



# Application of white mustard and oats in the phytostabilisation of soil contaminated with cadmium with the addition of cellulose and urea

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## Abstract

**Purpose** Determination of the effectiveness of white mustard and oats in immobilising cadmium as a soil contaminant and determining the role of cellulose and urea in restoring homeostasis in soil under pressure from  $\text{Cd}^{2+}$ .

**Materials and methods** Soil samples were contaminated with cadmium ( $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ ) at 0, 4, 8 and 16  $\text{mg Cd}^{2+} \text{ kg}^{-1}$ . In order to reduce the negative impact of  $\text{Cd}^{2+}$ , cellulose was introduced to the soil at the following rates: 0 and 15  $\text{g kg}^{-1}$  and urea at 80 and 160  $\text{mg N kg}^{-1}$ . The yield of the above-ground parts and roots was determined on days 40 and 80 of the experiment, along with the cadmium content in the plant material. The enzyme activity was also determined, and the physical and chemical properties of the soil were determined on the day of the oats' (aftercrop) harvest.

**Results and discussion** Contamination of soil with  $\text{Cd}^{2+}$  at 4 to 16  $\text{mg kg}^{-1}$  d.m. of soil reduced the yield of white mustard and oats. The tolerance index (TI) values indicate that oats (aftercrop) is more tolerant than white mustard of soil contamination with  $\text{Cd}^{2+}$ . Cadmium accumulated more intensely in roots compared with the above-ground parts of the plants. The translocation index (TF) indicates smaller  $\text{Cd}^{2+}$  translocation from roots to above-ground parts, as it was below 1 in both plants. An addition of cellulose and nitrogen offsets the adverse impact of cadmium on plants. Arylsulphatase was the most sensitive to soil contamination with  $\text{Cd}^{2+}$ , followed by dehydrogenases, catalase,  $\beta$ -glucosidase and urease, and alkaline phosphatase and acid phosphatase were the least sensitive. Contamination of soil with  $\text{Cd}^{2+}$  changed its physical and chemical properties only slightly.

**Conclusions** White mustard and oats have phytostabilisation potential with respect to soil contaminated with cadmium. Cellulose introduced to the soil and fertilisation with urea alleviated the negative impact of cadmium on the growth and development of plants.

**Keywords**  $\text{Cd}^{2+}$  · Physical and chemical properties · Plant yield · Tolerance index (TI) · Translocation factor (TF) · Soil enzymes

## 1 Introduction

Cadmium is a common element in the earth's crust. Although from a chemical point of view, it is similar to zinc; unlike zinc, cadmium is not biologically essential. The use of cadmium in industry (e.g. for the production of car batteries, fluorescent materials, rubber, paint, pigments, nickel-cadmium batteries) and the development of civilisation and urbanisation may lead

to the significant exposure of the natural environment to contamination with cadmium (Satarug et al. 2010). Cadmium salts are relatively soluble, which is why this element can migrate in the natural environment. Moreover, cadmium forms soluble complexes with organic compounds, which increases its mobility (Prca et al. 2012; Lorenc-Plucińska et al. 2013; Aghababaei et al. 2014). Therefore, solutions are being sought to counteract these processes. Areas contaminated with heavy metals require decontamination action. Therefore, phytoremediation may be one of the measures taken in such areas. It is not costly and it has no negative impact on the environment (Roccotiello et al. 2010; Cheraghi et al. 2011). Phytostabilisation, i.e. the ability of plants to take up, store and immobilise heavy metals by binding them to bioactive molecules, is one of the methods of phytoremediation (Sas-Nowosielska et al. 2008; Cheraghi et al. 2011; Dalvi and

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Bhalerao 2013). The mechanism of immobilisation involves prevention of metal ion transport across the cellular membrane and their inactivation by binding within the cell or modification to less toxic species. Interception of toxic heavy metal ions in plant cell cytosol and their transport is mediated by polypeptides rich in –SH groups, such as phytochelatins, metallothioneins and glutathione. Transport of metals bound in complexes along the tonoplast to the vacuoles is mediated mainly by the metal/H<sup>+</sup> antiport or the ABC transporter (ATP-binding cassette), or the ZIP transporter family (iron-, zinc-regulated transporter-like proteins). Heavy metals are immobilised in the tonoplast by their binding to organic acids (mugineic acid, phytic acid) or to sulphides (Kabata-Pendias and Mukherjee 2007; Wu et al. 2010; Dalvi and Bhalerao 2013). Phytostabilisation can be boosted by applying heavy metal immobilising additives to soil. Such additives introduced to the soil may include inorganic or organic substances which increase the soil pH, enhance the heavy metal binding effect, decrease bioavailability and toxicity by complex formation or metal precipitation, and increase the organic matter content and the ability to retain water (Masto et al. 2008; Mench et al. 2010; Gómez-Sagasti et al. 2012; Burns et al. 2013; Bolan et al. 2014). A significant element in the phytostabilisation process is the selection of suitable plant species. Such plants should be characterised by tolerance to high cadmium soil contamination, the ability to form a compact plant cover on a soil surface, have a well-developed root system, increased accumulation of cadmium in the roots, and low nutritional and habitat requirements (Mench et al. 2010; Zou et al. 2012). Grasses exhibiting most of the above properties are most frequently used in heavy-metal phytostabilisation. Moreover, some crops such as wheat, oats, maize and white mustard are used in phytoremediation (Knox et al. 2001; Yoon et al. 2006; Zhang et al. 2010). Therefore, a study was conducted aimed at determining the effectiveness of white mustard and oats in immobilising cadmium as a soil contaminant. Additionally, nitrogen and cellulose was introduced to the soil to support phytostabilisation. Cellulose is a polysaccharide and the main component of the plant cell wall structure. Post-harvest plant residues in soil contain approx. 45% of cellulose. To achieve intensive cellulose decomposition in soil by microorganisms, appropriate requirements must

be met (the C/N ratio, water content in the soil, soil pH). An adequate amount of nitrogen should be supplied to the soil to ensure a desired C/N ratio. Following the introduction of cellulose into the soil, the availability of carbon is in abundance. Under such conditions, microorganisms multiply and reduce the mineral nitrogen pool, while the deficiency in mineral nitrogen retards cellulose transformation (Haddad et al. 2019). In order to determine the condition of soil contaminated with cadmium, activity of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase,  $\beta$ -glucosidase and arylsulphatase, and the physical and chemical properties of soil were determined in the soil samples. Since cadmium has a negative impact on the soil microbiome, a study hypothesis was put forward that plants can reduce the adverse effect of cadmium on soil biological properties by ‘trapping’ the metal in their roots and above-ground parts. Cellulose and nitrogen were additionally introduced to the soil to support the plants in it.

## 2 Materials and methods

### 2.1 Study area characterisation

The land used for the study is situated in Tomaszkowo near Olsztyn in the Voivodship of Warmia and Mazury in the north-east of Poland, central Europe (53.7161° N, 20.4167° E). The soil for analyses was collected from the 0–20-cm layer. It was subsequently dried at room temperature and sieved through a 1-cm mesh sieve. In terms of granulation, it was classified as sandy clay loam; detailed characteristics are included in Table 1.

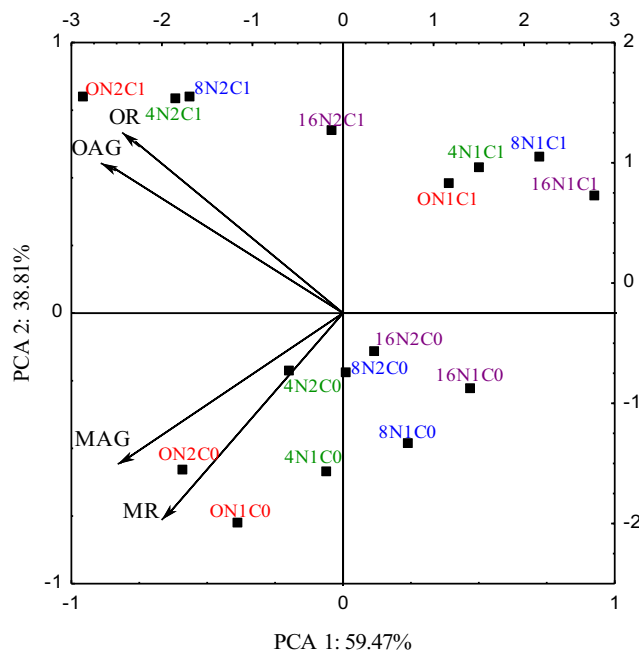
### 2.2 Pot experiment

The experiment was carried out in the vegetation hall of the University of Warmia and Mazury, Olsztyn, in north-eastern Poland. Soil was placed in 3.5-kg pots; earlier, cadmium chloride was added to the soil at 4, 8 and 16 mg Cd<sup>2+</sup> kg<sup>-1</sup>, cellulose at 15 g kg<sup>-1</sup>, and urea at 80 and 160 mg N kg<sup>-1</sup>. Pots with soil uncontaminated with cadmium and without cellulose addition were control objects. Fertilisation was also applied: N

**Table 1** Physicochemical properties of the soils used in the experiment

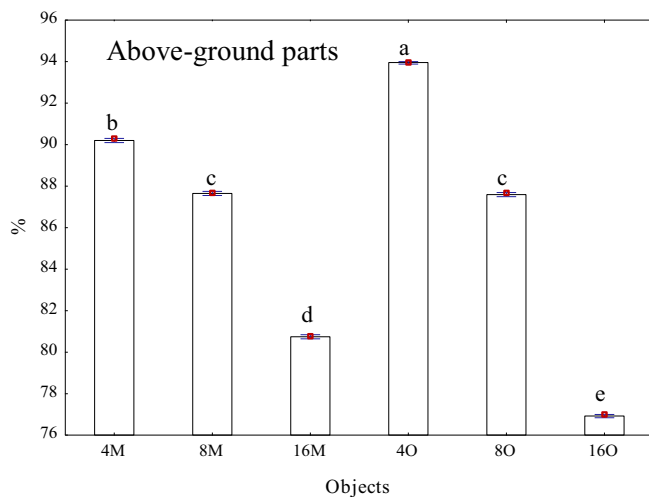
Type of soil	Granulometric composition (w mm)			$C_{org}$ g kg <sup>-1</sup>	$N_{total}$	pH <sub>KCl</sub>	HAC	EBC	CEC	BS (%)
	< 0.002 %	0.002– 0.050	0.050– 2.000							
scl	1.49	17.95	80.56	14.30	0.98	7.00	6.40	165.90	172.30	96.29

scl sandy clay loam,  $C_{org}$  total organic carbon,  $N_{total}$  total nitrogen, HAC hydrolytic acidity, EBC total exchangeable cations, CEC total exchange capacity of soil, BS basic cations saturation ratio in soil



**Fig. 1** Principal component analysis (PCA) of plant yield in soil contaminated with cadmium. Dose  $Cd^{2+}$  mg  $kg^{-1}$  d.m. of soil: 0–0 mg, 4–4 mg, 8–8 mg and 16–16 mg. Cellulose: C0 without cellulose, C1 with cellulose. Dose N mg  $kg^{-1}$  d.m. of soil: N1–80 mg; N2–160 mg. Plant species: M white mustard, O oat. Plant parts: AG above-ground parts, R roots. ◀ – indicates variables; ■ – indicates cases

80 and 160 mg  $[CO(NH_2)_2]$ , P 21 mg  $[KH_2PO_4]$ , K 73 mg  $[KH_2PO_4 + KCl]$ , and Mg 15 mg  $[MgSO_4 \cdot 7H_2O]$ . The first nitrogen dose was adjusted to suit the plants’ nutritional requirements. Being the simplest organic substance in terms of molecular structure, cellulose was used in the experiment as an additional source of carbon for microorganisms. The content of total organic carbon and total nitrogen was taken into account when establishing the cellulose and nitrogen rate. The soil was subsequently mixed thoroughly and placed in the



**Fig. 2** Percentage reduction of the above-ground parts and roots of plants, regardless of cellulose addition and nitrogen rate,  $n = 3$ . Dose  $Cd^{2+}$  mg  $kg^{-1}$  d.m. of soil: 0–0 mg, 4–4 mg, 8–8 mg and 16–16 mg.

pots. Twenty seeds of white mustard (*Sinapis alba*) var. Rota were sown. After germination, the plant number was reduced to eight per pot. After 40 days, above-ground parts and roots were harvested, and oats (*Avena sativa* L) var. Bingo was sown as the aftercrop. After germination, the number of plants was reduced to 12 per pot and they were harvested after another 40 days. The soil moisture content was maintained at 50% of the capillary water capacity to ensure the proper growth and development of the plants. The experiment was performed in four replicates.

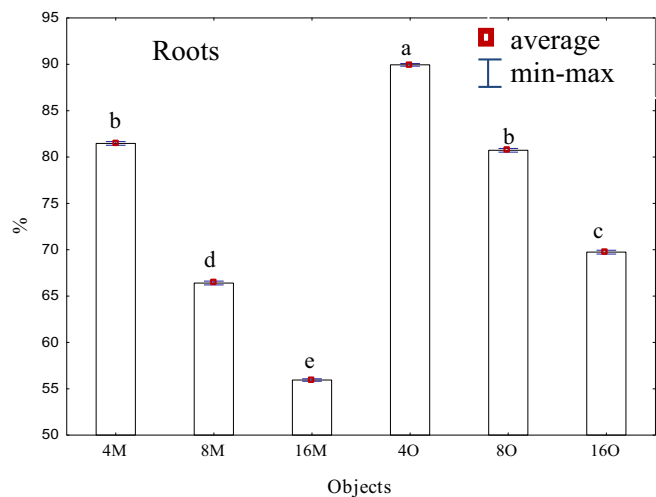
### 2.3 Characteristics of cellulose and urea

The cellulose tested in the study was obtained from ACROS ORGANICS. It was extra pure cellulose, with a particle size of 90  $\mu m$ . The nitrogen was introduced to the soil as analytically pure urea  $CH_4H_2O$  which had been obtained from the P.P.H ‘STANLAB’, Poland.

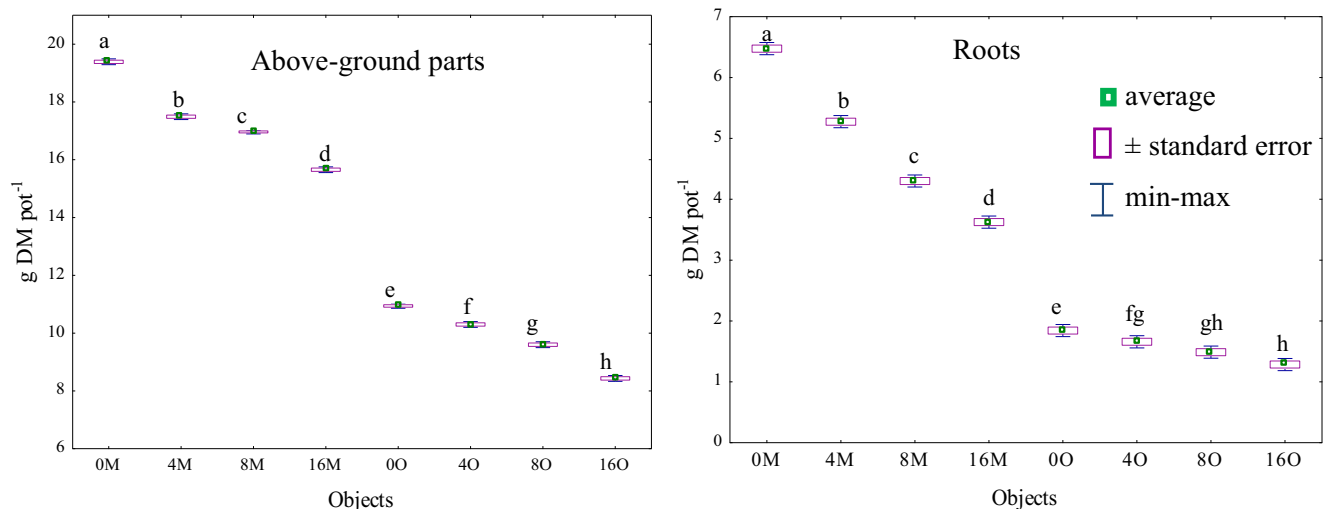
### 2.4 Methodology of determination of the soil physical and chemical properties and cadmium level in plants

Activity of the following soil enzymes was determined in soil samples after harvesting white mustard (day 40) and oats (day 80):

- Dehydrogenase ( $\mu mol$  TFF  $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- Catalase ( $mol$   $O_2$   $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- Urease ( $mmol$  N- $NH_4$   $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- Acid phosphatase ( $mmol$  PNP  $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- Alkaline phosphatase ( $mmol$  PNP  $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- $\beta$ -Glucosidase ( $mmol$  PNP  $kg^{-1}$  d.m.  $h^{-1}$  of soil)
- Arylsulphatase ( $mmol$  PNP  $kg^{-1}$  d.m.  $h^{-1}$  of soil)



Plant species: M white mustard, O oat. The same letters (a–e) are assigned to the same homogeneous groups



**Fig. 3** Effect of soil contamination with cadmium on plant yield, regardless of cellulose addition and nitrogen rate,  $n = 3$ . Dose  $Cd^{2+}$   $mg\ kg^{-1}$  d.m. of soil: 0–0 mg, 4–4 mg, 8–8 mg and 16–16 mg. Plant species: M white mustard, O oat. The same letters (a–h) are assigned to the same homogeneous groups

The detailed procedures by which the activity of these enzymes is determined are described by Boros-Lajszner et al. (2018).

The following physical and chemical properties of soil were determined after harvesting oats (aftercrop):

- pH of the soil, total organic carbon, hydrolytic acidity, total exchangeable base cations, total cation exchange capacity of soil and soil saturation with base cations. The detailed procedures by which these determinations were made are described by Boros-Lajszner et al. (2018).
- Cadmium content in above-ground parts and roots was determined by atomic absorption spectroscopy (AAS) after microwave mineralisation as per the standard PN-EN 14084:2004 (N).

### 2.5 Calculations and statistical analysis

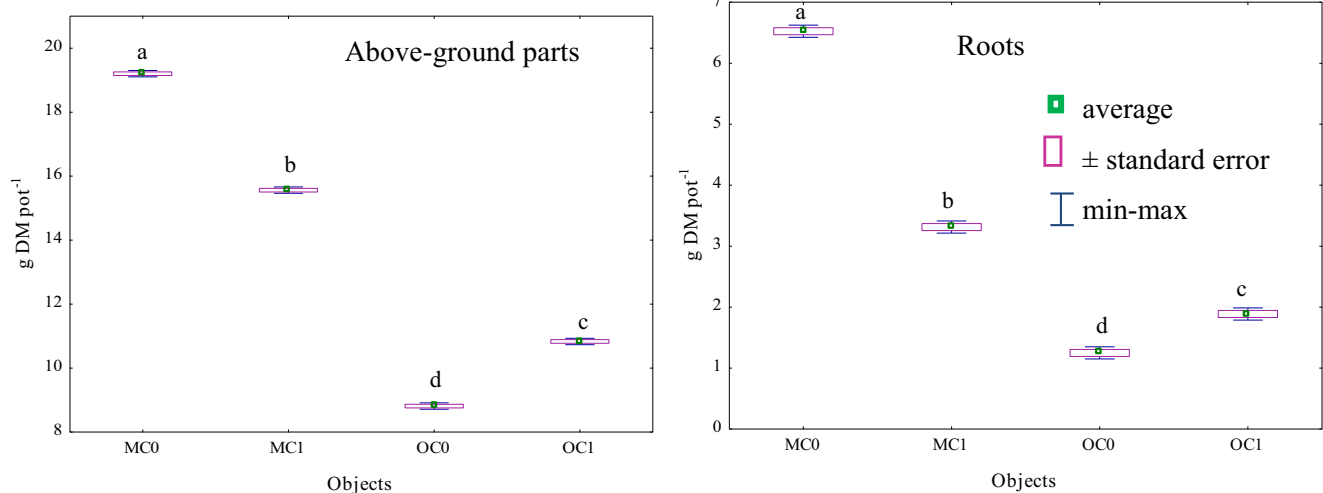
The tolerance of white mustard and oats to an excess of  $Cd^{2+}$  was compared using a tolerance index, denoted as TI. It was calculated as the ratio of the plant yield from contaminated soil to the plant yield from non-contaminated soil (control).

They were calculated from the following formula:

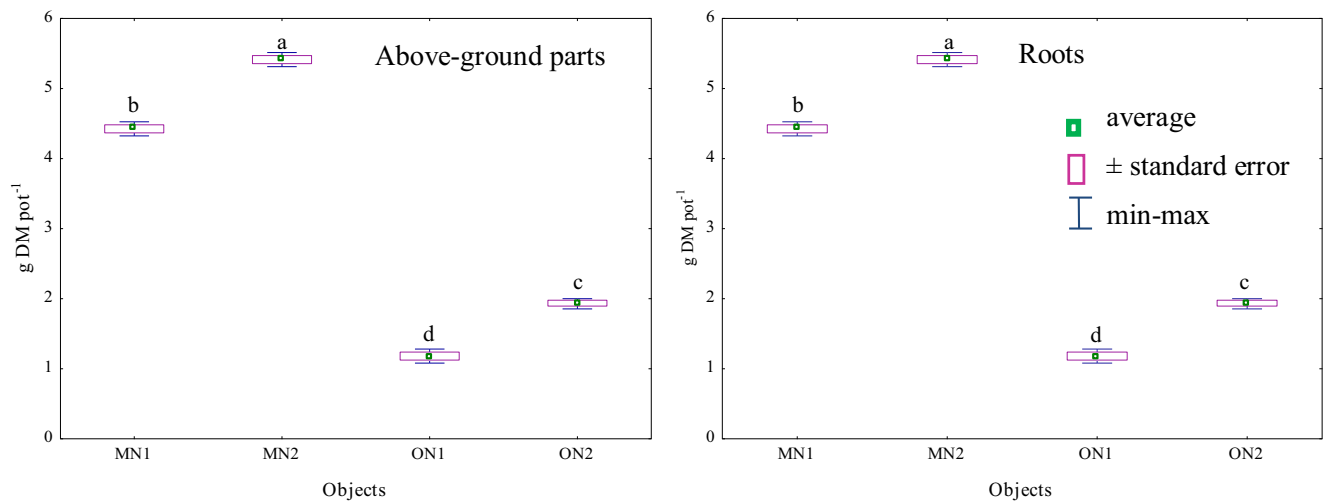
$$TI = \frac{Y_{Cd}}{Y_C}$$

where:

$Y_{Cd}$  mean plant yield from soil contaminated with  $Cd^{2+}$   
 $Y_C$  mean plant yield from soil non-contaminated with  $Cd^{2+}$



**Fig. 4** Effect of cellulose addition on plant yield, regardless of cadmium and nitrogen rate,  $n = 3$ . Cellulose: C0 without cellulose; C1 with cellulose. Plant species: M white mustard, O oat. The same letters (a–d) are assigned to the same homogeneous groups



**Fig. 5** Effect of nitrogen rate on plant yield, regardless of cadmium rate and cellulose addition,  $n = 3$ . Dose N mg kg<sup>-1</sup> d.m. of soil: N1–80 mg and N2–160 mg. Plant species: M white mustard, O oat. The same letters (a–d) are assigned to the same homogeneous groups

The following was calculated from cadmium content in the above-ground parts and roots:

$$TF = \frac{Cd_{AG}}{Cd_R}$$

where:

Cd<sub>AG</sub> cadmium content in the above-ground parts of plants  
 Cd<sub>R</sub> cadmium content in the plant roots

The most important phytoremediation parameter is the tolerance factor (TF), whose value for plants should not exceed 1. Low values of this parameter reflect a poorly functioning system of cadmium transport from the roots to the above-ground parts.

STATISTICA 13.1 software (StatSoft 2018) was used to analyse the results statistically with the analysis of variance ANOVA at  $P \leq 0.05$ . Homogeneous groups were determined with the Tukey test. The above-ground and root yield is shown using principal component analysis (PCA).

### 3 Results

#### 3.1 Plant growing

The phytotoxicity of cadmium, which showed as a decrease in the biomass yield, varied depending on the plant species and the level of soil contamination with the metal. The plants reacted to Cd<sup>2+</sup> by reducing both the above-ground and root biomass (Fig. 1). The principal components explain a total of 98.28% of the variance of the primary variables; PCA 1 explains 59.47% and PCA 2 38.81%. It was observed that two homogeneous groups were formed around the principal components. The first group is made up of vectors representing the above-ground parts and the root yield of white mustard and others consisting of the above-ground parts and the root yield of oats. Vectors deployed along the coordinate axes indicate that there is a negative impact of cadmium on the plant yield, despite the application of cellulose and nitrogen. Biomass of

**Table 2** Tolerance index (TI) based on plant yield

Plant parts	Plant species			
	White mustard		Oat	
	Dose of nitrogen (mg kg <sup>-1</sup> )			
	N <sub>80</sub>	N <sub>160</sub>	N <sub>80</sub>	N <sub>160</sub>
Without cellulose				
Above-ground parts	0.827 <sup>b</sup> ± 1.253	0.817 <sup>b</sup> ± 0.564	0.872 <sup>b</sup> ± 0.633	0.879 <sup>a</sup> ± 0.743
Roots	0.690 <sup>d</sup> ± 1.202	0.703 <sup>c</sup> ± 0.143	0.695 <sup>d</sup> ± 0.452	0.848 <sup>b</sup> ± 0.222
With cellulose				
Above-ground parts	0.874 <sup>a</sup> ± 0.611	0.947 <sup>a</sup> ± 0.412	0.883 <sup>a</sup> ± 0.844	0.832 <sup>c</sup> ± 1.532
Roots	0.701 <sup>c</sup> ± 0.504	0.623 <sup>d</sup> ± 0.123	0.807 <sup>c</sup> ± 0.651	0.825 <sup>d</sup> ± 0.191

The same letters (a–d) in columns are assigned to the same homogeneous groups,  $n = 3$

**Table 3** Cadmium content ( $\text{mg kg}^{-1}$  d.m.) in above-ground parts and roots of plants and translocation factor (TF)

Dose $\text{Cd}^{2+}$ ( $\text{mg kg}^{-1}$ of soil)	Above-ground parts	Roots	TF
White mustard			
Without cellulose			
0	$0.770^e \pm 0.101$	$5.137^f \pm 0.671$	$0.150^d \pm 0.012$
16	$22.503^a \pm 2.433$	$54.881^b \pm 7.682$	$0.410^a \pm 0.011$
With cellulose			
0	$0.449^f \pm 0.062$	$1.371^g \pm 0.192$	$0.327^b \pm 0.013$
16	$11.407^b \pm 1.363$	$43.690^c \pm 6.733$	$0.261^c \pm 0.012$
Oat			
Without cellulose			
0	$0.073^h \pm 0.014$	$0.652^h \pm 0.093$	$0.112^e \pm 0.013$
16	$5.719^c \pm 0.563$	$79.119^a \pm 8.511$	$0.072^h \pm 0.014$
With cellulose			
0	$0.185^g \pm 0.033$	$5.590^e \pm 0.662$	$0.033^i \pm 0.011$
16	$3.446^d \pm 0.152$	$34.338^d \pm 5.731$	$0.100^{fg} \pm 0.013$

The same letters (a–i) in columns are assigned to the same homogeneous groups,  $n = 3$

the above-ground parts of the plants under study decreased compared to the control with increasing heavy metal content in soil. Reduction of the above-ground biomass of white mustard and oats was similar. White mustard biomass decreased significantly by 10%, 12% and 19% for 4, 8 and 16  $\text{mg Cd}^{2+} \text{ kg}^{-1}$  of soil, regardless of the addition of cellulose and nitrogen as urea (Fig. 2). Biomass of the oats' above-ground parts decreased by 6%, 12% and 23%. The root biomass decreased to a greater extent than the above-ground parts for all plants under study. The greatest yield reduction was observed for white mustard. The biomass of white mustard roots decreased significantly by 19% ( $-4 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ ), by 34% ( $-8 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ ) and by 44% ( $-16 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ ) compared to the control, respectively. The respective values for oats as the aftercrop were the following: 10%, 19% and 30%. Figure 3 presents the effect of soil contamination with cadmium on the plant yield, which illustrates how increasing metal doses decreased the above-ground parts and roots' biomass of white mustard and oats, regardless of the addition of cellulose or nitrogen. This was particularly manifested in the case of roots. Cellulose introduced to the soil alleviated the negative impact of the heavy metal on the growth and development of oats (aftercrop), as the yield of the above-ground parts and roots in pots with an addition of cellulose was higher by 19% and 34% compared to the pots without the sorbent addition. It was the reverse in pots with white mustard, because the yield of above-ground parts and roots was higher in the pots without an addition of cellulose by 19% and 33%, respectively (Fig. 4). The application of nitrogen in two rates, i.e. 80 and 160  $\text{mg N kg}^{-1}$ , was also important, as a higher yield of the above-ground parts of white mustard (by 16%) and oats (by 33%) was observed, and that of roots (by 20% and 50%, respectively) in pots with a higher nitrogen rate compared to

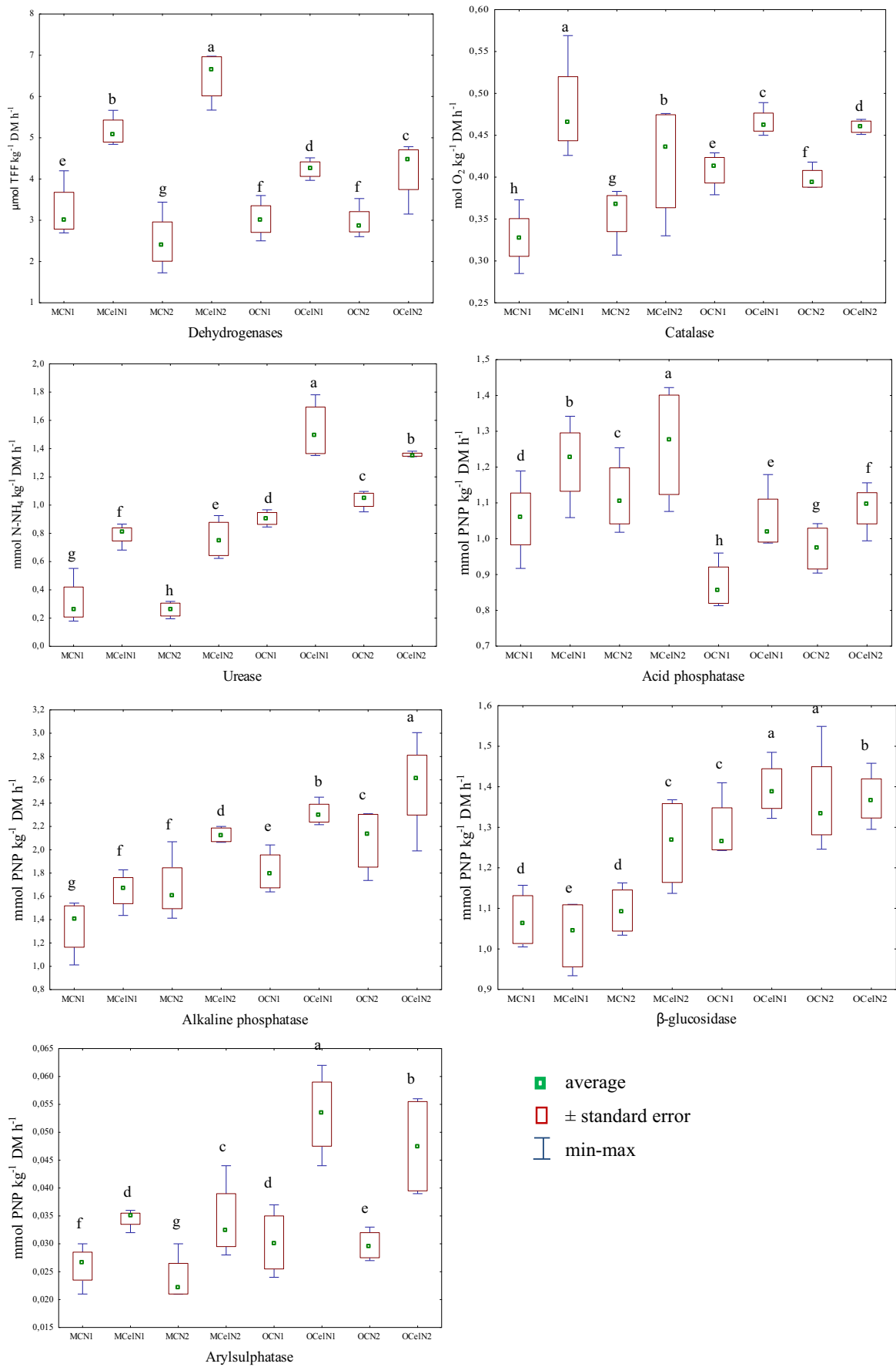
pots in which 80  $\text{mg N kg}^{-1}$  was applied (Fig. 5). The tolerance index (TI) values indicate that oats (aftercrop) were more tolerant than white mustard of soil contaminated with  $\text{Cd}^{2+}$ . They ranged from 0.695 to 0.883 for oats, and from 0.623 to 0.874 for white mustard, except the pot with the addition of cellulose and nitrogen at 160  $\text{mg N kg}^{-1}$ , where the TI was calculated as 0.947 (Table 2).

### 3.2 Cadmium content evaluation

The cadmium content in the above-ground parts and roots of white mustard and oats increased with the heavy metal rate (Table 3). More  $\text{Cd}^{2+}$  accumulated in the above-ground parts and roots of the plants in pots without the addition of cellulose than with an addition. Irrespective of the cellulose addition and cadmium contamination, more of this metal accumulated in pots with oats than with white mustard. More cadmium was accumulated in roots than in above-ground parts of the plants, regardless of the plant or cellulose addition.

The metal mobility in white mustard and oats was determined by means of the translocation factor (TF), which was calculated from cadmium content in the above-ground parts and roots of the plants (Table 3). For white mustard, it was higher by 36% in pots without cellulose with the cadmium rate of 16  $\text{mg kg}^{-1}$  compared to the pots with cellulose. It was the reverse in pots with oats. This indicates an increase in the heavy metal mobility from roots to the above-ground parts caused by the cellulose application. Higher values of TF were

**Fig. 6** Activity of enzymes in soil contaminated with cadmium,  $n = 3$ . M white mustard, O oat, C control soil, Cel cellulose, N1 nitrogen dose 80  $\text{mg kg}^{-1}$  d.m. of soil, N2 nitrogen dose 160  $\text{mg kg}^{-1}$  d.m. of soil. The same letters (a–h) are assigned to the same homogeneous groups



observed in pots with white mustard than in those with oats. This may be attributed to the fact that oats were the aftercrop. The translocation factor was  $< 1$ , and it was much lower for oats than for white mustard.

### 3.3 Enzymatic assays

Cadmium changed the biochemical properties of the soil throughout the experiment, decreasing the activity of the enzymes under study (Fig. 6). The highest cadmium rate ( $16 \text{ mg Cd}^{2+} \text{ kg}^{-1}$ ) decreased the activity of arylsulphatase by 28%, dehydrogenases by 26%, catalase by 25%,  $\beta$ -glucosidase by 23%, urease by 19%, acid phosphatase by 13% and alkaline phosphatase by 6% compared to the control, regardless of the plant and the nitrogen rate. The addition of cellulose to the soil improved the soil biochemical properties. It was particularly manifested in soil non-contaminated with cadmium. The effect of cellulose added to the soil on the activity of dehydrogenases, catalase and acid phosphatase was more manifested in pots with white mustard than in those with oats as the aftercrop. It was the reverse in the case of the other enzymes, i.e. the enzyme activity was higher in soil in which oats grew. Nitrogen fertilisation had no effect on the enzyme activity. In general, higher activity of dehydrogenases, catalase, urease and acid phosphatase was observed in soil with white mustard growing in it than in soil with oats, and the activity of alkaline phosphatase,  $\beta$ -glucosidase and arylsulphatase was higher in soil with oats growing in it, regardless of the level of soil contamination with cadmium.

### 3.4 Soil physicochemical investigations

Physicochemical properties of soil contaminated with increasing doses of cadmium are shown in Table 4. The soil pH changed only slightly with increasing cadmium contamination, regardless of the cellulose and nitrogen addition. The content of organic carbon in pots with cellulose was higher by ca. 5% compared to pots without cellulose, regardless of the nitrogen rate. Total exchangeable base cations also decreased with increasing contamination with the heavy metal. It was higher in pots with cellulose, particularly in those with the lower nitrogen rate (from  $122.45$  to  $127.50 \text{ mmol}^{(+)} \text{ kg}^{-1}$  d.m. of soil). Hydrolytic acidity increased with increasing level of soil contamination with cadmium. As with total exchangeable base cations, hydrolytic acidity was higher by 33% in pots with cellulose and  $80 \text{ mg N kg}^{-1}$  compared to pots without cellulose. Hydrolytic acidity and total exchangeable base cations were used to calculate the total cation exchange capacity of soil (CEC) and soil saturation with base cations (BS). These parameters decreased with increasing contamination with  $\text{Cd}^{2+}$ . Higher values of CEC were observed in pots with cellulose compared to those without it. It was the reverse for BS.

## 4 Discussion

### 4.1 Plants

Higher plants have developed the ability to absorb and neutralise heavy metals (Rascio and Navari-Izzo 2011; Zheng et al. 2011). The literature (McGrath and Zhao 2003; Ali et al. 2013; Dalvi and Bhalerao 2013) describes two methods employed by plants to cope with such contaminants. In one of them, the absorption of heavy metals is blocked by roots. The other is the opposite of the first one, as it involves taking up, storing and immobilising heavy metals by binding them to bioactive molecules. The latest research focuses on the latter method of heavy metal neutralisation, including cadmium, by growing white mustard and oats as an aftercrop. The study has shown that oats are more tolerant of soil contamination with  $\text{Cd}^{2+}$  compared to white mustard. Rascio and Navari-Izzo (2011), and Zheng et al. (2011) report that there are plant species which are more tolerant of soil contamination with cadmium and which do not exhibit any symptoms of toxicity. This may result from the formation of phytochelatins, i.e. proteins which neutralise cadmium phytotoxicity by its binding. Studies conducted by these authors have shown that more cadmium is accumulated in roots than in the above-ground parts of plants. According to Masarovicova et al. (2010), this may be attributed to the fact that cadmium transport from the underground to above-ground parts was, to some extent, inhibited as a result of triggering the mechanisms of defence against stress caused by an excess of cadmium in the soil. Calculated in this study, TF indicates that white mustard and oats have the potential for phytostabilisation, as plants with low translocation factor ( $\text{TF} < 1$ ) are suitable for phytostabilisation (Roccotiello et al. 2010; Cheraghi et al. 2011). Padmavathiamma and Li (2007) found that plants which can be used for phytostabilisation must be characterised by a low translocation factor, which was confirmed in a study by the authors of this paper. In the process of phytostabilisation, cadmium is accumulated in plant roots, adsorbed on their surface and precipitates in the rhizosphere area. In this way, the negative effect of cadmium on soil is reduced. Immobilisation of heavy metals may also be enhanced by an addition of organic matter, clay minerals, carbonates or phosphates to the soil as well as by a decrease in soil acidification (Schnoor 2000; Jabeen et al. 2009). In this study, cellulose alleviated the negative impact of  $\text{Cd}^{2+}$  on the growth and development of white mustard and oats. It reduced the cadmium content in the above-ground parts and roots of the plants. Although a positive effect of cellulose was observed by these authors, according to literature reports (O'Connell et al. 2008; Sud et al. 2008; Hashim et al. 2011), non-modified cellulose has a low capacity for heavy metal adsorption and variable physical stability. Therefore, studies are being conducted of chemical cellulose modification in



**Table 4** Physicochemical properties of the soil

Dose Cd <sup>2+</sup> (mg kg <sup>-1</sup> of soil)	pH <sub>KCl</sub>	C <sub>org</sub> g kg <sup>-1</sup> d.m. of soil			HAC mmol <sup>(+)</sup> kg <sup>-1</sup> d.m. of soil			EBC			CEC			BS %			
		80	160	80	160	80	160	80	160	80	160	80	160	80	160	80	160
<b>Without cellulose</b>																	
0	7.13 <sup>b</sup> ± 0.05	7.07 <sup>bc</sup> ± 0.05	13.25 <sup>bc</sup> ± 0.04	13.70 <sup>b</sup> ± 0.08	6.50 <sup>c</sup> ± 0.18	6.13 <sup>c</sup> ± 0.18	124.17 <sup>ab</sup> ± 5.14	122.33 <sup>a</sup> ± 5.44	130.67 <sup>c</sup> ± 5.04	128.46 <sup>a</sup> ± 5.31	95.03 <sup>a</sup> ± 4.87	95.23 <sup>a</sup> ± 4.95					
4	7.07 <sup>b</sup> ± 0.05	6.97 <sup>c</sup> ± 0.05	13.10 <sup>cd</sup> ± 0.03	12.70 <sup>d</sup> ± 0.08	6.88 <sup>c</sup> ± 0.18	6.63 <sup>bc</sup> ± 0.18	120.33 <sup>ab</sup> ± 2.36	118.33 <sup>bc</sup> ± 2.87	127.21 <sup>d</sup> ± 2.14	124.96 <sup>b</sup> ± 2.52	94.59 <sup>ab</sup> ± 2.01	94.69 <sup>ab</sup> ± 2.26					
8	7.07 <sup>b</sup> ± 0.05	6.97 <sup>c</sup> ± 0.05	12.80 <sup>e</sup> ± 0.02	12.60 <sup>d</sup> ± 0.08	7.00 <sup>c</sup> ± 0.18	6.88 <sup>b</sup> ± 0.18	117.83 <sup>ab</sup> ± 4.37	116.67 <sup>cd</sup> ± 2.36	124.83 <sup>e</sup> ± 2.99	123.55 <sup>c</sup> ± 2.21	94.39 <sup>b</sup> ± 2.81	94.43 <sup>b</sup> ± 2.02					
16	7.02 <sup>b</sup> ± 0.01	6.69 <sup>d</sup> ± 0.09	12.20 <sup>f</sup> ± 0.08	12.10 <sup>e</sup> ± 0.08	7.09 <sup>c</sup> ± 0.07	6.94 <sup>b</sup> ± 0.15	116.67 <sup>b</sup> ± 0.94	115.59 <sup>d</sup> ± 0.36	123.76 <sup>e</sup> ± 0.56	122.53 <sup>cd</sup> ± 0.11	94.27 <sup>bc</sup> ± 0.39	94.34 <sup>b</sup> ± 0.09					
<b>With cellulose</b>																	
0	7.57 <sup>a</sup> ± 0.05	7.37 <sup>a</sup> ± 0.05	13.60 <sup>a</sup> ± 0.03	14.30 <sup>a</sup> ± 0.08	8.50 <sup>b</sup> ± 0.18	8.75 <sup>a</sup> ± 0.18	127.50 <sup>a</sup> ± 2.04	120.83 <sup>ab</sup> ± 3.12	136.00 <sup>a</sup> ± 2.69	129.58 <sup>a</sup> ± 2.89	93.75 <sup>c</sup> ± 2.32	93.25 <sup>c</sup> ± 2.76					
4	7.53 <sup>a</sup> ± 0.05	7.33 <sup>a</sup> ± 0.05	13.35 <sup>b</sup> ± 0.04	13.75 <sup>b</sup> ± 0.04	10.50 <sup>a</sup> ± 0.10	8.88 <sup>a</sup> ± 0.18	125.17 <sup>ab</sup> ± 2.66	117.00 <sup>c</sup> ± 4.08	135.67 <sup>a</sup> ± 3.45	125.88 <sup>b</sup> ± 3.97	92.26 <sup>cd</sup> ± 3.59	92.95 <sup>c</sup> ± 3.68					
8	7.53 <sup>a</sup> ± 0.05	7.33 <sup>a</sup> ± 0.05	13.15 <sup>bcd</sup> ± 0.04	13.65 <sup>b</sup> ± 0.04	11.00 <sup>a</sup> ± 0.18	8.88 <sup>a</sup> ± 0.18	123.50 <sup>ab</sup> ± 1.23	114.50 <sup>de</sup> ± 0.41	134.50 <sup>ab</sup> ± 1.03	124.38 <sup>bc</sup> ± 0.37	91.82 <sup>d</sup> ± 0.89	92.06 <sup>d</sup> ± 0.24					
16	7.40 <sup>a</sup> ± 0.08	7.20 <sup>ab</sup> ± 0.02	13.03 <sup>d</sup> ± 0.13	13.46 <sup>c</sup> ± 0.04	11.05 <sup>a</sup> ± 0.25	8.98 <sup>a</sup> ± 0.04	122.45 <sup>ab</sup> ± 1.94	112.47 <sup>e</sup> ± 1.59	133.50 <sup>b</sup> ± 1.58	122.45 <sup>d</sup> ± 1.25	91.72 <sup>d</sup> ± 1.24	91.85 <sup>d</sup> ± 1.19					

C<sub>org</sub> total organic carbon; HAC hydrolytic acidity; EBC total exchangeable cations; CEC total exchange capacity of soil; BS basic cations saturation ratio in soil, n = 3

The same letters (a–f) in columns are assigned to the same homogeneous groups

order to achieve sufficient structural strength and to make it better able to adsorb heavy metal ions. Higher yield of the above-ground parts and roots of white mustard and oats were observed in pots where nitrogen was applied at 160 mg N kg<sup>-1</sup> d.m. of soil. This may result from the fact that nitrogen is a nutrient needed by plants as a structural element of proteins and nucleic acids. Its presence is a condition of growth and development of crops, it stimulates growth of roots and the above-parts of plants, making them intensely green, i.e. it is the most important nutrient from a practical point of view (Hesse et al. 2004; Dubousset et al. 2009; Kaur et al. 2010; Carfagna et al. 2010; Gill et al. 2012).

#### 4.2 Biochemical, physical and chemical properties of the soil

Biochemical activity of soil is very important for the proper functioning of soil ecosystems, whereby it is regarded as an appropriate indicator in estimating changes caused by soil contamination with heavy metals (Wu et al. 2010; Lombard et al. 2011; Kucharski et al. 2011). This study demonstrated a negative effect of cadmium on enzymatic activity of soil. Zaborowska et al. (2017) also noted an adverse effect of cadmium applied at 40 to 200 mg kg<sup>-1</sup> d.m. of soil on the activity of arylsulphatase, which deepened with an increasing level of soil contamination with Cd<sup>2+</sup>. A toxic effect of cadmium on individual enzymes was also demonstrated by Mikanova (2006) and Zaborowska et al. (2015). As in this study, arylsulphatase proved to be more sensitive to the presence of the metal under study than urease. According to the findings of a study by Wyzkowski and Wyzkowska (2009), soil contamination with cadmium decreased the activity of hydrolases, such as urease, acid phosphatase and alkaline phosphatase. Kavamura and Esposito (2010) demonstrated that the effect of heavy metals, including cadmium, on changes of soil enzyme activity is a result of disturbed function or denaturation of proteins or destruction of the microorganism cell membrane integrity. According to Gu and Yeung (2011), the toxic effect of Cd<sup>2+</sup> on the enzyme structure and their effect changing the soil pH both reduces the enzyme-secreting microorganism count and decreases enzyme activity.

As Muhammad et al. (2012) reported, good parameter soils succumb to a lesser extent to the effect of stress factors, such as the presence of cadmium. The findings of this study confirm it. According to literature reports (Bolan et al. 2014; Stritsis et al. 2014; Li et al. 2016), the level of cadmium in a soil solution depends on the balance between processes of mobilisation and the immobilisation of metals. These processes are controlled by the physicochemical properties of soil (such as pH, exchangeable cation capacity), activity and diversity of soil microorganisms, and the growth and development of plants.

## 5 Conclusions

White mustard and oats exhibited phytostabilisation potential towards cadmium-contaminated soil because the criterion (reducing cadmium transport from roots upwards) was met. White mustard and oats accumulated significantly larger amounts of Cd<sup>2+</sup> in the roots than in the above-ground parts. Cellulose introduced to the soil and fertilisation with nitrogen alleviated the negative impact of cadmium on the growth and development of plants. The highest yield of the above-ground parts and roots of the plants was observed in pots where nitrogen was applied at 160 mg N kg<sup>-1</sup>. Soil contamination with cadmium decreased the activity of all enzymes under study (dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase,  $\beta$ -glucosidase, arylsulphatase). Excessive doses of cadmium decreased the soil pH, total organic carbon, total exchangeable base cations, total cation exchange capacity of soil and soil saturation with base cations, whereas it increased the hydrolytic acidity.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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