SUMMARY

Alders (*Alnus* spp.) are widely used for bioremediation of polluted land and are well known for positive effects of their cultivation in degraded areas. An example is the Legnica-Głogów Copper Region, where copper ores have been mined intensively for many decades.

This study was aimed to compare responses of black alder (*Alnus glutinosa* (L.) Gaertn.) and grey alder (*Alnus incana* (L.) Moench) of three provenances (Krzyż, Rzepin, Wolsztyn) to short-term and long-term exposure (1–2 growing seasons, i.e. 23–72 weeks) to phytotoxic concentrations of trace metals (Cu, Cd, Zn, and Pb). The substrate was collected in the buffer zone of Copper Smelter Głogów in Żukowice. Also effects of an additional stress factor were studied, namely of periodical flooding of the substrate (resulting from exposure to floods caused by neighbouring rivers) on the growth and development of grey and black alder grown in the substrate collected in the buffer zone of Copper Smelter Głogów in Wróblin.

The above aims were realised by investigation of properties, mineral composition, and biochemical parameters (concentration of total soluble phenolics) and enzymatic ones (activity of dehydrogenases, phosphomonoesterases, proteases, and urease) of the applied substrates. The evaluation involved various characteristics of plant material: morphological (mineral composition), physiological (chlorophyll content), and biochemical (analyses of oxidative stress: $O_2^{\bullet-}$, H_2O_2, degree of lipid peroxidation; enzymatic reactions: SOD, CAT, APX, GPX, GR; and non-enzymatic antioxidants: ascorbate). Also the symbiosis of alder roots with actinobacteria (*Frankia* sp.) was studied: the efficiency of N_2 binding was analysed on the basis of acetylene reduction assay, as a crucial process affecting alder growth and development.

Short-term (23 weeks) and long-term (72 weeks) exposure of two alder species to phytotoxic concentrations of trace metals (Cu, Cd, Zn, and Pb) in the soil showed that alders accumulated toxic concentrations of Cu and high levels of Zn, Cd, and Pb in fine roots. Besides, in above-ground parts (shoots), concentrations of Cu, Pb, Cd, and Zn were increased. Thus alders can be classified as species capable of stabilization of metals (Cu, Pb, and Cd) in the rhizosphere and phytoextraction of Zn from polluted soil.

During cultivation of both alder species, many important enzymatic processes were slowed down (including those catalysed by dehydrogenases, phosphomonoesterases, proteases, or urease) in the rhizosphere of the substrate polluted with Cu, Cd, Zn, and Pb. The intensity of N_2 binding by actinobacteria in root nodules was also reduced. After one growing season, no remarkable changes in seedling biomass were observed. After two growing seasons, the biomass of shoots and the whole seedling significantly decreased. Cell membrane integrity

in fine roots of alders did not seem to be affected, in spite of the observed lower efficiency of enzymatic antioxidants (SOD, APX, CAT, and GPX).

A probable defence strategy of alder in response to the toxic trace elements in the soil was the increased secretion of root exudates, rich in phenols, neutralizing the negative effects of phytotoxic levels of Cu, Cd, Zn, and Pb. It resulted in a reduction of plant biomass after long-term exposure to stress.

The experiment investigating the response of alders to the additional stress factor, i.e. periodical flooding of the substrate polluted with toxic trace metals (cumulative stress), revealed that the decrease in intensity of many enzymatic processes in the soil was even greater (dehydrogenases, alkaline phosphomonoesterases, proteases, urease). Similar morphological and physiological changes were recorded in both alder species (decreased rate of shoot and root biomass growth, lowered total chlorophyll content) but simultaneously an effective symbiosis with actinobacteria was maintained. The observed increased levels of reactive oxygen species under the influence of flooding of the substrate polluted with trace metals did not disturb cell membrane integrity in fine roots of grey alder thanks to maintenance of an effective system of enzymatic defence. By contrast, in fine roots of black alder, membrane lipid peroxidation increased. The experiment with periodical flooding of the substrate confirmed earlier observations (from the experiments conducted for 1–2 growing seasons) about the influence of root exudates on alder tolerance to the stress caused by toxic trace metals.

The presented results indicate that alders are useful for phytoremediation of industrial wastelands, additionally exposed to the risk of floods caused by neighbouring rivers.