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COPPER – ESSENTIAL AND TOXIC

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Copper is a well-known chemical element, widely used both in the past and nowadays. Although in ancient Egypt people believed copper had anti-inflamatory properties, it had later on proven to be extremly toxic. Electrical wires, pipes, roofs, brass, bronze, who can imagine everyday life without copper? And yet, liver cirrhosis, dysarthria, dystonia, mental problems, here are some of the toxic effects of the metallic element on the human body.

Keywords: copper toxicity, Wilson disease, cuproptosis, ceruloplasmin, superoxid dismutase.

INTRODUCTION

Copper was the first known and widely used metal in the humankind history. It gave its name to an entire period called the Eneolithic Age. In Romania, this period lasted approximately between 2500 and 2000 B.C. Especially in the Cucuteni culture, in an area that stretches from the Hășmaș Mountains beyond the Nistru river, several tools, ornaments and weapons made out of copper have been discovered[1]. The name of this metal comes from the Cyprus Island, which was the main source of copper supplies for the Roman Empire.

The processing and the use of this metal presented some disadvantages. Its low hardness, 3 on the Mohs scale, posed problems regarding tools and weapons use. Also, its high melting point, 1084^0C, created difficulties in reaching such a temperature. Other problems were caused by the high cooling rate of the molten copper as well as the absorption of many gases during cooling, which created difficulties regarding its casting into molds[2]. These inconveniences have been removed by obtaining bronze, an alloy of copper and tin, containing 90% Cu and 10% Sn. In Romania, the bronze age lasted for about 1000 years, until 1200 – 1100 B.C., although numerous bronze objects were also found in the first part of the Iron Age.

COPPER IN THE ENVIRONMENT

In the earth's crust, copper is the 26th element by weight, its concentration ranging from 27 to 68 ppm[3,4]. Certain studies estimate that copper is found in the amount of 3×10^{11} tons in the ores of the crust, while in the continental crust, estimated at 1.52×10^{19} tons, copper content is 4×10^{14} tons[4]. The main minerals of copper are sulphides: chalcozine (Cu_2S), covellite (CuS) and chalcopyrite ($CuFeS_2$). Also, copper exists in the form of oxide compounds, cuprite (Cu_2O), or basic carbonates, malachite ($CuCO_3.Cu(OH)_2$) and azurite ($2CuCO_3.Cu(OH)_2$). The metal content in ores usually ranges between 2–4%, but it can reach and exceed 7%[5]. Global copper mining production was 21 million tons in 2021. Chile was in first place – 5600 tons, followed by Peru – 2200 tons, Congo and China – 1700 tons each. Total reserves have been estimated at 880 million tons, while undiscovered resources are estimated at 3.5 billion tons[6].

The atmospheric concentration of copper is generally low. The main natural source of pollution is represented by volcanic emissions, while the main anthropogenic sources are the metallurgical industry, the cement industry and the burning of fossil fuels. In 1995, global emissions of copper into atmosphere were 35,000 tons[7]. A 2016 study in a rural area in northern China found that the average level of copper in suspended matter less than 2.5 microns in diameter ($PM_{2.5}$) was 0.041 µg/m^3. This was higher than Beijing's 0.026 µg/m^3 in 2016, but lower than another Chinese city, Bandong's 0.11 µg/m^3 in 2014[8]. Nowadays, the greenhouse gas emissions associated with the copper production is 0.3% of the total, but it is estimated to increase to 2.7% by 2050. This trend is due to increase in the global demand for copper, which will reach 62 million tons by 2050[9].

In the ocean water, copper concentration is estimated at 0.25 µg/L, while in rivers it reaches 1.48 µg/L. Depending on the pH, it can be found in a dissolved or precipitated state. Up to a pH value of 6, it is found as Cu^{2+}, while at basic pH it is present as carbonate, $[Cu(CO_3)_2]^{2-}$, and hydroxo complexes, $[Cu(OH)_3]^-$ and $[Cu(OH)_4]^{2-}$. In natural waters, in a proportion of 98% and even more, Cu(II) forms complexes with dissolved organic compounds. As a precipitate, copper is found in freshwaters as $Cu(OH)_2$ and $CuCO_3$, while in seawater mainly as $Cu(OH)_2$. In deep waters with low oxygen content, it is found in the form of sulphides[7]. Also, it can be immobilized by the sediments, by forming bonds with clay, hydrous manganese oxides and iron oxides[10]. A study of the entire Swedish sector of the Baltic Sea, including 13 monitoring stations, found that the average level of copper in sediments was 39 mg/L, with a 90th percentile of 63 mg/L. This values are above the background level set by the Swedish EPA, 15 mg/L[11]. Mainly, the Cu(II) source for the Baltic Sea is anthropogenic – 80%, while the calculated input is 1300 tons from river waters and 1200 tons from atmospheric deposition[7]. In drinking water, copper concentration varies between <5 µg/L and >30 mg/L, the major source of pollution being the stagnation of water in copper pipes, allowing leaching of the metal[12]. Storing tap water in copper containers leads to the increase of the copper level. A study reveals that a sample of such water stored in a 1 L container for 12 h has a concentration of 0.327 mg Cu/L, while after 168 h of storage the level reaches 0.813 mg Cu/L. The initial copper level, before deposition, was 9 µg/L[13]. According to EU legislation, the maximum allowed level of copper in drinking water is 2 mg/L, while in USA the same value, established by EPA, is 1.3 mg/L[14,15].

The estimated global average copper content of the soil is 14 mg/kg. The lowest values are recorded in sandy soils, 3 – 30 mg/kg, while the highest are specific to loamy soils, 7 – 140 mg/kg[16]. In 27 EU countries, a study was carried out on the level of copper in vegetable soils, the 22000 samples being taken from a depth of up to 15 cm. Of these, more than 16,000 (75%) showed a copper content of less than 20 mg/kg, nearly 200 revealed a concentration of 100 – 200 mg Cu/kg, and only 52 had concentrations more than 200 mg Cu/kg. A level of 100 mg Cu/kg soil represents an alert threshold for human health, while a value above 150 mg Cu/kg is a risk factor. The average concentration of copper in the soil of some European countries where its higher values were recorded is shown in the table below[17].

Table 1

The average level of copper (mg/kg) in the soil of some EU countries

Țară	Conc. Cu
Cyprus	53.41
Italy	41.22
Malta	33.11
Greece	27.97
Bulgaria	27.71
Romania	23.38
Ireland	17.49
Hungary	15.39

In Romania, an area rich in metalliferous ores is the Maramureș county, covering an area of about 6300 km^2, where around 460,000 inhabitants are curently living. For many years, in Baia Mare, the main city of the region, a copper smelter represented an important source for environmental contamination. The analysis of agricultural and forest soil samples revealed a copper concentration ranging between 5.6 – 48 mg/kg, with a mean value of 20 mg/kg, below the value reported in other agricultural Romanian sites[18].

Anthropogenic sources of soil contamination include the use of copper compounds as fungicides, the use of sewage sludge and liquid manure in agriculture, mining activities, atmospheric deposition, particles from car braking systems. In the EU, around 150,000 tonnes of fungicides are sold yearly, around 4 million tons of treated sewage sludge is used as fertilizer and 150 million pigs are fed with 6.2 million tons of copper via foodd additives. Since 1885, the use of an anti-fungal substance, consisting of copper sulphate and lime, in order to combat mildew in grapes vines, resulted in an increase of the metal concentration in those soils. Some data in this regard, listed in the table below, are relevant[19].

Table 2

Mean copper concentration (mg/kg) in vineyards of some EU countries

Country	Cu conc.	Percent samples with concentration higher than 100 mg Cu/kg soil
France	91.29	41.65%
Italy	71.90	19.8%
Romania	64.87	18.75%
Germany	54.69	9.1%
Cyprus	39.07	–
Portugal	23.76	5.5%

It is important to specifiy that in France, in Aquitaine, Languedoc-Roussillon and Provence-Alpes-Cote d'Azur, half of the soil samples have a copper level above 100 mg/kg. Also, in the Trento province of Italy, the analysis of six soil samples shows a mean copper concentration equal to 220.9 mg/kg, while in Emiglia-Romana the mean value is 110.44 mg/kg[19].

COPPER IN PLANTS AND ANIMALS

Copper is essential for normal plants development. The deficiency of this microelement reduces water transport in plants, causes chlorosis, i.e. yellowing of young leaves, curling of their edges and the lignification of certain tissues. Also, copper deficiency affects seed and pollen viability as well as fruit and seed formation. On the other hand, excessive amounts of copper have toxic effects on plants as well as on soil microorganisms. The damage to the roots is manifested by inhibiting their growth, affecting the volume, changing their structure and the blackening and thickening of the cell walls, as a result of the decrease in cell division. Another sign of poisoning is the yellowing of the leaves, as a consequence of the reduction in the content of photosynthetic pigments, such as carotenoids and chlorophyll. The high concentration of copper in the soil disturbs K and P absorption[20]. A study made on the *Arabidopsis* plant showed that exposure to concentrations of 5µM Cu for 15 days led to the decrease of nitrogen uptake and accumulation. The cause was the toxic copper effect, that led to the decrease in the expression of some genes encoding *bZIP* transcription factors, *NR1* nitrate reductase and *NRT-1* low-affinity nitrate transporters. In turn, they regulate the expression of nitrate transporters in the roots, *NRT* family[21]. Also, excessive amounts of copper disturb the uptake of some other essential elements, as it was shown in the citrus roots for Mn, Zn and Ca, and Mn, Ca and Zn in the leaves. In the citrus orchards, the input of Cu as fungicide reaches 15–30 kg/ha annually[22]. The critical concentration of copper in the leaves and shoots of some cereals and vegetables, beyond which the yield decline occurs, is shown in the Table 3[23].

Table 3

The critical copper concentration (mg Cu/kg dry mass) in the shoots and leaves of some plants

Plant	Critical Cu concentration
Barley	14 – 25
Wheat	11 – 18
Maize	5 – 21
Lettuce	8 – 23
Beans	15 – 30
Cabbage	25
Spinach	25 – 35

In animals, as in humans, the highest levels of cooper can be found in the liver and kidneys, and the lowest in muscles and skin. Also, the concentration depends on the environment in which the animals live. For exemple, in Poland, in the liver of twenty organic fed pigs, the mean concentration was 7.42 mg Cu/kg liver fresh weight (FW), while in the kidneys the corresponding value was 9.52 mg/kg. In the muscles, the microelement was not detected[24]. Other data show that for cows the cooper concentrations were (mg/kg FW tissue) 8.15 in the liver and kidneys and 1.41 in muscles. For lambs, the same values were 89.8 (liver), 5.39 (kidneys) and 1.47 (muscles)[7].

Table 4

Copper concentration (mg/kg FW) for some aquatic organisms

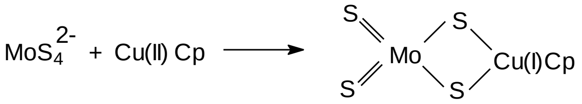
Species, organs	Country or geographic area	Mean value, range
Alga, whole Oysters, soft part Fish, muscle	India	12.3 4.2 < 0.09
Mollusks, soft parts Bivalves Gastropods	Israel	9.4 – 13.3 31 – 48
Perch, liver Perch, muscle	Norway, small lakes	1.5 – 1.6 0.16
Crayfish, whole	USA	56.2 – 77.7
Mussels, soft parts	Italy, Goro Bay	6.9
Cod, muscles Cod, liver	Southern Baltic	0.01 – 1.3 0.3 – 1.5
Herring, muscles Herring, liver	Southern Baltic	8.83 5.35

In the case of aquatic organisms living in waters contaminated with copper, its content can reach 102 mg/kg for detritivores, 54 mg/kg for predators and 43 mg/kg for omnivores. The factors which influence the copper level in their body are water salinity, pH, age, the size of the aquatic organism, etc.

The copper content in some aquatic species is presented in the Table 4[7].

A study conducted in China, in the country's third largest lake, revealed that in the carp the copper content is reduced. The copper concentration in the lake was 0.71 µg/L both in the summer and winter. In summer, in the muscle tissue, the level of the microelement was 0.037 mg/kg, while in the winter it was somewhat higher, reaching 0.097 mg/kg[25].

Dietary copper deficiency affects ruminants more than non-ruminants. The deficiency is related to the simultaneous presence in food of molybdenum and sulfur, which form thiomolybdates. These compounds have the general formula $MoS_nO^{2-}_{4-n}$, where n = 1–4, their formation depending on the pH and the S:Mo ratio. Generally speaking, tetramolybdates, MoS_4^{2-}, predominate in the animal body. Once at the cellular level, MoS_4^{2-} binds the copper in the structure of the enzymes of which it is a part, reducing their activity. In the case of ceruloplasmin, the most encountered cuproenzyme, the complexation is done without moving the metal element from the enzyme structure, according to the reaction below[26]:



where Cp stands for ceruloplasmin. For cattle, the symptoms of copper deficiency are less specific: loss of appetite, reduced milk yield, changes of the coat colour, loss of hair around the neck and eyes, on the forelimbs and thorax. In advanced stages osteoblasts malfunction appears and, as a consequence, spontaneous fractures. Also, ataxia, hypomyelination of the spinal cord and degeneration of the myocardium occur[27]. In young lambs, copper deficiency causes a disease called neonatal enzootic ataxia, which include symptoms such as muscular incoordination and partial paralysis of the hindquarters. In adult sheep, the deficiency can be noted in the form of steely wool, elasticity and affinity for dyes and, for the black sheep, a lack of pigmentation[28].

The animals most often affected by copper poisoning are sheep, the difference between the required amount and the toxic dose being small. The symptoms of poisoning are jaundice, haemoglobinuria, hemolysis, dark-colored kidneys and liver[28]. For cattle, the accumulation of copper in the body is favoured by a high magnesium to calcium ratio and high levels of Ni, Fe, Co and Cr in the soil. When the storage capacity of the liver for the toxic is exceeded, the signs of the poisoning appear. For the acute phenomenon, the effects are massive degradation of the red blood cells, haemoglobinuria and haemoglobinemia, while the clinical manifestations include the lack of appetite, apathy, weakness, thirst. For the chronic poisoning, the symptoms are less specific and include hair loss, decreased milk yield, fertility disorders[27].

COPPER IN THE HUMAN BODY

For the humans, copper is an essential trace element, the amount of copper in the adult's body being between 100 and 150 mg. The daily requirement of this element is calculated taking into account its plasma concentration and the activity of some cuproenzymes. In the USA, the recommended daily allowance (RDA) ranges from 340 µg in the case of newborns to 900 µg, for over 19 years old[29]. In the EU, based on the idea of an intake that compensates for losses and does not alter the body's copper reserves, the adequate daily intake is considered to be 1.6 mg Cu for men and 1.3 mg Cu for women[30]. The main source of copper is the food and, secondarily, the consumption of drinking water.

Table 5

Copper content in some foods (mg/kg)

Food	Cu concentration
Milk, plain	0.1 – 0.88
Milk, dried skim	11
Fresh cheese	0.03 – 0.4
Eggs	0.8
Pork chop, cooked	1
Chicken, cooked	0.7
Beef, ground	1
Muscle meat, beef	0.1 – 1.8
Oysters	0.3 – 16
Tuna	0.1 – 1.2
Salmon	0.5 – 0.8
Shrimps	2 – 2.9
Maize products	0.6 – 16.6
Wheat bread	2.9
Wheat bread, whole grain	3.4
Soybeans	16
Bananas	0.7 – 3
Apples	0.1 – 2.3
Oranges	0.8 – 0.9
Broccoli	0.1 – 0.87
Carrots	0.37 – 0.62
Peas	1.9 – 2.4
Cabbage	0.1 – 7.7

The Table 5 shows the concentration of copper in various foods[28,31].

The data in the table, unless otherwise stated, refer to unprocessed foods.

Copper enters the body mainly through the digestive tract, as a result of the consumption of food and drinking water. It is absorbed mainly in the stomach and the proximal zone of the small intestine, i.e. the duodenum. The quantity that enters the blood is inversely proportional to the dose that reaches the digestive tract. Thus, at a daily consumption of 0.78 mg Cu, the percentage absorbed is 55.6%, while at a value of 7.53 mg/day the percentage decreases at 12.4%[32]. Probably, the maximum percent of absorbed copper ranges between 63 – 67%, the average value being 30–40% for diets specific to industrialized countries[33]. From the small intestine, copper passes into enterocytes as Cu^{2+} or bound to amino acids. The mechanism of absorption involves the activity of the high-affinity copper transporter, CTR1. Prior to be bound and transported across the apical membranes of the enterocytes by CTR1, Cu(II) is reduced to Cu(I) under the action of some reductases. Through the sulfur atoms belonging to methionine fragments in the transporter structure, Cu(I) is translocated in the cytosol, where it is taken up by three proteins of the chaperones class: ATOX1, CCS and COX17. ATOX1 transfers copper to an ATPase, ATP7A, which transports the metal element inside Golgi apparatus, being used here for the synthesis of some enzymes: tyrosinase, lysyl oxidase, etc. On the other hand, by translocating to the basolateral membrane of the enterocyte, ATP7A allows copper to enter the blood circulation and distribution in the body[34,35].

After entering the blood, through the portal venous system, about 75% of the copper is transported to the liver in the form of complexes with albumin, α2-macroglobulin or amino acids such as histidine. In the liver, the trace element enters the constitution of the main cuproenzyme, ceruloplasmin, in this form reentering the blood circuit in proportion of 65–90%. Probably, the kidneys are also a source of ceruloplasmin in the blood, as it’s been demonstrated that this organ expresses the corresponding mRNA. The largest amounts of copper in the body are found in the skeletal (46 mg) and muscular (26 mg) systems. The organs that contain higher copper concentrations are the liver (approximately 6.2 µg/g, 10 mg total), the brain (5.2 µg/g, 8.8 mg total), the heart (4.8 µg/g) and the kidneys (4–12 µg/g)[36,37]. The copper turnover was studied by restricting copper in the diet of rats for 8 weeks. After 56 days of restriction, the copper level decreased in the plasma with 99%, in the liver with 62%, in the kidneys with 66% and in the brain with less than 1%[38]. The main excretion route for the copper is through the bile (approximately 2.5 mg/day) and, consequently, through the feces, while in the urine the trace element content is low, being contained between 0.01 and 0.025 mg/day[15].

Part of enzymes, hormones and vitamins, copper plays an important role in the human body. To date, more than 25 cuproenzymes are known, the most common being ceruloplasmin and superoxide dismutase (SOD). Ceruloplasmin belongs to the family of oxidases that contain several ions – multicopper oxidases (MCO). This category also includes hepstetin, present in enterocytes and contributing to the absorption of iron, and zyklopen, an enzyme accomplishing a similar role in the placenta. Synthesis of ceruloplasmin is carried out in the liver, its plasma level being between 0.15–0.96 mg/mL[39]. The enzyme consists of a polypeptide chain with 1046 amino acid fragments, having a molecular mass of 132 kDa. Structurally, it can be divided in six domains. Three copper ions belong to even domains, 2, 4 and 6, and the other three form a trinuclear cluster at the interface of the domains 1 and 6[40].

Ceruloplasmin has multiple functional roles. It participates in the oxidation of Fe^{2+} to Fe^{3+}, thus preventing the formation of hydroxil radicals through Fenton-type reactions, in the oxidation of Cu^{+} and NO to nitrites, the decomposition of hydrogen peroxide in the presence of GSH (glutathione in reduced form), etc. Iron enters enterocytes in divalent form, but the incorporation into its plasma transporter, transferrin, requires its oxidation to Fe^{3+}. This process takes place through the intervention of ceruloplasmin, hepstetin, as it can also be non-enzymatic[41]. The oxidation of Fe^{2+} under the action of ceruloplasmin is carried out as follows[42]:

$$4Fe^{2+} + CP - Cu^{2+} \rightarrow 4Fe^{3+} + CP - Cu^{+}$$
$$4Fe^{3+} + 2Apo - TF \rightarrow 2TF - Fe^{3+}$$
$$4Cu^{+} + O_2 + 4H^{+} \rightarrow 4Cu^{2+} + 2H_2O$$

where CP stands for ceruloplasmin, TF for transferrin and Apo-TF for apo-transferrin. It is important to mention that each transferrin molecule binds two Fe^{3+} ions.

Superoxide dismutase (SOD) is a widely distributed enzyme in microorganisms, plants and animals. It is one of the most antioxidant enzymes, disproportioning superoxide radical ($O_2^{\cdot -}$) in H_2O_2 and O_2. $O_2^{\cdot -}$ and H_2O_2 have a physiological role for redox signalling in cells, but in excessive amounts they lead to the oxidative stress, which targets DNA and produce lipids peroxidation. In scavenging superoxide radicals, SOD requires metal ions as cofactors: Cu, Mn, Zn. There are three types of SODs in eukaryotes, depending on the cellular location and the type of cofactor. Two of them, SOD1 and SOD3 are Cu/Zn enzymes, while SOD2 requires as cofactor Mn. SOD1 is widely distributed in all cells of the human body, being located in cytoplasm as well as in the nucleus and cell membrane. It is a homodimer, each of the two structural units being made up of eight polypeptide chains, and having a molecular mass of 32 kDa. Its catalytic activity depends on Cu, while Zn has only a structural role. SOD2, containing Mn, is localized in the mitochondrial matrix, while SOD3 can be found in lymph, blood, synovial fluid. SOD3 is a homotetramer, dimers being linked by two-disulfide bridges, with a molecular mass equal to 135 kDa[43].

The mechanism of action of Cu/Zn SODs involves two steps. In the first, the oxidation of $O_2^{\cdot -}$ to O_2 is carried out due to the reduction of Cu(II) to Cu(I). In the second one, the restoration of Cu(II) is also done by the reaction of the enzyme with $O_2^{\cdot -}$ and results in the formation of H_2O_2. This mechanism is presented below[44]:

$$\begin{array}{c} O_2^{\cdot -} + \mathrm{Cu(II)\ SOD} \rightarrow O_2 + \mathrm{Cu(I)\ SOD} \\ O_2^{\cdot -} + 2H^+ + \mathrm{Cu(I)\ SOD} \rightarrow H_2O_2 + \mathrm{Cu(II)\ SOD} \\ \hline 2\,O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2 \end{array}$$

Although copper is an essential trace element, disturbance of homeostasis and increase of its intracellular concentration leads to cell death. There many forms of cell death, including apoptosis (programmed cell death), necroptosis (having necrosis features), pyroptosis (an inflammatory programmed cell death), ferroptosis (iron-dependent cell death). The mechanism by which copper leads to cell death is distinct from the others and is called cuproptosis. It was studied using small molecules, called copper ionophores, which transport the metallic element inside the cells. A pulse treatment with one of this ionophores, elesclomol, for 2 hours, at a concentration of 40 nM, led to a 15 to 60-fold increase of the intracellular copper level, followed by cells death more than 24 hours later[45]. Morphological manifestations of cuproptosis are endoplasmatic reticulum injury, cell membrane rupture, mithocondrial shrinkage. The specific mechanisms of cuproptosis are: triggering of the oxidative stress, inhibition of the ubiquitin-proteasome system (UPS) and binding to the lipoylated proteins of the TCA (tricarboxylic acids) cycle[46]. Increasing of the intracellular copper level promotes the increase of ROS (reactive oxygen species) concentration, Cu^+ acting as catalyst in Fenton reaction. This leads to inner mithocondrial membrane damage, deoxynucleotide depletion, inhibition of DNA synthesis and, finally, causes cell death. UPS participates in the degradation process of about 80% of the proteins in the cell. It was shown that disulfiram – Cu^{2+} complex, where disulfiram is another copper ionophore, decreases the proteasome activity, damaging the breast cancer cells. Finally, probably the most important cuproptosis mechanism is linked to copper action on lipoylated proteins. Lipoylation is a posttranslational modification of a lysine fragment, which consists in the attachement of a lipoamide cofactor through an amide bond. Lipoamide is an organocompound with eight carbon atoms, having a pentatomic ring with a sulfur-sulfur bond and a side chain[47]. There are known only four lipoylated proteins in mammals, all of them being linked to TCA cycle. One of these proteins is PDH (pyruvate dehydrogenase), which catalyzes the biotransformation of pyruvate into acetyl-coenzymeA, compound that enters the TCA cycle. By binding to lipoylated proteins, copper causes their oligomerization and, also, it was observed the loss of Fe-S cluster proteins. Hence, it occurs protein toxicity stress and cell death[45,46].

CONCLUSIONS

Copper has been known and used since the dawn of the human history. For living organisms, it is a doubled-edged sword. Entering the constitution of more than 25 enzymes, it is essential for life. However, in excess, it can cause serious health problems of genetic etiology, such as Wilson’s disease. Also, it should not be forgotten that one of the first ecological disasters occured at the end of the 19th century, at the Ashio mining complex in Japan, where copper was extracted. Essential or toxic: it often depends on us.

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