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EFFECT OF CADMIUM, COPPER AND ZINC ON PLANTS, SOIL MICROORGANISMS AND SOIL ENZYMES *

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Abstract

Heavy metals when present in amounts equal to the geochemical background do not interfere with the soil metabolism, which is associated with the growth and development of soil microorganisms as well as the processes of synthesis and re-synthesis, governed by intra- and extracellular enzymes. In the said concentrations, heavy metals do not cause undesirable changes in the development of plants. On the contrary, such elements as copper and zinc are essential constituents of physiological processes in all living organisms, including microorganisms and plants. Some soils suffer from zinc and copper deficits, which is why they are enriched with fertilizers containing copper or zinc to satisfy the nutritional requirements of crops. Cadmium is different in that its essential role in the proper functioning of living organisms has not been proven yet.

In Poland, soils contaminated with heavy metals, including cadmium, copper and zinc, occur only locally. The purpose of this study has been to discuss the characteristics of these elements in terms of the chemical properties and the role in the natural environment, the effect they produce on plants when present in excessive concentrations in soil and the response of soil microbes and enzymes to such contaminants.

Crops cultivated on soil with an elevated content of heavy metals typically present inhibited growth, reduced transpiration, chlorosis of leaves, limited germination of seeds and deformations of the root system. The effect induced by heavy metals is more pronounced in the early development of plants. Mobility and plant availability of heavy metals depend on a series of factors, for example the soil pH, content of organic matter, grain-size composition of soil, content of iron and manganese oxides, soil sorption capacity and the type of metal. Higher bioavailability of heavy metals is observed in soils with a low content of humic acids. As the soil pH increases (within 6.5-7.5), metals, especially zinc and – to a lesser degree – copper become less toxic to plants.

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The mechanism building tolerance of plants to heavy metals is closely connected with processes which reduce the uptake and transport of metals and with detoxification on cellular membranes and inside cells. An increase in the concentration of metals induces the synthesis of phytochelates, whose main function is to sustain the homeostasis of metals in the cell. These proteins also transport metal ions to vacuoles, where they can be bound by oxalates.

Excessive amounts of cadmium, copper and zinc disrupt the homeostasis of soil by interfering with the control mechanisms on the level of genes, thus inhibiting the activity of microbial enzymatic proteins. They cause damage to metabolic pathways, often resulting in the apoptosis of cells. Consequently, the counts and species diversity of soil microorganisms suffer. Such process as nitrification and ammonification are inhibited, alongside the activity of soil enzymes. The adverse influence of cadmium, copper and zinc on microorganisms and enzymes can be alleviated by application of organic and natural fertilizers. For soil phytoremediation, microorganisms resistant to these metals but enhancing their availability can be used.

Key words: cadmium, copper, zinc, plants, microorganism, enzymes.

ODDZIAŁYWANIE KADMU, MIEDZI I CYNKU NA ROŚLINY, DROBNOUSTROJE I ENZYMY GLEBOWE

Abstrakt

Metale ciężkie, gdy stanowią tło geochemiczne, nie powodują zakłóceń metabolizmu glebowego związanego z rozwojem organizmów glebowych oraz z procesami syntezy i resyntezy, o których decydują zarówno enzymy wewnątrzkomórkowe, jak i zewnątrzkomórkowe. Nie powodują także niekorzystnych zmian w rozwoju roślin, a wręcz przeciwnie, takie pierwiastki jak miedź i cynk są mikroskładnikami niezbędnymi w procesach fizjologicznych wszystkich organizmów, w tym także drobnoustrojów i roślin. W niektórych glebach stwierdza się niedobór cynku i miedzi, dlatego by uzupełnić potrzeby pokarmowe roślin wprowadza się nawozy zawierające miedź lub cynk. Inny charakter ma kadm, którego niezbędności w prawidłowym funkcjonowanie organizmów jak na razie nie udowodniono.

W Polsce lokalnie występują gleby zanieczyszczone metalami ciężkimi, w tym kadmem, miedzią i cynkiem, dlatego celem pracy było przedstawienie charakterystyki tych pierwiastków pod względem właściwości chemicznych i roli w środowisku przyrodniczym, ich oddziaływania na rośliny uprawiane w warunkach nadmiaru tych pierwiastków w glebie oraz odpowiedzi drobnoustrojów i enzymów glebowych na to zanieczyszczenie.

Rośliny uprawiane na glebach o podwyższonej zawartości metali ciężkich charakteryzują się zahamowaniem wzrostu, ograniczeniem transpiracji, chlorozą liści, ograniczeniem kiełkowania nasion oraz deformacją systemu korzeniowego. Odziaływanie to jest silniejsze we wczesnych fazach rozwojowych. Mobilność i dostępność metali ciężkich dla roślin jest uzależniona m.in. od pH gleby, zawartości materii organicznej, składu granulometrycznego gleby, zawartości tlenków żelaza i manganu, pojemności sorpcji oraz rodzaju metalu. Większą biodostępność metali ciężkich dla roślin obserwuje sie w glebach o niskiej zawartości kwasów humusowych. Wraz ze wzrostem pH gleb (6,5-7,5) zmniejsza się fitotoksyczne działanie, szczególnie cynku, w mniejszym stopniu miedzi.

Mechanizm tolerancji roślin na metale ciężkie związany jest z procesami ograniczającymi pobieranie i transport metali, procesami detoksykacji na błonach komórkowych i wewnątrz komórki. Wzrost stężenia metali indukuje syntezę fitochelatyn, których główną funkcją jest utrzymanie homeostazy metali w komórce. Białka te przenoszą również jony metali do wakuoli, gdzie mogą być wiązane przez szczawiany.

Nadmierne ilości kadmu, miedzi i cynku naruszają homeostazę gleby, zaburzając mechanizmy kontroli na poziomie genów, przez co hamują aktywność białek enzymatycznych drobnoustrojów. Wywołują uszkodzenia szlaków metabolicznych, niejednokrotnie prowadząc do apoptozy komórki. W konsekwencji zmianie ulega liczebność oraz różnorodność gatunkowa mikroorganizmów. Hamowane są takie procesy, jak nitryfikacja i amonifikacja oraz aktywność enzymów glebowych. Negatywne działanie kadmu, miedzi i cynku na drobnoustroje i enzymy można łagodzić stosując nawozy organiczne i naturalne, szczególnie efektywna jest substancja organiczna zasobna w kwasy huminowe. W fitoremediacji można wykorzystywać także drobnoustroje oporne na te metale, a jednocześnie zwiększające ich przyswajalność.

Słowa kluczowe: kadm, miedź, cynk, gleba, rośliny, mikroorganizmy, enzymy.

INTRODUCTION

Some heavy metals play an extremely important role in biochemical reactions which are significant for the growth and development of microorganisms, plants and animals (Kavamura, Esposito 2010). However, when present in excessive concentrations, then can form non-specific compounds, causing a cytotoxic effect (Nies 1999). Moreover, these metals perform a variety of functions. Such metals as cerium, tin, gallium, thorium and zircon do not play any biological roles. Iron, manganese and molybdenum are important micronutrients and are low in toxicity. Cobalt, copper, chromium, nickel, wolfram, vanadium and zinc belong to essential micronutrients but are toxic. Antimony, arsenic, cadmium, lead, mercury, silver and uranium are highly toxic. Their metabolic role is rather insignificant.

The IUNG Institute in Puławy (Siebielec et al. 2012) has proposed the following division into six degrees based on threshold levels of heavy metals in the 0 to 20 cm soil horizon:

0 – natural content,
I – elevated content,
II – weak contamination,
III – moderate contamination,
IV – strong contamination,
V – very strong contamination.

Depending on the soil grain-size distribution, pH and content of organic matter, the zinc threshold levels were established as follows: the 0 degree comprises soils with 50 to 100 mg Zn kg^{-1}, I – from 100 to 300 mg Zn kg^{-1}; II – from 300 to 1,000 mg Zn kg^{-1}, III from 700 to 3,000 mg Zn kg^{-1}, IV from 3,000 to 8,000 mg Zn kg^{-1} and V from over 3,000 to over 8,000 mg Zn kg^{-1}. For copper, the respective limits are: the 0 degree – from 15 to 40 mg Cu kg^{-1}, I – from 30 to 70 mg Cu kg^{-1}, II – from 50 to 100 mg Cu kg^{-1}, III – from 80 to 150 mg Cu kg^{-1}, IV – from 300 to 750 mg Cu kg^{-1} and V – from over 300 to over 750 mg Cu kg^{-1}. Finally, for cadmium, the set values are: 0 – from 0.03 to 1 mg Cd kg^{-1}, I – from 1 to 3 mg Cd kg^{-1}, II – from

2 to 5 mg Cd kg^{-1}, III – from 3 to 10 mg Cd kg^{-1}, IV – from 5 to 20 mg Cd kg^{-1}, V – from over 5 to over 20 mg Cd kg^{-1} (SIEBIELEC et al. 2012). The lower limits apply to very light soils, and the upper ones – to medium and heavy ones.

According to the Regulation of the Minister for the Environment of 9 September 2002 (Journal of Law 165, item 1359), the allowable concentrations of heavy metals in the topmost layer of soil from 0 to 30 cm are 4 mg Cd kg^{-1}, 150 mg Cu kg^{-1} and 300 mg Zn kg^{-1} of soil. In Poland, soil contamination with heavy metals is localized and appears predominantly in industrialized regions (SIEBIELEC et al. 2012). Globally, pollution of the natural environment has increased dramatically over the past century (FAIZ et al. 2009). The increase can be attributed to the rapid economic development, urbanization and industrialization (KELLY et al. 2003, MIKANOVA 2006, LIAO, XIE 2007, HELMREICH et al. 2010, DUONG, Lee 2011, KHAN et al. 2011, QIAO et al. 2011).

Ecologically, the accumulation of heavy metals in soils is extremely hazardous because soil is a major link in the natural cycling of chemical elements; it is also a primary component of the trophic chain, composed of soil – plants – animals – humans (FAIZ et al. 200, TAKÁČ 2009, BIELIŃSKA, MOCEK-PŁÓCINIAK 2010, LIU et al. 2012, SAGI, YIGIT 2012). Any disruption of the equilibrium between these components may have serious consequences in any of the links of this chain.

CHARACTERIZATION AND PRESENCE OF CADMIUM IN SOIL

Cadmium was discovered in 1817 by Stromeyer (CIBA et al. 1996, TRAN, POPOVA 2013). It is a transition metal, which belongs to group 12 of elements. Its atomic number is 48 and the relative atomic weight is 112,411. The density of the metal is 8,64 g cm^{-3}. The melting point is 321.11°C, and the boiling points equals 767°C. It appears in three oxidation states: Cd^0, Cd^+ and Cd^{2+}. There are 31 isotopes of cadmium with the atomic mass from 99 to 124. Its total content in the Earth's crust is $2 \cdot 10^{-5}\%$ by weight. Cadmium occurs as the mineral called greenockite (CdS). In addition, it appears as an impurity in zinc ores. It is a by-product of zinc metallurgic processes, added as a component to alloys for making telephone and telegraph cables. As a metal, cadmium is also used in nuclear reactors control rods to absorb neutrons. Cadmium compounds find applications in the manufacture of anti-corrosive coatings on plastic materials. The geochemical properties of cadmium are very similar to those of zinc, although it has a greater affinity to sulphur. Cadmium appears in simple and complex compounds. Its predominant form is bivalent, and the metal forms various complex ions (e.g. $CdOH^+$, $CdHCO_3^-$, $CdCl^-$, $Cd(OH)_4^{2-}$). It dissolves in mineral acids but is resistant to the effect of alkalines. It is a toxic element to humans and animals, acting as a strong carcinogenic agent. Cadmium accumulates in the body, in which it is responsible for skeletal deformations, causes kidney disorders and is a factor in diseases of the blood circulation system and in neoplasms (Ciba et al. 1996). The International Agency for Research on Cancer has classified cadmium to the first category of carcinogenic factors in human cancers. Because cadmium ions have a configuration of electrons analogous to that of zinc ions, they are able to supplant zinc in proteins (Beyersmann, Hartwig 2008, Tran, Popova 2013). Cadmium is transported by membrane transporters and cellular channels, where it forms complexes with thiol groups of biomolecules (Bridges, Zalups 2005).

In line with the Directive of the European Parliament and Council no 2006/11/EC, cadmium in included in the list I of families and groups of substances most toxic, persistent and most readily bioaccumulated (Official Journal of the EC 4.3.2006). The content of cadmium in soils worldwide ranges from 0.4 mg to 167 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001), and in the Polish soils its level varies from 0.04 mg to 57.50 mg kg^{-1} of soil, with an average content measured in 2010-2012 as 0.56 mg kg^{-1} of soil. In total, 98.6% of soils in Poland have a natural content of cadmium. Its highest amounts are detected in the southern parts of the country, encompassing the Provinces of Lower Silesia, Opole, Silesia and Małopolska (Siebielec et al. 2012).

CHARACTERIZATION AND PRESENCE OF COPPER IN SOIL

Copper is a metal known since ancient times (Ciba et al. 1996). It belongs to group 11 of elements. It is red in colour, but when exposed to humid air, it acquires a green layer of verdigris [$Cu(OH)_2$]. The atomic number of copper is 29 and atomic mass equals 63.546. The melting point is 1,084.87°C, and the boiling point is 2,567°C. The density of the metal is 8.92 g cm^{-3}. Copper occurs in five oxidation states: Cu^0, Cu^+, Cu^{2+}, Cu^{3+}, Cu^{4+}. There are 18 copper isotopes with the atomic masses from 58 to 73. Copper creates simple and complex compounds. It is resistant to hydrochloric and hydrofluoric acids, but dissolves in oxidizing acids (e.g. sulphuric and nitric acids). Its total content in the Earth's crust is $1 \cdot 10^{-2}$% by weight. It rarely occurs as native copper, but is more often found as a component of such minerals as chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and malachite ($Cu(OH)_2CuCO_3$). In Poland, the largest copper deposits are in Lubin. As a metal, copper is used to make electric wires and parts of various machines. There are many copper alloys, e.g. brass (Cu + Zn), bronze (Cu + Sn) and cupronickel (Cu + Ni). Copper compounds are used in manufacture of plant protection chemicals, dyes, pigments, artificial fertilizers (as a micronutrient) and catalysts. They are also employed for electroplating. Copper is an essential element to the proper functioning of all organisms. Copper belongs to the elements which are required by living organisms. It participates in photosynthesis and respiration of plants (Ashworth, Alloway 2004). *"Copper deficiency in humans causes anaemia, disorders of the nervous and circulation systems. Its excess may damage the liver, kidneys, cardiovascular vessels and brain tissue"* (Ciba et al. 1996).

The Directive of the European Parliament and Council no 2006/11/EC classifies copper into list II of families and groups of substances, which contains substances harmful to the aquatic environment, but whose influence can be limited to a given area (Official Journal of EC 4.3.2006). The content of copper in soils worldwide ranges from 13 mg to 3,700 mg kg^{-1} of soil (KABATA-PENDIAS, PENDIAS 2001), and in Poland, it varies from 1.53 mg to 271.73 mg kg^{-1} of soil, with the average concentration in Polish soils equal 10.2 mg kg^{-1} of soil. Most soils in Poland are characterized by the natural copper content. The first degree contamination (4 sites) occurs in the provinces of Małopolska, Lublin and Lower Silesia; the third degree contamination (1 site) can be detected in the Province of Lower Silesia and the fourth degree contamination (1 site) appears in the Province of Lower Silesia (SIEBIELEC et al. 2012).

CHARACTERIZATION AND PRESENCE OF ZINC IN SOIL

Like copper, zinc was known in the ancient times (CIBA et al. 1996). This metal belongs to group 12 of elements. Its atomic number is 30 and the atomic mass equals 65.39. The melting point is 419.58°C, and the boiling point is 907°C. The density of the metal is 7.14 g cm^{-3}. Zinc occurs in three oxidation states: Zn^0, Zn^+, Zn^{2+}. Zinc forms simple and complex compounds. There are 23 zinc isotopes with the atomic weights from 57 to 78. Zinc dissolves in acids and alkalines, releasing hydrogen. It occurs in the following minerals: sphalerite (ZnS), smithsonite ($ZnCO_3$) and willemite (Zn_2SiO_4). Deposits of these minerals in Poland can be found in the area between Olkusz, Chrzanów and Bytom. The content of zinc in the Earth's crust is $7 \cdot 10^{-3}$% by weight. Zinc is used to make zinc sheets or zinc coating on iron and steel products. It is also a component of alloys, reducer in the metallurgy of noble metals and in organic chemistry. Zinc compounds are used to make paints, varnishes, cosmetics, plastics, artificial fertilizers (micronutrient). Zinc is an essential element for all living organisms (CIBA et al. 1996). It plays an important role in catalyzing biochemical reactions by participating in the formation of an enzyme-substrate system, protein translation, gene copying and multiplication of a genetic chain (SEKLER 2007). Zinc deficiency causes changes in the bone system and in the chemical composition of blood, and may lead to the cardiac insufficiency or brain developmental defects; the excess of zinc is harmful to the body (CIBA et al. 1996). The toxicity of zinc stems from its interaction with other heavy metals. Zinc is responsible for disturbing the functions of mitochondria (SEKLER et al. 2007).

The Directive of the European Parliament and Council no 2006/11/EC classifies zinc list II of families and groups of substances, containing substances harmful to the aquatic environment, whose effect can be limited to a given area (Official Journal of EC 4.3.2006).

The content of zinc in soils worldwide varies within a broad range from 35 mg to 12,400 mg kg^{-1} of soil (Kabata-Pendias, Pendias 2001); in the Polish soils, the range of zinc concentrations is from 10.27 mg do 5,805 mg kg^{-1} of soil, and its average content is 79.81 mg kg^{-1}. At present, there are three sites classified as the 2nd degree soil contamination and one site determined to represent the 4th soil pollution degree in Poland (Siebielec et al. 2012).

SOURCES OF HEAVY METALS IN SOIL

The natural content of heavy metals in soils is known as the biogeochemical background (Coskun et al. 2006, Castaldi et al. 2009, Takáč 2009, Kobierski, Dąbkowska-Naskręt 2012). The occurrence of heavy metals in soils is closely dependent on the chemical composition of parent rock (Kobierski, Dąbkowska-Naskręt 2012, Siebielec et al. 2012). Their concentrations in soils are negatively correlated with the depth of a soil profile. (Glina, Bogacz 2013). A higher content of heavy metals is observed in soils developed from flysch rocks and delluvial deposits (Tran, Popova 2013). The content of heavy metals in soils is shaped by both natural and anthropogenic factors. The natural conditions affecting the content of heavy metals in soils are: the parent rock, soil formation processes, grain-size distribution of a given soil, content of humus, oxydation/reduction potential, soil sorption capacity, soil reaction, plant cover (Burt et al. 2003, Covelo et al. 2007, Dragović et al. 2008, Jaworska, Dąbkowska-Naskręt 2012, Nadgórska-Socha et al. 2013, Skwierawska 2013, Tran, Popova 2013). Moreover, metals like cadmium, copper and zinc as soil pollutants can originate from geochemical processes evoked by volcanic eruptions or the weathering of parent rock (Kabata-Pendias 2004), from emissions by industries and motor transport (Kelly et al. 2003, Mikanova 2006, Liao, Xie 2007), from landfills (Szymańska-Pulikowska 2012), sewage sludge and all types of fertilizers made from waste (Xie et al. 2009, Achiba et al. 2010, Jakubus 2012, Sienkiewicz, Czarnecka 2012). Other sources of contamination include some mineral fertilizers and plant protection chemicals (Qiao et al. 2011). The highest quantities of heavy metals enter soils from the metallurgic and mining industries (Maiz et al. 2000, Wong 2003, Vásquez-Murrieta et al. 2006), and from transportation routes and emission of fumes (Liao, Xie 2007, Helmreich et al. 2010, Duong, Lee 2011, Khan et al. 2011, Qiao et al. 2011). Moreover, some amounts of heavy metals can permeate the environment from tyre manufacturing plants (Khan et al. 2011), petroleum refineries, leaks of petroleum products and lubricants from motor vehicles (Christoforidis, Stamatis 2009). Copper and zinc come from the same sources (Kobza 2005).

The fate of these contaminants depends on two groups of events. The first group comprises processes which aim at depressing their solubility and mobility; the other one encompasses processes stimulating the mobility of heavy metals, thus increasing their toxicity (Achiba et al. 2010).

EFFECT OF CADMIUM, COPPER AND ZINC ON PLANTS

The proper growth and development of plants above all depend on the availability of adequate amounts of nutrients. Apart from macroelements, there are also microelements such as copper and zinc which are necessary for maintaining proper functions of an organism (McCall et al. 2000). These elements may play a role of building blocks or catalysts. There are also such elements in the natural environment which do not have any physiological role, e.g. cadmium (Waalkes 2003, Tran, Popova 2013).

The average concentration of cadmium in plants ranges from 0.03 to 0.70 mg kg^{-1} d.m., copper from 1 do 16 mg kg^{-1}d.m., and zinc from 10 to 86 mg kg^{-1} d.m. (Kabata-Pendias, Pendias 2001). Both deficit and excess of these elements have a negative effect on plants, although their tolerance of copper or zinc deficits in soils is relatively high (Nadgórska-Socha et al. 2013, Tran, Popova 2013).

Many references, e.g. Wyszkowska, Kucharski (2003a), Wyszkowska et al. (2005), Wyszkowski, Wyszkowska (2006), Wyszkowska et al. (2006a), Wyszkowski, Wyszkowska (2009), Wyszkowska et al. (2010), Nadgórska-Socha et al. (2013), Tran, Popova (2013), state that soil contamination with heavy metals has an adverse influence on the growth and development of plants. Plants growing on soil contaminated with heavy metals may tend to take up more of these elements, which are then transferred to subsequent links in the feeding chain (Ciećko et al. 2001, Królak 2003, Zalewska 2012, Nadgórska-Socha et al. 2013). Two mechanisms of the uptake of trace elements by plant roots are distinguished: passive one, by diffusion, and active one, running against the gradient of concentrations and powered by metabolic energy (Rajkumar, Freitas 2008). Although cadmium is not essential for the growth and development of plants, it is readily taken up by the root system (Ciećko et al. 2001, Renella et al. 2004, Tran, Popova 2013), and therefore disturbs the uptake of other elements (Ciećko et al. 2004a, 2005).

Plant species or even cultivars might differ from one another in the tolerance to excessive quantities of cadmium, copper and zinc and in their ability to absorb these elements (Vig et al. 2003, Ciećko et al. 2001, Sekler et al. 2007, Beyersmann, Hartwig 2008, Zalewska 2012, Nadgórska-Socha et al. 2013). The most sensitive are papilionaceous plants, hop, grapevine, fruit (citrus) trees, cereals and spinach. The phytotoxicity of excess heavy metals is caused through the disturbance of physiological processes due to disorders in the uptake of micro- and macroelements that are necessary for the proper functioning of plants (Nadgórska-Socha et al. 2013, Tran, Popova 2013). Crops cultivated on soils with an elevated content of heavy metals are characterized by inhibited growth, reduced transpiration, chlorosis of leaves, limited seed germination and deformations of the root system (Nadgórska-Socha et al. 2013, Tran, Popova 2013). These effects are stronger during the early development stages (Vig et al. 2003, Sekler et al. 2007, Beyersmann, Hartwig 2008, Tran, Popova 2013). The mobility and plant availability of heavy metals depend on the soil pH, content of organic matter, grain-size composition of soil, content of iron and manganese oxides, soil sorption capacity, type of a metal and others (SEKLER et al. 2007, TAKÁČ 2009, ZALEWSKA 2012, TRAN, POPOVA 2013, GUALA et al. 2013). Heavy metals are more easily available to plants in soils with a low content of humic acids (BARANČÍKOVÁ, MAKOVNÍKOVÁ 2003, BORŮVKA, DRÁBEK 2004). As the pH of soils increases (from 6.5 to 7.5), the phytotoxic effect of heavy metals subsides, especially that of zinc and - to a lesser degree - of copper. The process is more intensive in soils containing elevated levels of these metals (CIEĆKO et al. 2001, FINŽGAR 2007, TAKÁČ 2009, WYSZKOWSKI,WYSZKOWSKA 2009, GUALA et al. 2013, NADGÓRSKA-SOCHA et al. 2013, TRAN, POPOVA 2013).

The mechanism building the tolerance of plants to heavy metals is associated with processes which restrain the uptake and transport of metals, processes of detoxication on cellular membranes and analogous processes inside cells (SEKLER et al. 2007, BEYERSMANN, HARTWIG 2008). An increase in the concentration of metals induces the synthesis of phytochelates, whose main function is to maintain the homeostasis of metals in cells. These proteins also transfer metal ions to vacuoles, where they can be bound by oxalates (TEKLIĆ et al. 2008, TRAN, POPOVA 2013).

A useful test indirectly demonstrating changes in the microbiological and biochemical properties is simultaneous assessment of the effects of heavy metals on the growth and development of plants (BELYAEVA et al. 2005, TRAN, POPOVA 2013). Such complex comparisons enable us to appreciate fully the harmful impact of heavy metals on the soil environment (WYSZKOWSKA, KUCHARSKI 2003a, WYSZKOWSKI, WYSZKOWSKA et al. 2006a, WYSZKOWSKA 2009).

Plants became more sensitive as the degree of soil contamination with copper increased. The said sensitivity was a species-specific trait. Yellow lupine was the most sensitive to excess copper; spring canola, especially grown on a more compact soil, i.e. on sandy loam, was the least sensitive. Oat demonstrated an intermediate sensitivity, regardless the soil on which it had been sown (WYSZKOWSKI, WYSZKOWSKA 2004, WYSZKOWSKA et al. 2010). Also, WYSZKOWSKA et al. (2009) confirmed empirically that an excessive content of copper in soil had a negative influence on yields of oat, spring canola and yellow lupine.

The assimilability of heavy metals by plants is also shaped by the antagonistic and synergistic effects of elements. According to BADORA (2002), zinc inhibits the accumulation of cadmium, whereas aluminum is an essential element for the process of zinc immobilization in soil. RENGEL (2000) showed that more intensive fertilization of soil with zinc not only raised the content of that element in *Holcus lanatus*, but also lowered its concentrations of iron, manganese and copper. In turn, CIEĆKO et al. (2004b and 2006) concluded that soil contamination with cadmium depressed the content of lead in aerial parts of maize and roots of radish, or potassium in oat grain and in the aerial parts and roots of yellow lupine and radish.

In treatments where copper had been applied simultaneously with another heavy metal, toxicity of the applied mixtures of metals decreased in the following order: CuNi >CuPb >CuZn>CuCr>CuCd; with two metals: CuZnNi >CuZnPb>CuZnCd >CuZnCr; and with three metals: CuZnNiCr>CuZnNiPb>CuZnNiCr (WYSZKOWSKA et al. 2006a). In another study, WYSZKOWSKA et al. (2007) concluded that the yield of oat had declined significantly on soil polluted with a mixture of metals (NiZnCuPbCdCr).

EFFECT OF CADMIUM, COPPER AND ZINC ON SOIL MICROORGANISMS

Among the factors which influence life in soils are heavy metals (HUANG, SHINDO 2000), which permeate into the soil environment from a variety of sources (KHAN, SCULLION 2002) and substantially modify soil properties. For this reason, they are a severe problem to the whole ecosystem and to organisms which live in it (BELYAEVA et al. 2005).

Ecologically, the accumulation of elements in soil is dangerous because of their possible delayed re-mobilization (OLIVEIRA, PAMPULHA 2006, DE BRUWERE et al. 2007, MERTENS et al. 2007). Toxicity and bioavailability of heavy metals depends on their chemical form and quantities present in a given habitat (LEIROS et al. 1999, LOSKA, WIECHUŁA 2000). Other factors are the temperature, oxidative and reductive potential, presence of anions and cations of other metals and pH (SŁABA, DŁUGOŃSKI 2002).

The results reported by EGLI et al. (2010) and JIANG et al. (2010) indicate that cadmium, copper and zinc can disrupt the microbiological equilibrium of soil. Diverse effects produced by these heavy metals on individual groups of microbes result from specific physiological, morphological and genetic characteristics of the former (CHMIELOWSKI, KŁAPCIŃSKA 1984, BINET et al. 2003, RENELLA et al. 2006, PAUL et al. 2007).

Regarding the metals essential for the proper course of cellular processes, such as copper, zinc or iron, there are mechanisms which regulate their cellular capture. Toxic metals, however, like mercury, cadmium or lead, do not have any specific transport methods. BRIDGES and ZALPUS (2005) explain the mechanism engaged in the penetration of some heavy metals into cells according to the concept of molecular mimicry, in which metal ions are bound by biomolecules. Molecular mechanisms depend strongly on proteins characterized by specific affinity for copper and cadmium.

Disturbances of the biological balance of soil caused by excess of cadmium, copper and zinc might be attributed to the disruption of physiological functions, denaturation of proteins and destruction of cellular membranes of soil microorganisms (CHMIELOWSKI, KŁAPCIŃSKA 1984, ULBERG 1997, KUCHARSKI et al. 2000, LEDIN 2000, BINET et al. 2003, KUCHARSKI, WYSZKOWSKA 2004, RENELLA et al. 2006, ZABOROWSKA et al. 2006). On the one hand, soil bacteria immobilize heavy metals. On the other hand, they contribute to the enhanced mobility of heavy metals, a result caused mainly by microbial metabolites (GILLER et al. 1998, DUMESTRE et al. 1999, LUGAUSKAS et al. 2005, KUFFNER et al. 2008, HE et al. 2010a, b), which is why certain strains of microorganisms are increasingly often employed in phytoremediation (PAUL et al. 2007, RAJKUMAR, FREITAS 2008, HE et al. 2010a, b).

Soil pollution with heavy metals in different quantities and forms causes changes in the counts of microorganisms and activity of microbial enzymes, which is a true reflection of the actual microbiological condition of soil (DICK et al. 2000, TRASAR-CEPEDA et al. 2000, WYSZKOWSKA et al. 2007).

Heavy metals create abiotic stresses (GILLER et al. 1998, LUGAUSKAS et al. 2005, HE et al. 2010a, b) by inducing disorders in the metabolism of microorganims. They can cause the denaturation of proteins and disintegration of cellular membranes (BROOKES et al. 1984). The destructive effect of metals involves some damage to the control systems regulated by regulatory and signal proteins, including the cell's development, apoptosis and regulation of the cellular cycle (BEYERSMANN, HARTWIG 2008). According to NIES (1999) and SCHMIDT et al. (2005), the toxic effect of metals could be due to the blocking of enzymatic active centres and driving away cations important for the functioning of a cell, supplanting their functions, e.g. discontinuation of the cell-to-cell adhesion (cadmium), direct binding with the DNA (chromium), interacting with the binding sites of protein phosphatases (vanadium) (BEYERSMANN, HARTWIG 2008). According to SKŁODOWSKA (2000), cadmium can supplant zinc, while zinc can replace magnesium in cellular structures of microorganisms. These processes might cause mutations. This effect was verified for cadmium acting on *Bacillus subtilis* (PACHA, GALIMSKA-STYPA 1986).

Microorganisms are characterized by high adaptability to undesirable environmental conditions. Tolerant species demonstrate higher resistance to stress factors than sensitive ones (RENSING et al. 2002). Their tolerance is associated with such metabolic functions as:

1) specific transport of metal ions which involves permeases localized in the cytoplasmic membrane (CHMIELOWSKI, KŁAPCIŃSKA 1984, ULBERG 1997, BINET et al. 2003);
2) synthesis and excretion to the environment chelating compounds, which bind and transport ions dissolved in the environment (CHMIELOWSKI, KŁAPCIŃSKA 1984, RENELLA et al. 2006, PAUL et al. 2007);
3) non-specific accumulation of metals: sorption of ions onto mucosal surfaces and the binding by bio-polymers of the wall and membrane complex (CHMIELOWSKI, KŁAPCIŃSKA 1984, LEDIN 2000);
4) presence of plasmids in a bacterial cell, which enable it to acquire resistance to toxic elements: Ag, As, Cd, Cr, Hg, Ni, Sb, Te (ZHANG et al. 2001, MEGURO et al. 2005). The *Rhizobium* bacteria possessing more plasmids are more tolerant to heavy metals than cells of the same species with fewer plasmids (LAKZIAN et al. 2002).

There can be different target sites of accumulation inside cells. Blue-green algae and yeasts accumulate metals in vacuoles, often in the form of polyphosphate granules (CHMIELOWSKI 1991, BINET et al. 2003). The transfer of metals in the form of a PC (*phytochelatina*) - CdS complex into vacuoles is associated with the occurrence of an ABC family transporter, coded by the htm (heavy metal tolerance) 1 gene (OW 1996). Microorganisms can also disarm heavy metals by changing their oxidation degree or converting into a volatile form through methylation (CHMIELOWSKI 1991, BINET et al. 2003).

Many researchers (GILLER et al. 1998, KUCHARSKI, WYSZKOWSKA 2004, LUGAUSKAS et al. 2005, ZABOROWSKA et al. 2006, WYSZKOWSKA et al. 2008, BOROS et al. 2011) demonstrated that cadmium, copper and zinc, when present in excessive quantities in soil, cause disorders in the microbiological balance of soil. Most common are decreasing counts and diminishing diversity of microorganisms (KUCHARSKI 1992, MOFFETT et al. 2003, RENELLA et al. 2005a,b, KHAN et al. 2006, LORENZ et al. 2006, XIE et al. 2009, WYSZKOWSKI, WYSZKOWSKA 2009, WAKELIN et al. 2010). MOFFET et al. (2003) found 25% lower biodiversity of taxonomic groups in soil contaminated with 400 mg Zn kg^{-1} versus soil with the natural content of zinc (57 mg kg^{-1} of soil). Several references, e.g. GILLER et al. 1998, SINGHA et al. 1998, SAUVE et al. 1999, CELA, SUMNER 2002, WYSZKOWSKA et al. 2006a, WYSZKOWSKA et al. 2007, indicate that nitrifying bacteria, symbiotic nitrogen-fixing bacteria and *Azotobacter* spp are the microorganisms most susceptible to heavy metals. Similar conclusions were drawn by LUGAUSKAS et al. (2005), BOROWIK et al. (2013) as well as OLIVEIR and PAMPULH (2006). Heavy metals produce a stronger effect on *Azotobacter* cells than organotrophic bacteria mainly because richer communities of microbes are more resistant to heavy metals than single species and genera (LOC, JANSSEN 2005, MERTENS et al. 2010).

SINGHA et al. (1998) report that cadmium is a 6- to 8-fold stronger inhibitor of nitrification and ammonification than zinc. The power of nitrification was reduced to 86.1%, under the influence of heavy metals while ammonification was depressed down to 44.2% of the unaffected level. GUPTA and CHAUDHRY (1994) proved that conversion of $N\text{-}NH_4$ to $N\text{-}NO_3$ was inhibited by metal ions in the following order Hg > Zn > Ni > Pb. The negative influence of zinc on nitrification is most probably due to the direct toxic effect of excess zinc on nitrifying bacteria (RUYTERS et al. 2010) as well as the toxic influence of the metal on enzymes responsible for nitrification (TREVISAN et al. 2012). The adverse effect of zinc, copper and cadmium on autochthonic soil microorganisms is confirmed by a wealth of references (KUCHARSKI, WYSZKOWSKA 2004, ZABOROWSKA et al. 2006, WYSZKOWSKA et al. 2007, WYSZKOWSKA et al. 2008, RUYTERS et al. 2010).

According to WYSZKOWSKA and KUCHARSKI (2003a), the inhibitory effect of heavy metals on soil microorganisms can be represented as follows:

oligotrophic bacteria: (Ni > Pb > $Cr_{(III)}$ > Cu > Zn > Cd),

copiotrophic bacteria: (Cd > Ni > $Cr_{(III)}$ > Zn > Cu), ammonifying bacteria: (Ni > Pb > $Cr_{(III)}$ > Cd > Zn > Hg),

nitrogen immobilizing bacteria: (Zn > $Cr_{(III)}$ > Hg > Cu),

actinomycetes: (Cu > $Cr_{(III)}$ > Ni > Zn > Pb).

Also, SMYŁŁA (1995) established series of metals according to their toxic effect on actinomycetes of the genus *Streptomyces* (Hg > Cd > Cu > Zn > Ni > Pb).

There are also reports (LOC, JANSSEN 2005, LIU et al. 2007) suggesting that under certain conditions heavy metals can stimulate higher counts of microbial cells in soil, which may be a result of the succession of microorganisms. Such contrary effects of heavy metals on microorganisms can be a result of the varied composition of metabolites produced by microorganisms (MEGURO et al. 2005, WYSZKOWSKA et al. 2007). Products of metabolism form chelates with different metals or change into permanent deposits.

According to LIU et al. (2007), the way heavy metals act depends on their type and rate. In an experiment reported by the cited authors, cadmium applied in rates from 1 to 200 mg kg^{-1} raised counts of actinomycetes and fungi, while depressing numbers of bacteria. According to LIU et al. (2007) and KHAN et al. (2010), bacteria are more sensitive to heavy metals than actinomycetes or fungi. However, lead doses above 50 mg kg^{-1} decreased counts of bacteria, actinomycetes as well as fungi. WYSZKOWSKA and KUCHARSKI (2003a) demonstrated a significant increase in counts of fungi in soil contaminated with zinc and copper at a rate of 500 mg kg^{-1} of soil, while WAKELIN et al. (2010) identified changes in the structure of a bacterial community dwelling in soil with excess copper.

Many references (WYSZKOWSKA, WYSZKOWSKI 2002, YANG et al. 2007, CASTALDI et al. 2009) prove that counts of soil microbes can also be determined by species of grown crops. YANG et al. (2007), VOGELER et al. (2008) and CASTALDI et al. (2009) provide evidence that crops can moderate the influence of heavy metals on soil microbes. The experiments reported by the quoted researchers unquestionably show that crops improve the microbiological activity of soil, mainly owing to substances secreted by roots. RENELLA et al. (2006) created a simplified, artificial rhizosphere, which enabled them to demonstrate that root secreta such as glucose, glutamic acid, citric acid, oxalic acid and their mixtures have a significant albeit varied effect on the growth of microorganisms.

RAJKUMAR and FREITAS (2008) concluded that bacteria resistant to heavy metals can be more successfully used in phytoremediation because they add to a better solubility of heavy metals and can be taken up in larger quantities by plants known as hyperaccumulators. Similar conclusions have been arrived at by HE et al. (2010a, b) and PAUL et al. (2007).

YUANGEN et al. (2006) state that cadmium, copper and zinc cause disorders in the soil respiration and depress the biomass of microorganisms. These researchers suggest that both parameters can be useful for evaluation of the degree of soil contamination with the metals TEJADA (2009) demonstrates that the biomass of microorganisms, mass of earthworms and number of nematodes decrease in soils polluted with cadmium in doses from 100 to 1000 mg kg^{-1} of soil. The negative influence of cadmium on microorganisms was alleviated by application of organic and natural fertilizers. The latter research suggests that organic substance is a good, strategic element in remediation of soils polluted with heavy metals. Particularly helpful for soil remediation is organic matter with a high content of humic acids. The important role of organic substance in remediation of soils polluted with heavy metals has been pointed to by other scholars as well (MARZADORI et al. 2000, RENELLA et al. 2005a, RENELLA et al. 2006, PÉREZ-DE-MORA et al. 2006, TEJDA et al. 2008, TRASAR-CEPEDA et al. 2008, CASTALDI et al. 2009, LI et al. 2009, MORENO et al. 2009, EGLI et al. 2010).

A study conducted by KELLY et al. (2003) in the vicinity of a zinc plant proves that heavy metal contamination has a negative effect on mycorrhizal fungi, Gram positive bacteria and other fungi and actinomycetes. The results suggest that metals also change the structure of microorganisms, which can be restored by effective soil remediation.

LOC and JANSSEN (2005) prove that soil contamination with zinc causes disappearance of sensitive microorganisms, thus raising the counts of zinc tolerant cells. In their study, the physiological diversity of microbial communities decreased as the zinc contamination degree increased. According to WANG et al. (2010), Gram positive bacteria are more susceptible to heavy metals than Gram negative ones. These authors ranked heavy metals with respect to their toxicity towards microorganisms as follows: Cr > Pb > As > Co > Zn > Cd > Cu. On the other hand, GRABOWSKI et al. (1997) decided that the negative influence of heavy metals on microorganisms should be ordered in the following series, according to the increasing toxicity of each subsequent metal: Cu > Pb > Zn > Cd > Hg > Ni > Co > $Cr_{(VI)}$.

The above information univocally proves that excessive rates of cadmium, copper and zinc interfere with the homeostasis of soil, disturbing the control mechanisms on the level of genes, thus inhibiting the activity of enzymatic proteins. Rates of heavy metals above the norm cause damage to metabolic pathways, often resulting in apoptosis of cells. Consequently, counts and diversity of macro- and microorganisms change. Counts of microorganisms in soil are an indirect indicator of the soil's biological activity (WYSZKOWSKA, KUCHARSKI 2003a, LUGAUSKAS et al. 2005, RENELLA et al. 2006). Heavy metals decrease biomass of microorganisms and reduce their activity in soil (WYSZKOWSKI 2002, LUGAUSKAS et al. 2005, MIN et al. 2005, WYSZKOWSKA, WYSZKOWSKA et al. 2008). In cases when they do not lower counts of microorganisms, they still reduce their diversity (MOFFETT et al. 2003, RENELLA et al. 2005a, b, KHAN et al. 2006, LORENZ et al. 2006, WANG et al. 2007, XIE et al. 2009, WAKELIN et al. 2010). LOC and JANSSENA (2005) claim that although the tolerance of microorganisms to soil pollution with heavy metals is a new concept in ecotoxicology and the mechanism involved in this phenomenon has not been completely recognized, it is undeniable that the physiological diversity of microorganisms decreases as the contamination with heavy metals increases. The reason is higher morbidity of sensitive cells under stress conditions, which favours an increase in counts of more tolerant microbes. The research reported by LORENZ et al. (2006), KHAN et al. (2010) or WAKELIN et al. (2010) also shows that heavy metals alter the structure of bacterial communities in soils.

EFFECT OF CADMIUM, COPPER AND ZINC ON SOIL ENZYMES

Soil contamination with heavy metals alters counts and diversity of microorganisms, but also changes the enzymatic activity of soil, which – as many authors claim (KUCHARSKI 1997, DICK et al. 2000, TRASAR- CEPEDA et al. 2000, WYSZKOWSKA et al. 2005a, b, LIU et al. 2007, GULSER, ERDROGAN 2008, VOGELER et al. 2008, MORENO et al. 2009, FU et al. 2009, LEE et al. 2009, XIE et al. 2009, JIANG et al. 2010, WYSZKOWSKA et al. 2013) – is an objective manifestation of the biological status of soil. Among enzymes secreted by microorganisms to soil important are the ones which take part in degradation of plant residues and in transformations of nitrogen, phosphorus and sulphur compounds (KUCHARSKI 1997, WYSZKOWSKA, WYSZKOWSKI 2003, WYSZKOWSKA et al. 2005b, KUCHARSKI, WYSZKOWSKA 2010, BIELIŃSKA et al. 2013). In the environment, the most important functions are performed by the enzymes which belong to oxidoreducates: dehydrogenases and catalase, and to hydrolases: acid phosphatase, alkaline phosphatase, urease, arylsulphatase and β-glucosidase (KUCHARSKI 1997, RENELLA et al. 2006, KUMPIENE et al. 2009, WYSZKOWSKI, WYSZKOWSKA 2009, DICK et al. 2000).

Some heavy metals are essential for enzymes to function properly. Zinc appears in over 300 enzymes, which belong to six classes (MCCALL et al. 2000). Trace elements in enzymes play a triple function: catalytic, structural and regulatory. Many intracellular enzymes could not function well without zinc. Such enzymes include carbon anhydrase, carboxypeptidase, thermolysine, alkaline phosphatase, dehydrogenases (glyceraldehyde-3-phosphate, alcohol, glutamine), fructo-diphosphate aldolases, superoxide dismutase, DNA and RNA polymerase, tRNA transferase. Zinc can stabilize their protein structure, or else act as its activator or inhibitor (CORDOVA, ALVAREZ-MON 1995). The effect of heavy metals on soil enzymes can be direct or indirect. The direct influence consists in changing the activity of free, extracellular enzymes; the indirect influence is produced by affecting the biosynthesis of enzymes by microorganisms, composition of soil microorganisms, mycorrhizae, production of root excreta or release of enzymes from dead roots (CORDOVA, ALVAREZ-MON 1995, MCCALL et al. 2000, HINOJOSA et al. 2008).

These natural functions of zinc can be distorted when the metal appears in excessive amounts. Moreover, cadmium demonstrates a high degree of similarity to zinc ions, which means it can replace zinc in many biocomplexes and change their biological activity (VIG et al. 2003).

In general, heavy metals, including cadmium, copper and zinc, depress the activity of soil metals if present in excessive amounts (DJUKIC, MANDIC 2006, WYSZKOWSKA et al. 2006b, WYSZKOWSKI, WYSZKOWSKA 2006, GULSER, ERDROGAN 2008, VOGELER et al. 2008, KUCHARSKI et al. 2009, LEE et al. 2009, WYSZKOWSKA et al. 2010, JIANG et al. 2010, KUCHARSKI et al. 2011), although there are exceptions. For example, KÝZÝKAYA et al. (2004) reported an experiment in which the activity of dehydrogenases and catalase as well as respiration declined under the influence of excess cadmium and copper, but the activity of urease remained unchanged. CHAPERON and SAUVE (2008) showed that both cadmium and copper inhibit the activity of dehydrogenases as well as urease. Cadmium applied in doses of 1, 10, 20 and 50 mg Cd kg^{-1} and copper in doses of 50, 250, 500 and 1000 mg kg^{-1} produced a stronger inhibitory effect when applied singly than in conjunction. This observation is not always confirmed, as the study completed by YANG et al. (2006) revealed that cadmium and zinc produced a synergic effect on urease, catalase and alkaline phosphatase. Urease, in turn, is the most sensitive enzyme with respect to the tested metals. KHAN et al. (2006) noticed that cadmium and lead are of little importance in respect of the activity of soil enzymes (dehydrogenases, alkaline phosphatase, catalase). This conclusion was most probably formulated because of the small scale of contamination in the cited experiment, which was 1,5 mg, 3 mg and 5 mg Zn kg^{-1}, and 150 mg, 300 mg and 500 mg Pb kg^{-1}.

KUNITO et al. (2001) drew our attention to the fact that different metals inhibited enzymatic activity in different ways. The activity of dehydroegnases, urease and β-glucosidase was more strongly inhibited by zinc fractions extracted by nitric acid than by copper. In an experiment run by WANG et al. (2007), the activity of dehydrogenase declined in soil contaminated with 10 mg Cd kg^{-1}, whereas the same metal did not inhibit urease. SUVE et al. (1999) verified that soil contamination with lead and copper may also retard nitrification. Such undesirable events can be prevented by introducing to soil phytostabilizing substances, e.g. zeolite and lime (CASTALDI et al. 2009, KUMPIENE in. 2009).

According to RENELL et al. (2005a), the activity of enzymes in soils polluted with heavy metals depends on the structure of pollutants. They arrived at this conclusion based on experiments which involved soil fertilization with sludge polluted with nickel and cadmium or manganese and zinc. Sludge containing nickel and cadmium depressed the activity of phosphatases, β-glucosidase and arylsulphatase, while sludge polluted with manganese and zinc lowered the activity of arylphosphatase alone. Both types of sludge had a stimulating effect on protease, while the activity of urease was unaffected by either type of soil amending substance. The cited authors also noticed that presence of some heavy metals in sludge could be a serious obstacle to its utilization. Similar conclusions were presented by VIOG et al. (2003).

EPELDE et al. (2008) found out that by growing the hyperaccumulating plant called *Thlaspi caerulescens* on soil contaminated by zinc and cadmium, it was possible to attain a higher activity of β-glucosidase, arylsulphatase, acid phosphatase, alkaline phosphatase and urease, although the two heavy metals did not cause unambiguous inhibition of the mentioned enzymes in uncropped soil.

A study conducted by CASTALDI et al. (2004) proves that the activity of dehydrogenases, sulphatase, glucosidase and the respiratory activity in soil decreased exponentially as the content of heavy metals in soil increased, while the activity of protease and urease was not significantly correlated with the content of these metals.

WANG et al. (2006) observed that the activity of alkaline phosphatase, arylsulphatase, nitrification and respiration was significantly negatively correlated with the content of cadmium and zinc, or aluminum and manganese, while being positively correlated with the content of calcium and level of pH. The activity of acid phosphatase was negatively correlated with the content of calcium, magnesium and pH but positively – with the content of aluminum, cadmium, manganese and zinc. CHAPERON and SAUVE (2007) concluded that copper and zinc are inhibitors of dehydrogenases and urease, but were even more strongly affected by silver and mercury. KHAN et al. (2010) demonstrated that cadmium had a negative effect on both microorganisms and the activity of phosohatases and urease. In turn, SPEIR et al. (1999) proved that cadmium and nickel are stronger inhibitors than copper, zinc and chromium (III).

LIU et al. (2007) tested cadmium applied in doses from 5 to 200 mg kg^{-1} and noticed that it inhibited the activity of urease and phosphatases; however, in the doses of 5 and 10 mg kg^{-1}, the metal stimulated the activity of catalase, causing an evident inhibition of that enzyme only when introduced to soil at a rate of 200 mg kg^{-1}. Cadmium added to soil in doses from 5 to 100 mg kg^{-1} stimulated the activity of invertase, but inhibited that enzyme when applied in higher doses. LORENZ et al. (2006) and KUCHARSKI et al. (2011) claim that the adverse effect of heavy metals on the microbiological and biochemical activity of soil is persistent. Twenty-five years after polluting soil with cadmium in amounts of 50 and 250 mg kg^{-1}, it was found to contain 34 and 134 mg Cd kg^{-1}, respectively. The PCR analysis showed that the structure of bacteria was different from that observed in unpolluted soil. Under the influence of cadmium, the activity of alkaline phosphatase, arylphosphatase, protease and urease declined. In contrast, the activity of xylanase either did not change or increased, being correlated to the content of fungal quinones and *Proteobacteria*. Microorganisms were exceptional in that they did not respond negatively to the contamination (LORENZ et al. 2006).

MARZADORI et al. (2000) claim that the destructive influence of copper can be alleviated by humic acids with a high molecular weight (100-300 kDa). Such acids proved to be good stabilizers of the activity of urease. They also protected that enzyme from attacks by proteases. In general, the activity of soil enzymes was higher in cropped than in uncropped soil (PÉREZ-DE-MORA et al. 2006, Castaldi et al. 2009). This regularity is attributed to the positive role played by substances exerted by roots, which moderate effects of heavy metals on soil's enzymatic performance (Renella et al. 2005a, Pérez-de-Mora et al. 2006, Tejada et al. 2008, Wyszkowska et al. 2009). Another reason is the uptake of metals by plants (Rajkumar, Freitas 2008). The ameliorating influence of plants on heavy metals affecting the soil metabolism has been implied by other researchers (Renella et al. 2006, Epelde et al. 2008, Jiang et al. 2010, Wyszkowska et al. 2010). Also, a study by Chaudhuri et al. (2003) prove that introduction of organic substance to soil limits the extent of the negative effect of heavy metals on activity of dehydrogenases, urease, acid phosphatase and arylsulphatase.

A review of the relevant literature shows that many researchers (Welp 1999, Nowak et al. 2003, Wyszkowska, Kucharski 2003b, Wyszkowska et al. 2006a) have attempted, with a different degree of success, to determine series of enzymes with respect to their sensitivity to heavy metals. Below are some of the results. Sensitivity of:

– dehydrogenases, according to Welp (1999) is: Hg (2 mg) > Cu (35 mg) > Cr^{6+} (71 mg) > Cr^{3+} (75 mg) > Cd^{2+} (90 mg) > Ni^{2+} (100 mg) > Zn^{2+} (115 mg) > As^{3+} (168 mg) > Co^{2+} (582 mg) > Pb^{2+} (652 mg kg^{-1}), and according to Wyszkowska and Kucharski (2003b): Cu^{2+} > Zn^{2+} > Cr^{6+}> Hg^{2+} > Ni^{2+} > Cd^{2+} > Cr^{3+}, Wyszkowska et al. (2006a): Cr^{6+}>Cd^{2+}>Zn^{2+}>Pb^{2+}>Cu^{2+}>Ni^{2+}.;
– acid phosphatase – according to Nowak et al. (2003): Cu^{2+} > Al^{3+} > Cd^{2+} > Zn^{2+} > Fe^{3+}> Ni^{2+} > Pb^{2+} > Sn^{2+} > Fe^{2+} > Co^{2+}, and according to Wyszkowska and Kucharski (2003b): Cu^{2+} > Ni^{2+} > Zn^{2+} > Cd^{2+} > Cr^{3+} > Cr^{6+} > Hg^{2+}; Wyszkowska et al. (2006a): Cr^{6+}> Ni^{2+}>$Cu^{2+}$$Cd^{2+}$> Pb^{2+}>Zn^{2+};
– alkaline phosphatase – according to Nowak et al. (2003): Cd^{2+} > Al^{3+} > Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+} > Ni^{2+} > Fe^{2+} > Se^{2+} > Co^{2+}, and according to Wyszkowska and Kucharski (2003b): Zn > Cu^{2+} > Ni^{2+} > Hg^{2+} > Cr^{6+}, Wyszkowska et al. (2006a): Cd^{2+}>Ni^{2+}>Cu^{2+}> Zn^{2+}>Cr^{6++}>Pb^{2}.

Differences in the above series may have been caused by differences in the content of the silt fraction in analyzed soils, which absorbed different amounts of both heavy metals and some of the enzymes (Boyd, Mortland 1985). Another reason could have been the different degrees of soil contamination analyzed by the cited authors.

All the factors which alter the activity of soil enzymes also modify enzymes responsible for oxidation of ammonia nitrogen (Trevisan et al. 2012). The same effect is produced by heavy metals (Mertens et al. 2010), but in that case it is accompanied by the toxic influence of heavy metals on nitrifying bacteria (He et al. 2012). The adverse effect of heavy metals on the ammonifying process has also been indicated by Antil et al. (2001) or Hund-Rinke and Simon (2008), while Suve et al. (1999), Yin et al. (2003), Hund-Rinke and Simon (2008) and Vogeler et al. (2008) reported their adverse influence on nitrification.

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