* Maedica (Buchar), 6(4): 247-257.

Blaurock-Busch E., Amin O.R., Dessoki H.H., Rabah, T. 2012, *Toxic metals and essential elements in hair and severity of symptoms among children with autism.* Maedica (Buchar), 7: 38-48.

Brzóska M.M., Moniuszko-Jakoniuk J. 2001. *Interactions between cadmium and zinc in the organism.* Food Chem. Toxicol., 39: 967-980.

Elsheshtawy E, Tobar S., Sherra K, Atallah S, Elkasaby R. 2011. *Study of some biomarkers in hair of children with autism.* Middle East Current Psych., 18: 6-10.

Faber S., Zinn G.M., Kern J.C. 2ND, Kingston H.M. 2009. *The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders.* Biomarkers, 14: 171-180.

Fido A., Al-Saad S. 2005. *Toxic trace elements in the hair of children with autism.* Autism, 9(3): 290-298. DOI: 10.1177/1362361305053255

Gogia S., Sachdev H.S. 2012. *Zinc supplementation for mental and motor development in children.* Cochrane Database Syst Rev., 12:CD007991. DOI: 10.1002/14651858. CD007991.pub2

Gonzalez-Reimers, E., Martín-González, C., Galindo-Martín, L.M. Aleman-Valls R., Velasco-Vázquez J., Arnay-de-la-Rosa M., Pérez-Hernández O., Hernández Luis R. 2014. *Lead, cadmium and zinc in hair samples: relationship with dietary habits and urban environment.* Biol Trace Elem Res., 157: 205-210. DOI: 10.1007/s12011-014-9896-8.

Grandjean P., Landrigan P.J. 2006. *Developmental neurotoxicity of industrial chemicals.* Lancet., 368: 2167-2178.

Konikowska K., Regulska-Ilow B., Różańska D. 2012. *The influence of components of diet on the symptoms of ADHD in children.* Rocz Panstw Zakl Hig., 63(2): 127-134

LONSDALE D., SHAMBERGER R.J., AUDHYA T. 2002. *Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: A pilot study*. Neuroendocrinology, 23: 303-8.

MOUSAIN-BOSE M., ROCHE M., RAPIN J., BALI J.P. 2004. *Magnesium Vit B6 intake reduces central nervous system hyperexcitability in children.* J. Am. Coll. Nutr., 23: 545S-548S.

PRIYA M.D.L., GEETHA A. 2011. *Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism.* Biol Trace Elem Res., 142: 148-158.

SANDERS A.P., FLOOD K., CHIANG S., HERRING A.H., WOLF L., FRY R.C. 2012. *Towards prenatal biomonitoring in North Carolina: Assessing arsenic, cadmium, mercury and lead levels in pregnant women.* PLoS ONE 7, e31354. DOI: 10.1371/journal.pone.0031354

SERAJEE F.J., NABI R., ZHONG H., HUQ M. 2004. *Polymorphisms in xenobiotic metabolism genes and autism.* J. Child Neurol., 19(6): 413-417.

STAROBRAT-HERMELIN B., KOZIELEC T. 1997. *The effects of magnesium physiological supplementation on hyperactivity in children with attention deficit hyperactivity disorder (ADHD). Positive response to magnesium oral loading test.* Magnes. Res., 10: 149-156.

TABATADZE T., ZHORZHOLIANI L., KHERKHEULIDZE M., KANDELAKI E., IVANASHVILI T. 2015. *Hair heavy metal and essential trace element concentration in children with autism spectrum disorder.* Georgian Med News. 248: 77-8.2

TAKEDA A. 2003. *Manganese action in brain function.* Brain Res. Rev., 41: 79-87.

VAGHRI Z., BARR S., WONG H., CHAPMAN G., HERTZMAN C. 2008. A*ge-based differences in hair zinc of Vancouver preschoolers.* Biol. Trace Elem. Res., 126(1): 21-30. DOI: 10.1007/s12011-008--8215-7

VAGHRI Z., WONG H., BARR S., CHAPMAN G., HERTZMAN C. 2011. *Associations of socio-demographic and behavioral variables with hair zinc of Vancouver preschoolers.* Biol. Trace Elem Res., 143(3): 1398-1412.

WALSH T.J., USMAN A., TARPEY J. 2001. *Disordered metal metabolism in a large autism population.* Am. Psychiatric Association Meeting May, New Orleans.

YASUDA H, YASUDA Y, TSUTSUI T. 2013. *Estimation of autistic children by metallomics analysis.* Sci Rep., 3: 1199. DOI: 10.1038/srep01199

YASUDA H., YOSHIDA K., YASUDA Y., TSUTSUI T. 2011. *Infantile zinc deficiency: Association with autism spectrum disorders.* Sci. Rep. 1: 129. 10.1038/srep00129.

ZAWADZKI M., GAĆ P., PORĘBA R., ANDRZEJAK R. 2008. *Modification of the cardiovascular system in animals subjected to intoxication with manganese compounds.* Med. Pr., 59(5): 387-393. (in Polish)