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Abstract

Metal-polluted ecosystems usually contain several mixtures and amounts of their compounds, plants are exposed to respective interactions, which have shown to cause additive, synergistic or antagonist effects. The physiological feedback of plants exposed to excess of essential and non-essential metals includes alterations in a wide spectrum of physiological features, such as: water absorption and metal translocation through the plant, content of photosynthetic pigments and photosynthesis functioning, growth and biomass production.

The present study was aimed to assess the physiological responses and tolerance of *V. faba* seeds and seedlings to different experimental concentrations of $CdCl_2$ and $ZnSO_4$ salts and their mixtures. Seed germination capacity, root length and mitotic activity, fresh and dry weights of root and shoot, photosynthetic pigments content and respective tolerance indexes, were evaluated and compared.

The results demostrated metal and mixture dose-dependence of all screened endpoints, suggesting the following phytotoxicity range: Cd+Zn>Cd>Zn on faba seedlings. Concerning Zn and Cd interactions, Zn played a contrasting role against Cd, being antagonistic at a low concentration and synergistic at a high one. Contrariwise whatever the concentration, Cd was synergistic against Zn. Based on the data, the tolerance of faba bean under Cd and Zn stress was relatively moderate and the root appeared to be more sensitive than shoot. The findings should serve as an indicator and an alert of potential risk that biota and human health may incur by natural and anthropogenic discharge of heavy metals into the environment.

Keywords: Cadmium, Cd+Zn interaction, Physiological feedback, Stress tolerance, *Vicia faba* assay, Zinc

Introduction

Uncontrolled human activity has substantially modified the geochemical cycles and biochemical balance of heavy metals in the environment. Being accumulated in sediments, water, soil and ultimately in the biota through food chains, they represent an important eco-toxicological and generally biological concern. Metal-compounds can, when present in excess, or under the wrong conditions and in the wrong places, produce errors in the genetic information system (Patra et al., 2004).

Since soils and irrigation water sources usually contain several mixtures and amounts of heavy metals and their compounds, plants are rarely exposed in nature to the impact of a single heavy metal. As a consequence plants are affected by their respective interactions, which may adversely cause complex effects. The physiological feedback of plants exposed to the excess of essential and non-essential metals includes a wide spectrum of symptoms, such as: water absorption and upsets of mineral nutrition and translocation through the plant, hormonal status and membrane structure and permeability, content of photosynthetic pigments and photosynthesis functioning, growth and biomass production (Patra et al., 2004; Sunil et al., 2013). At a cytological and biochemical level heavy metals disturb redox homeostasis and induce oxidative stress, which damages proteins, membrane lipids and chlorophyll and even DNA leading to genotoxicity (Unyayar et al., 2006; Mithöfer et al., 2004; Wang et al., 2009b; Cuypers et al. 2010, Mesi and Kopliku, 2014).

Among heavy metals, cadmium is a rare but widely dispersed element, found naturally in the environment, mostly as cadmium sulfide or in association with zinc. Environmental pollution with cadmium results mainly from mining, metallurgic industries and manufactures of nickel-cadmium batteries, pigments and plastic stabilizers, Cd-containing phosphate fertilizers, burning fossil fuels and incineration of municipal waste (Bertin and Averbeck, 2006). Cd as a non-essential element is considered a trace pollutant for plants, animals and humans, due to its great solubility in water, long biological half-life and high toxicity (Prasad, 1995; Sandalio et al., 2001; Fargašová, 2004; Iqbal and Shazia, 2004; Nordberg, 2004; Demirevska-Kepova et al., 2006; Sharma and Dubey, 2006; Unyayar et al., 2006; Wang et al., 2009a; Tran and Popova, 2013). Zinc is classified as an essential micronutrient in all living systems, as required in various enzymatic and red-ox reactions and metabolic processes (Rout and Das, 2003; Broadley et al, 2007). As one of the most widely used metals in the world, various anthropogenic sources including: metal manufacturing industries, coal ash from electric utilities, sludge, Zn-based fertilizers and pesticides, atmospheric emissions from vehicles and galvanized products, have progressively increased Zn concentrations in several environments (Alloway and Ayres, 1993). Its toxicity has been documented in humans as well as in a wide range of plants and animals (Reichman, 2002; Paschke, et al., 2006; Nriagu, 2007; Wang et al., 2009b; Tkalec et al., 2014). Reported data concerning toxicity potential of Zn compounds are often contrasting mainly because this metal is redox-stable under physiological conditions.

As targeted physico-chemical analysis cannot normally control and predict the toxic and/or genotoxic properties of metals and their mixtures, they should be combined with biological assays. Higher plant-based assays have shown to be quick, simple, reliable, less expensive and similar in sensitivity to animals, making them ideal for the risk assessment of potential environmental mutagens. *Vicia faba* L. (2n = 12) has long been used as a model plant with multiple endpoints, providing a standardized method in evaluating the induced-toxicity from both organic and inorganic contaminants (Mesi et al., 2012; Barbério, 2013).

Although the discrete toxicity of Cd and Zn is well documented, their interdependent effects remain less understood and even contrasting, due to the complex relationships in biological systems. The present study was aimed to assess the physiological responses and tolerance of *V. faba* seeds and seedlings to different experimental concentrations of $CdCl_2$ and $ZnSO_4$ salts and their mixtures.

Materials and Methods

Vicia faba Assay and Heavy Metal Treatments

Vicia faba L. cv *Aguadulce* was chosen as the test plant. Healthy-looking seeds of uniform size (1.6±0.1 cm) were sterilized with NaOCl 50% and then soaked for 24 h in distilled water. The seeds were allowed to germinate in 18.5 cm Petri dishes between two layers of moist cotton with a Hoagland nutrient medium (used as well as negative control, NC). The tested heavy metals were applied in moderately high, but environmentally relevant concentrations: 5 and 15 μM for Cd as $CdCl_2$ and 20 and 60 μM for Zn as $ZnSO_4$ salts, which have been investigated in our former studies (Kopliku and Mesi, 2014a, b). Treatments included Cd and Zn salts alone and their combination, prepared by adding stock salt solutions to the nutrient medium in suitable amounts to achieve the selected concentrations.

The following endpoints: root length and mitotic activity, seed germination capacity, photosynthetic pigments content, fresh and dry weights of roots and shoots and respective tolerance indexes, were evaluated and compared. Individual sets of 50 seeds were utilized for each toxicity test. The first set was used to evaluate the germination capacity (GC) and the primary root length of the germinated seeds (MRL). The seeds were treated with salts for 72 h under controlled conditions in a thermostat (dark cultivation at 24 °C and 100% humidity). GC was expressed as a percentage of fully germinated seeds to the total number of seeds per champion.

For the cytotoxicity test, after the removal of the primary roots, the untreated 3 day-old seedlings were suspended in aerated growth containers for 4 days to let the secondary roots grow. Exposure time was 6 h for the treated groups followed by a 24 h recovery period. The newly emerged roots, being 1-

2 cm in length, were used for a microscopic analysis. Root tips (10 mm) randomly chosen from each treatment set, were placed on slides and the terminal root tips (1-2 mm) were cut off and used for further preparation. Five slides per treatment were prepared in accordance with the standard procedure for the Feulgen staining of squashed material. By scanning slides, data for the mitotic index (MI) were scored as a percent ratio of the number of dividing cells out of 1000 cells.

Two item champions per treatment were used for physiological investigations, following the normal procedure of the seedling growth. 10 days after sowing, uniform seedlings were transplanted to containers of 5 L covered by a foamed plastic plate. Heavy metal treatments started 7 days after transplanting the seedlings to the basic medium. The nutrient solution in the growth container was continuously aerated and renewed every five days. The first set of fresh seedlings, treated for 3 weeks with heavy metal salts, were used for the evaluation of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids content. 0.1 g shoot fresh weight was homogenized in 2 ml of chilled 80% acetone in a mortar and pestle for the extraction of photosynthetic pigments. Materials were filtered and spectrophotometrically measured at wavelengths of 452.5, 644 and 663 nm and the respective contents of photosynthetic pigments were calculated. After a 4-week treatment, the last set of plantlets was used to evaluate fresh and dry weights. The roots and shoots were separated and their respective fresh weight (RFW and ShFW) were determined. Then the plant materials were dried for 24h at 90°C and the respective dry weights (RDW and ShDW) per champion were recorded. Tolerance indexes of *V. faba* to Cd, Zn and Cd+Zn stresses were calculated by the following formula:

$$\text{Tolerance Index} = \frac{\text{Parameter value of treated seeds or seedlings}}{\text{Parameter value of control seeds or seedlings}} \times 100$$

Statistical Analysis

All experiments were set up in a completely randomized design and the results were expressed as the mean of three replicates per sample ± standard deviation (SD). Analysis of Variance (One-way ANOVA) was used to test for significant differences of evaluated parameters in *V. faba* seeds and seedlings, exposed to the chosen concentrations of Cd and Zn salts and their combinations. Differences between treatments and NC were assumed statistically significant at $P<0.05$ and $P<0.01$.

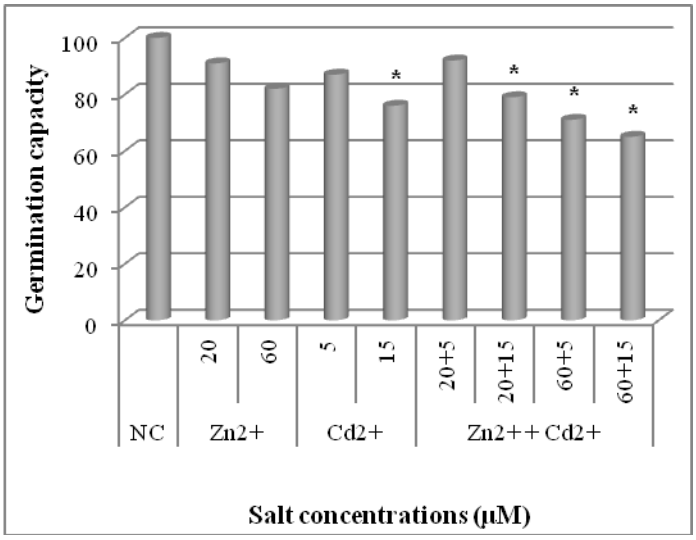
Results

Graphs A-J in Figure 1 and Table 1 summarize all the data related to the analyses of the physiological feedback of *V. faba* seeds and seedlings, treated with $CdCl_2$ (5 and 15 μM) and $ZnSO_4$ (20 and 60 μM) salts and their mixtures. The results revealed a certain dose- and mixture-dependence of all screened endpoints compared to NC (Hoagland nutrient medium), by using a One-way ANOVA test.

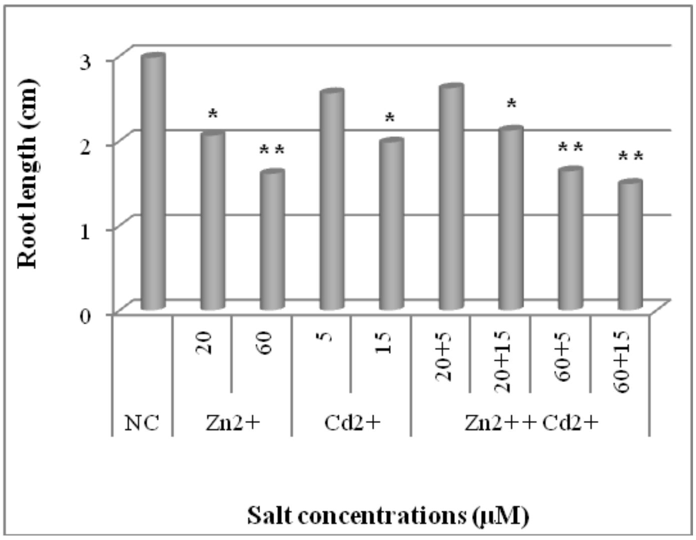
Germination Capacity, Root Length and Mitotic Index

86% of *V. fab*a seeds grown in a negative control solution fully germinated after three days. As illustrated in Figure 1A, the germination was obviously delayed as high as 24% and 21-35% of NC ($P<0.05$), by the application of respectively cadmium alone at the highest concentration and mixtures, except 20 µM Zn with 5 µM Cd. Primary roots of faba seedlings grown in NC had a mean length and mitotic index of 2.98 cm and 10.91%. The significant reduction of seedlings root length ($P<0.05$) was firstly detected at 20 µM of $ZnSO_4$ (31%) and 15 µM of $CdCl_2$ (33%), Figure 1B. The treatment of 60 µM Zn with 15 µM Cd induced the highest inhibitory effect (of 50%, $P<0.01$). On the other hand the addition of 20 µM Zn to both Cd concentrations reduced its adverse effect (of 2-4%). A similar reaction of the faba seedlings that were treated with Zn and Cd heavy metals was observed, related to the mitotic activity of secondary root tip meristem (Figure 1C). The lowest concentrations of both individual treatments did not significantly reduce the mitotic index, while 15 µM Cd treatment substantially suppressed the proliferation activity of meristematic tissue (38%) as compared to NC. Concerning to metal mixtures the strongest mito-depressive effect (of 52%, compared to NC, $P<0.01$) was induced by 60 µM Zn with 15 µM Cd treatment.

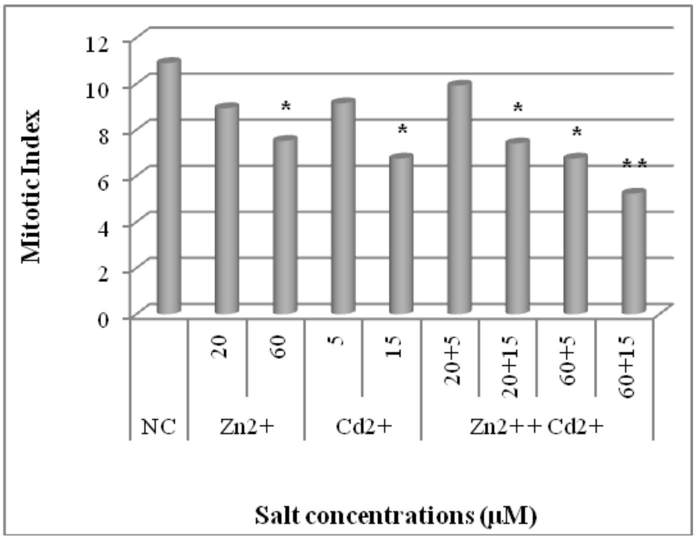
Figure 1. *Physiological Feedback of V. faba Seeds and Seedlings to Different Concentrations of $CdCl_2$ and $ZnSO_4$ Salts and their Mixtures*
Means labeled with asterisks are significantly different from control according to One-Way ANOVA test, respectively: * $P<0.05$; ** $P<0.01$; NC- negative control; FW – fresh weight; RFW – root fresh weight; RDW – root dry weight; ShFW – shoot fresh weight; ShDW – shoot dry weight; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; car – carotenoids.



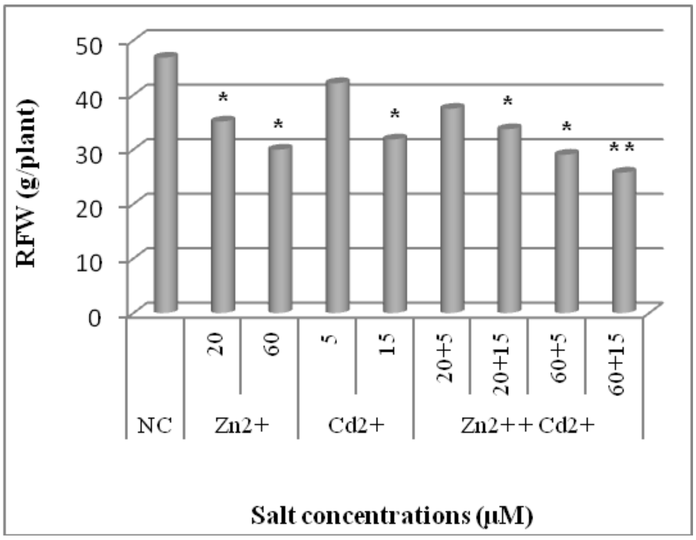
A


Root length (cm)
0
1
2
3
*
**
*
*
**
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

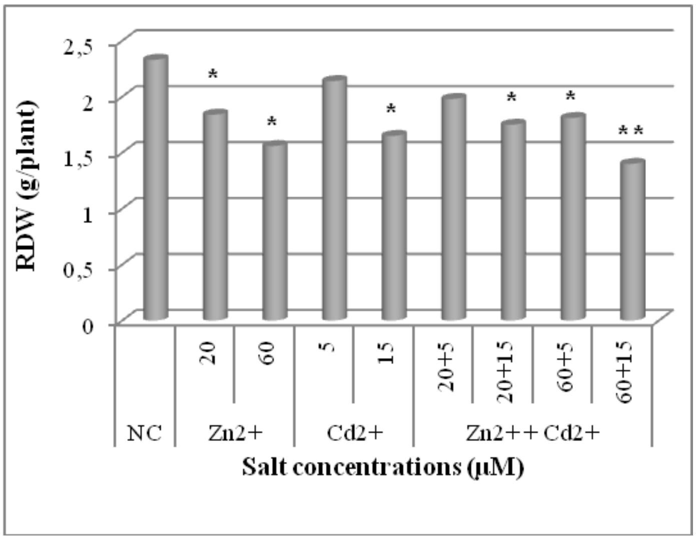
B


Mitotic Index
0
2
4
6
8
10
12
*
*
*
*
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

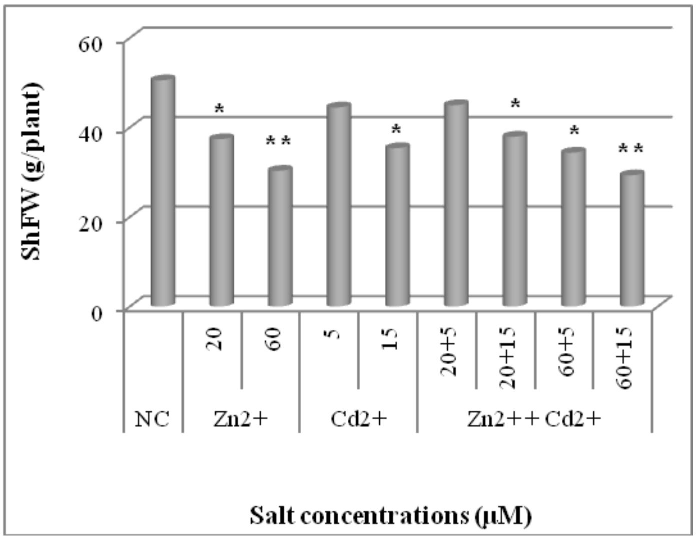
C


RFW (g/plant)
0
10
20
30
40
50
*
*
*
*
*
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

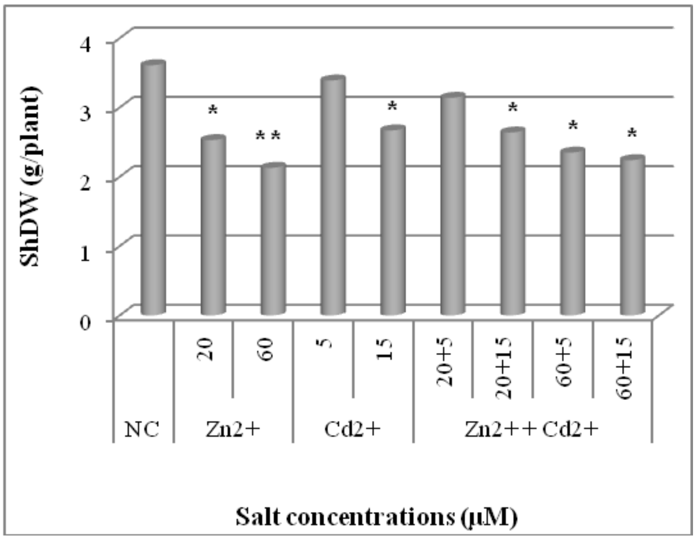
D


RDW (g/plant)
2,5
2
1,5
1
0,5
0
*
*
*
*
*
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

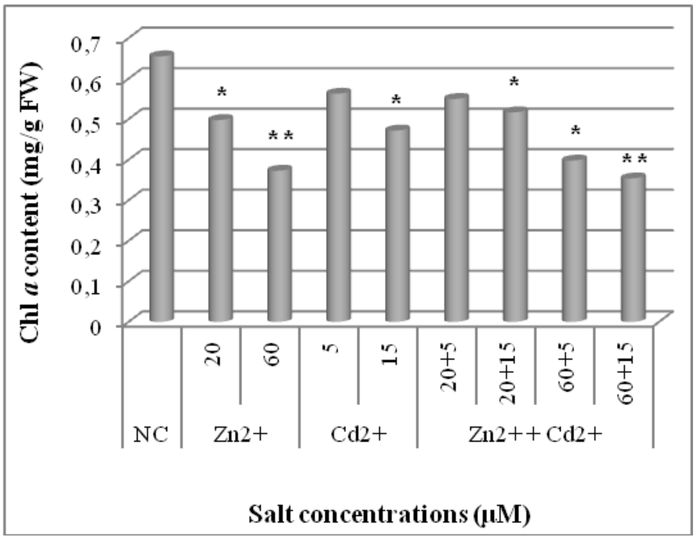
E


ShFW (g/plant)
60
40
20
0
*
**
*
*
*
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

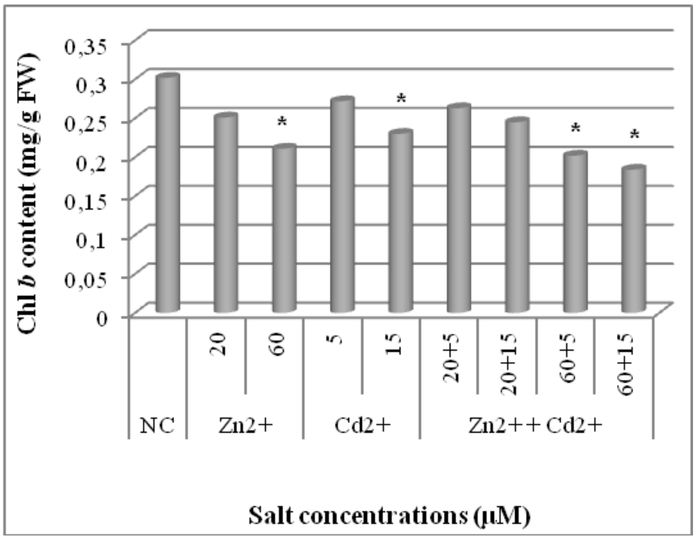
F


ShDW (g/plant)
4
3
2
1
0
*
**
*
*
*
*
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

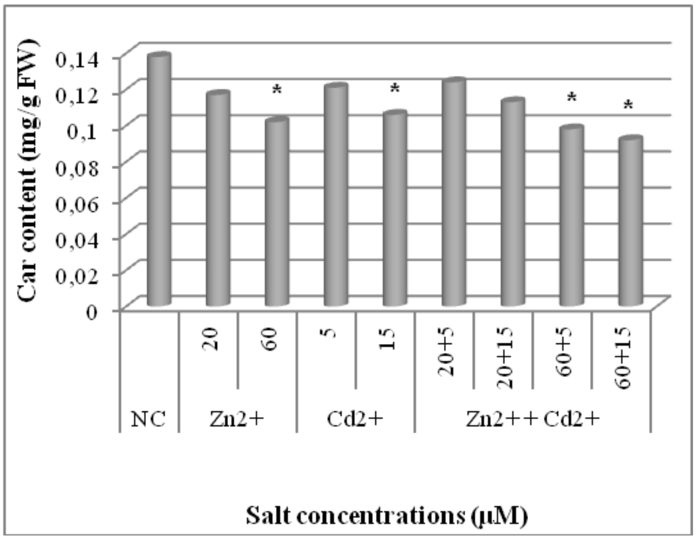
G


Chl *a* content (mg/g FW)
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
**
*
*
*
**
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

H


Chl *b* content (mg/g FW)
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
*
*
*
*
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

I


Car content (mg/g FW)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
*
*
*
*
20
60
5
15
20+5
20+15
60+5
60+15
NC
Zn2+
Cd2+
Zn2++ Cd2+
Salt concentrations (µM)

J

Root and Shoot Fresh and Dry Weights

Figure 1D-G indicates the effects of the examined Zn and Cd heavy metals and their mixtures on the root and shoot biomass of four week old-seedlings of *V. faba*. Fresh and dry root and shoot weights of seedlings grown in Hoagland medium resulted respectively: 46.83, 2.33, 50.54 and 3.61 g/plant. On the other hand Zn treatments caused a greater decline than Cd addition in the examined four weight parameters. The whole weight reduction (as compared to NC) through individual Zn and Cd concentrations and mixtures ranged as follow: 10-45% (RFW), 12-42% (RDW), 8-40% (ShFW) and 6-41% (ShDW). The lowest weight was obtained in fresh biomass of seedling roots under 60 μM Zn and 15 μM Cd mixture stress (of 25.75 g/plant, $P<0.01$). Meanwhile the highest one resulted in shoot dry biomass of plantlets treated with 5 μM $CdCl_2$ (of 3.39 g/plant), with no significant difference from the corresponding NC.

Photosynthetic Pigments

The respective quantities of photosynthetic pigments (chlorophyll *a* and *b* and carotenoids), scored in three week old-seedlings treated with a nutrient solution resulted: 0.654, 0.302 and 0. 138 mg/g fresh weight. As shown in Figure 1H, Chl *a* content was significantly reduced as high as 24% ($P<0.05$), 43% ($P<0.01$) and 28% ($P<0.05$) of corresponding NC, in seedlings exposed to both individual Zn concentrations and the highest Cd treatment concentration, respectively. The most drastic quantitative diminution was detected in the plantlets treated with a mixture of 60 μM Zn and 15 μM Cd (46% of control sample). A lower decline of chl *b* and car content was observed on the whole Zn and Cd heavy metal treatment, as compared to Chl *a* (Figure 1I and J). Statistically significant reduction of these physiological endpoints compared to NC was recorded only under the treatments of 60 μM Zn combined with 5 and 15 μM Cd addition: 33-39% and 29-33% ($P<0.05$), respectively.

Discussion

To our knowledge, the available information from the literature according to the physiological feedback of *Vicia faba* and other plant species to the combined action of heavy metal mixtures is limited. Seed germination is a crucial phase of the plant life-cycle, especially in crops and is generally accompanied with greater stress-sensitivity. Related to the root and shoot growth and the combination of cell division and elongation, the significant reduction of length is a true indicator of toxicity and is a general phenomenon caused by most heavy metals. Additionally the mitotic index reflects the frequency of cell division, being a confident endpoint in identifying the presence of cytotoxic pollutants in the environment. An MI reduction of 50% is considered a threshold value: a decrease below 50% (of control) indicates a sublethal effect, while below 22% denotes a lethal effect on the test organism. The recorded data in this investigation showed that zinc and cadmium, alone or mixed, strongly affected both the root length and the mitotic activity of *V. faba* root tip meristem. The mixtures of 60 μM Zn with 5 and 15 μM Cd additions were the most rhizotoxic, inducing even a sublethal effect to the faba seedlings. Detected reduction of these endpoints could be positively correlated, showing that the inhibition of root length may be a consequence of a mito-depressive effect of Cd and Zn excess. The reduction in root length has been reported to be caused by the accumulation of these heavy metals in the cell wall, which negatively modifies the metabolic activities and limits the cell wall elasticity (Chakravarty and Srivastava, 1992; Kiran and Şahin, 2006). Otherwise the suppression of seed germination was more evident only in sets imposed under the highest Zn and Cd concentration mixture as compared to other treatments. Second, different authors (Bonifacio and Montano, 1998; Bahmani et al., 2012) the reduced seed germination can be attributed to the changes of selection permeability properties of cell membranes and an accelerated breakdown of stored food materials in seeds due to the application of heavy metals.

Most heavy metals inhibit plant growth either by damaging the roots or causing crop failure. The evaluation of plant fresh and dry weight can give helpful information about the dynamics of growth, which is firmly dependent by environmental quality and chemical pollution. In addition the main prerequisite for a higher yield in crops is an increase in biomass production in terms of dry matter. In the current work the Cd and Zn-induced decline in fresh weight of both root and shoot of faba seedlings resulted comparatively more pronounced than corresponding dry weights. This fact confirmed the assumption that dry weight is an important indicator of an adaptations mechanism to chemical pollution in plants (Kabir et al., 2010). Additionally the fresh and dry biomasses of the oots appeared more affected than shoot ones, especially by Zn alone and its mixtures with Cd (Figure 1D-G).

It is considered that the growth parameters do not always provide enough objective information to phytotoxicity of the environment and therefore in plant bioassays, it is advisable to include functional indicators. Chlorophyll and carotenoid content can directly determine photosynthetic potential and the primary production of plants, giving also an indirect estimation of their nutritional state. Moreover it has important potential implications for the detection of crop stresses, agricultural field management, and especially for precision of agriculture practices (Peñuelas and Filella, 1998; Zarco-Tejada et al., 2004). Being considered an early symptom of metal toxicity, the decrease of photosynthetic pigments content directly influences the chloroplast development and the inhibition of photosynthesis. This paper’s data revealed an interesting variation of significant differences between the content of chlorophyll *a* and *b*, carotenoids and the total photosynthetic pigments in faba seedlings grown under heavy metal stress. Zn and Cd treatments, either independently or combined, exerted a negative impact particularly in chlorophyll *a*. In this context the observed decrease of the Chl content (*a* + *b*) may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis. During the process of chlorophyll degradation, Chl *a* is converted in Chl *b* (Fang et al., 1998). This might have caused the higher reduction observed for the Chl *a* compared to the Chl *b* content. Singh et al. (1996) emphasized that metal compounds generally affect chlorophylls more than carotenoids, and this statement was in agreement with our results obtained for both the analyzed metals.

Recent eco-toxicological studies have substantially improved the understanding of metal toxicity mechanisms at physiological level, particularly in plants. As the object of the present investigation, cadmium is considered one of the most toxic pollutants, representing a serious environmental and agricultural concern. It has been proposed that this heavy metal may interact with DNA directly or indirectly by inducing oxidative stress. On the other hand, Zn has reached high concentrations in many soils, becoming the most extensive phytotoxic microelement (after Al/Mn in arable acidic soils) far more deleterious than Cd, Cu, Ni, Co or other metals (Chaney, 1993; Liu et al., 1996). Zinc excess in plants has been shown to inhibit many metabolic processes, which can lead to limited growth and development of roots in particular and inducement of senescence (Rout and Das, 2003). Second Reichman (2002) says that plant responses to metals are generally dose-dependent and for essential metals such as zinc, these responses cover the phases from deficiency through sufficiency/tolerance to toxicity. Our results confirmed that individually cadmium chloride and zinc sulphate induced evident toxic effects in *V. faba* at low concentrations (5 and 20 μM, respectively), being in accordance with earlier researches (Demirevska-Kepova et al., 2006; Paschke, et al., 2006; Sharma and Dubey, 2006; Unyayar et al., 2006; Wang et al., 2009b; Zhang et al., 2009; Kopliku and Mesi, 2014a, b; Tkalec et al., 2014).

In nature plants are almost always exposed to complex mixtures of metal compounds, which may act independently or interact to produce additive, synergistic, or antagonistic effects. Despite the limited information, the interactions between non-essential and essential elements in general affect plant nutrition and induce physiological disorders and consequently growth restriction. Besides being usually associated in soils, cadmium and zinc can be simultaneously up taken by plants, even because of their similarity as divalent cations. The present data revealed that the mixture of Zn at its lowest concentration (20 μM) and Cd (5 and 15 μM) generally ameliorated the adverse effect of these heavy metals alone across all tested physiological parameters in faba seeds and seedlings. It may be noted that this antagonistic role of Zn against Cd resulted more pronounced in root length (MRL). On the other hand the mixture of the lowest cc of Cd (5 μM) with the highest cc of Zn (60 μM) significantly induced synergistic effects particularly in length and fresh weight of root and chlorophyll *a* content. Moreover the synergistic interaction of Zn and Cd was less prominent under the application of the highest doses mixture. Our findings on *V. faba* mostly suggested the following phytotoxicity range: Cd+Zn>Cd>Zn, generally being in agreement with former studies using other plant bioassays (Fargašovã, 2001; Tkalec et al., 2014).

Plant species differ in the level of tolerance to different elements for growth but excessive amount can lead to toxicity. These differences may be attributed to variable ion translocation to the aerial parts of the plants (Iqbal and Shazia, 2004). Concerning the tolerance index of *V. faba* to Cd and Zn (Table 1) seed germination and root length resulted to the most and less tolerant endpoints to separate action and respective interaction of cadmium and zinc.

Table 1. *Respective Tolerance Indexes of V. faba Plant to Different Concentrations of $CdCl_2$ and $ZnSO_4$ Salts and their Mixtures*

Treatments (μM)		**Tolerance Indexes (%)**									
		GC	MRL	MI	RFW	RDW	ShFW	ShDW	Chl*a*	Chl*b*	Car
Zn^{2+}	20	91	69	82	75	79	74	70	76	83	85
	60	82	57	69	64	67	60	59	57	70	74
Cd^{2+}	5	87	86	84	90	92	88	94	86	90	88
	15	76	67	62	68	71	70	74	72	78	77
Zn^{2+} + Cd^{2+}	20+5	92	88	91	80	85	89	87	84	87	90
	20+15	79	71	68	72	75	75	73	79	81	82
	60+5	71	55	62	62	78	68	65	61	67	71
	60+15	65	50	48	55	60	58	62	54	61	67

GC – germination capacity; MRL – mean root length; MI – mitotic index; RFW – root fresh weight; RDW – root dry weight; ShFW – shoot fresh weight; ShDW – shoot dry weight; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; car – carotenoids.

The fresh and dry weight tolerances had a gradual decrease through metal concentrations and mixtures, being lower for fresh biomass. Additionally shoot appeared more tolerant than root. The production of photosynthetic pigments was moderately tolerant, as well. The reason for decreasing tolerance against heavy metal mixtures might be due to changes in the physiological mechanism in seed germination and seedling growth of the faba plant. Based on root tolerance indices, our data could demonstrate that Cd and Zn are highly accumulated and remain chiefly in roots of *V. faba*. This fact sustained the statement that plants under heavy metal stress may activate mechanisms, which inhibit physiological and biochemical damages in the shoots by reducing root-to-shoot translocation of heavy metals (Prasad, 1995; Fargašovã, 2001; Sharma and Dubey, 2006; Kabir et al., 2010; Rascio and Navari-Izzo, 2011).

Conclusions

The present investigation confirmed that cadmium and zinc individually induced evident and cumulative toxic effects on *Vicia faba* seeds and seedlings. Concerning Zn and Cd interactions, Zn played a contrasting role against Cd, being antagonistic in low concentration and synergistic at a high one. Contrariwise whatever the concentration, Cd was synergistic against Zn. Based on data, the tolerance of the faba bean under Cd and Zn stress was relatively moderate and the roots appeared to be more sensitive than shoot. Seed germination and root length resulted to the most and less tolerant parameters to both separate action and respective interaction of the analyzed heavy metals. Such research studies with combined metal stresses can have a peculiar value particularly for developing countries as Albania, because they can be helpful in the solution of various problems associated with metal pollution in agricultural areas.

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