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Assessment of metals (Cu, Ni) and metalloids (As) induced stress responses in Barley *(Hordeum vulgare)* and wheat (*Triticum aestivum*)

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Abstract
Soil contamination by metals and metalloids is known to treat human beings and cause a serious damage of the ecosystem and destroy the environment. The present study was performed to evaluate the effect of Cu, Ni and As stress on seed germination and early seedling growth of barley and wheat. The plant seeds were treated with 0, 10, 50, 100 and 200 mg L^{-1} of the chosen elements. Based on the results, no effect was observed on seed germination whatever the metal used and the concentration applied (ranged between 96.6 and 100 % of barley and wheat final germination). Arsenic was found to decrease significantly root and shoot length, Tolerance index, root toxicity index and Seeds vigor index of seedlings of both plant species. By contrast, increasing Ni concentrations had a reduced effect on the parameter tested of barley seedlings. Results showed that barley was more sensitive to increasing levels of As and a high tolerance to Ni toxicity. Furthermore, the decreasing order effect of the tested elements on barley and wheat seedlings was As > Cu > Ni.

1. Introduction
Soil pollution by trace elements becomes a major world concern due to their harmful effects on human health, animals and plants and due to their high cleanup coast (billions of dollars) [1–3]. Trace elements are mostly realized into soil because of intensive human activities such as mining, industry, irrational use of pesticide and fertilizers [4–7]. Because of their persistence in the environment and non-degradability by chemicals or microbial activities [5,8], trace elements might enter to food chain [9] by direct contact or consumption of contaminated plants and animals [2,10,11] causing several diseases such as morphological abnormalities, physiological disorder and molecular perturbation [12–14]. Among trace elements, Cu and Ni are considered as essential micronutrient for plant growth and development but toxic at higher levels [15–18] while As is the most abundant and toxic metalloid in the environment [19,20]. Numerous works have reported the toxic effects of Cu, Ni and As on seed germination, early seedling growth and biomass of several plant species such as fenugreek, alfalfa, sorghum, mustard and wheat [7,20–23] in different growth conditions. In addition, plants in presence of excess of metals and metalloids might suffer from several symptoms such as germination inhibition, growth reduction, photosynthesis and water uptake disturbance, cellular damage, reactive oxygen species generation and biochemical and physiological disorder [7,24–28].

Due to the harmful effect of trace elements on environment, there has been a growing interest on ecological risk test for environmental bio-monitoring of trace elements [21]. Actually and due to the scientific research evolution, the interest was given to physiological, biochemical and molecular biomarkers such as enzyme activities, chlorophyll fluorescence, lipid peroxidation, genotoxicity and changes in the genomic DNA [21,24,29]. However, testing the effect of trace elements in the level of seed germination and seedling growth is primordial and highly required [30] since seed germination is the initial step in the plant life cycle [28,31]. This test have been reported to be a rapid and simple test assess and evaluate metals toxicity [32–34] and considered as a suitable indicator of metals stress [7,35] because of the sensitivity of seeds at this stage of cycle life to the metals [25] and to the environmental changes conditions [36].

The objective of the present experiment was to test the impact of Cu, Ni and As exposure on germination and early seedling growth of barley (*Hordeumvulgare*) and wheat (*Triticum aestivum*). Seed germination, growth, dry biomass as well as seeds vigor index, tolerance index and root toxicity index were assayed under increasing concentrations of above elements. In developing countries like Morocco, mining activities and agriculture are important in the national economy, while they produce high amount of wastes highly contaminated with trace elements [37,38] causing a major environmental problems. Moreover, the selection of barley and wheat species for this test because these crops are highly cultivated in Morocco even at arid and semi-arid areas [39–41] and used mostly for feed of both animals and humans.

2. Material and Methods

2.1. Plant seeds and Metal(loids) treatments

Dry, healthy and uniform size seeds of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) were surface sterilized by soaking them in 1% Sodium Hypochlorite solution for 10 min. The seeds were immerged during 1min in 70% Ethanol then rinsed with distilled water. This procedure was used to eliminate the possible fungi growth and bacterial contamination.

Increasing concentrations (0, 10, 50, 100 and 200 mg / L) of Cu, Ni and As were prepared by dissolving the metal elements Cu ($CuSO_4.5H_2O$), Ni ($NiSO_4.6H_2O$) and As ($Na_2HAsO_4.7H_2O$) in distilled water.

2.2. Germination experiment

The selected seeds were gently added to Petri dish covered with one layer of filter paper and previously imbibed with 5 ml of each treatment. Distilled water was used as a control. 3 replicates (Petri dishes) per treatment were prepared with 10 seeds of barley or wheat equally spaced (1cm) in each Petri dish. The dishes were then transferred to the oven for five days at temperature of 23 ±1°C.

2.3. Measurement of biological responses

Germination percentage (GP) was calculated by dividing the germinated seeds in each Petri dish over total seeds. Seeds were considered germinated when the radical reached at least 2 mm. Both root length (RL) and shoot elongation (SL) of both plant seedlings were measured using a ruler. Tolerance Index (TI), Root Toxicity Index (RTI) and Seeds Vigor Index were estimated using the formulas by Iqbal and Rahmati (1992), Wierzbicka et al. (2015) and Abdul-Baki and Anderson (1973), respectively:

$$TI = (RL_{treatment} / RL_{control}) \times 100$$
$$RTI = ((RL_{control} - RL_{treatment}) / RL_{control}) \times 100$$
$$SVI = (GP \times TPL) / 100$$

With: GP = Germination percentage, $RL_{control}$ = Root length in control, $RL_{treatment}$= Root length in treatment and TPL= Total plant length (TPL = roots + shoots length)

Production of dry biomass was calculated by drying seedlings in the oven for 48 h at 80 °C.

2.4. Statistical analysis

In order to meet the normality assumptions of ANOVA, percentage data were arcsine-transformed. One-Way Analysis of Variance (ANOVA) followed by *dunnett's post-hoc* test was applied to evaluate significant variations on the biological responses under metal treatments relatively to those observed under the respective controls ($p<0.05$). The SPSS software (version 20.0) was used to perform the statistical analysis.

3. Results and discussion

3.1. Germination percentage

The obtained results of the present study showed that the stress caused by Cu, Ni and As had no significant effect on barley and wheat seed germination ($p>0.05$) in comparison to control (Fig.1). In fact, whatever the metal used and the concentration applied both plant seeds reached almost 100% of final germination.

Germination assay is a rapid and easy test used to assess and evaluate metals phytotoxicity [32,44]. Phytotoxic effect of metals and metalloids on seeds germination is well documented. However, responses of plant seeds depends on the plant species, the metal itself and its concentration [20,27]. Several studies were carried out on barley [45–51] and wheat [21,26,52–56] seeds using both essential and non-essential elements under different experimental conditions. Sanal et al. (2014) found that both forms of arsenic (arsenate and arsenite) inhibited significantly germination seeds of barley. Different elements were found to have negative effects on barley such as Cu, Cd and Pb [46,58]. Cd was found to reduce germination percentage of wheat [59] while Cr had no negative effects on this parameter [60]. In soil, 10 plant species seeds (mung bean (*Phaseolus radiatus*), cucumber (*Cucumis sativus*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*), Chinese cabbage (*Brassica campestris var. chinensis*), broccoli (*Brassica oleracea*), mustard (*Brassica nigra*), kale (*Brassica campestris*) and pea (*Pisum sativum*)) were exposed different forms of arsenic [20]. The authors found that germination of barley was not affected by arsenate (As^{V}) while wheat germination was negatively affected. Moreover, germination of wheat seeds was found reduced under Cr, Cd, Mn and Zn stress [56].

In our study, barley and wheat seeds showed a high tolerance to increasing levels of Cu, Ni and As and germinate even under the highest concentrations applied of the studied elements. This difference of findings might be due to concentration variation, cultivar differences and laboratory conditions.

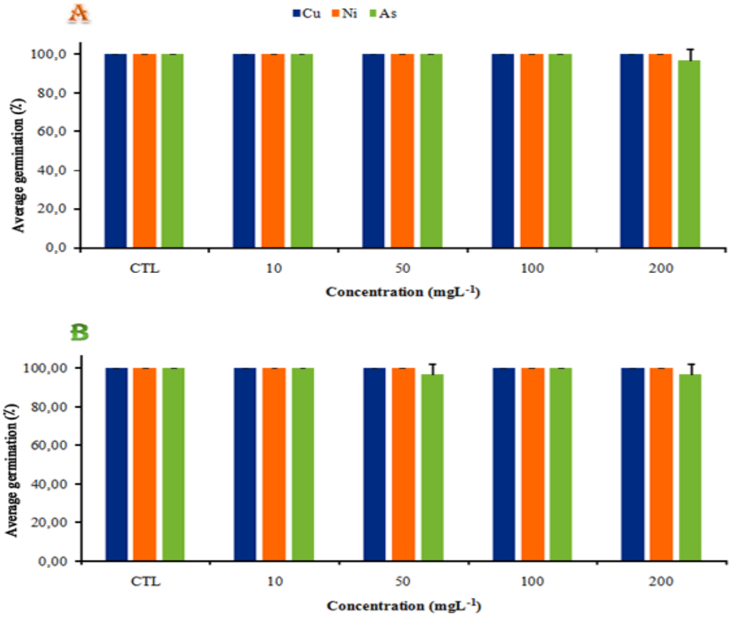

Fig.1. Effect of different concentrations of Cu, Ni and As on the average percentage (%) of germination of barely (A) and Wheat (B) grown in metal(loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by "star *". Statistical differences were tested at $p<0.05$.

3.2. Root and shoot length

Increasing concentrations of the studied elements had different effects on barley and wheat root and shoot elongation according to the elements used and the dose applied (Fig. 2). Copper and arsenic reduced significantly both root and shoot length of barley seedlings compared to the control, with the most pronounced effect was observed when As levels was increased followed by Cu. Wheat root and shoot length showed the same pattern as that of barley. The most pronounced effect was found in presence of As especially in the highest concentration. For Ni, increasing concentrations have almost no effect on barley roots and wheat shoots (except in 200 mg L^{-1}

where the inhibition is significant). However, concentrations from 50 to 200 mg L^{-1} reduced significantly both barley shoots and wheat roots. Mahmood et al. (2007) found that increasing Cu levels reduced significantly root length and a clear positive effect on shoot height of barley seedlings. In our experiment, As treatment deceased gradually the length of barley shoot which is in accordance with Sanal et al. (2014) that found that arsenate and arsenite reduced negatively both root and shoot of barley seedlings. The same findings were found when wheat (*Triticum aestivum*) were exposed to increasing As levels [54,62]. Root and shoots inhibition was found increased with increasing concentrations of As and Cd [55] as well as other elements such as Cd, B and Zn [63–65]. Arsenic is known to be toxic to several plant species from different families such as Fabaceae: *Trigonella foenum-graecum, Lathyrus sativus, Medicago sativa, Pisum sativum* [23,27,66] and Asteraceae: *Helianthus annuus* [67]. This effect of Arsenic might be due to their ability to be transported to plants via roots similarly to phosphate (Pi) using the same transporters [19,68] especially in *Holcuslanatus* and barley (*Hordeum vulgare*) plants [69]. Barley seedlings showed a high tolerance to Ni than Cu and As. It is worthy to note that low concentration of Cu ($Cu_{10\ mg/L}$= 12.11cm) and concentrations of Ni had a positive effect on barley and produced longer root ($Ni_{10\ mg/L}$= 12.95 cm, $Ni_{50\ mg/L}$= 12.54 cm and $Ni_{100\ mg/L}$= 12.13 cm) comparing to the control (10.93cm). For wheat, only the concentration of 10 mg $L^{-1}$of Cu and Ni and both 10 mg L^{-1} and 50 mg L^{-1} of Ni that increased the root length as compared to the control.

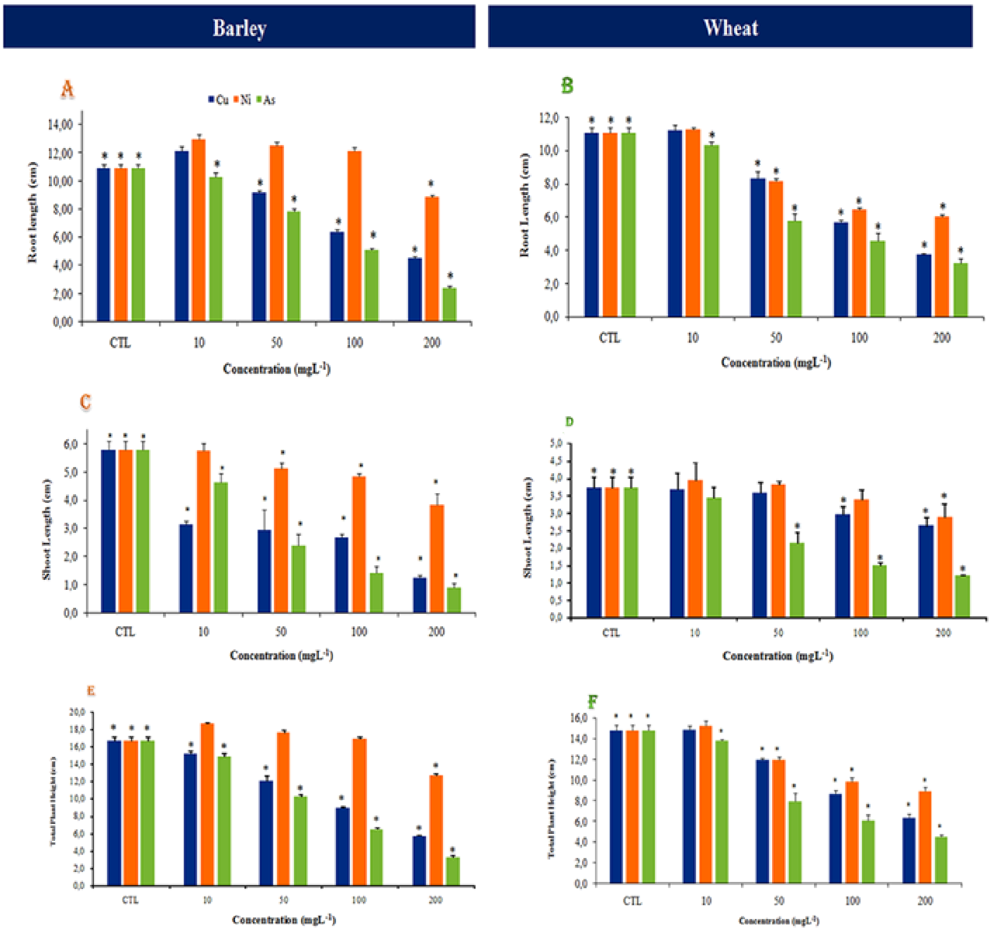

Fig.2. Effect of different concentrations of Cu, Ni and As on the root length, shoots length and total plant height of barely (A, C and E) and wheat (B, D and F) grown in metal (loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by "star *". Statistical differences were tested at $p<0.05$.

3.3. Tolerance index and root toxicity index

Roots developments of seedlings are very sensitive stage of plant growth and extremely sensitive to environmental changes [70] which make of them a rapid and useful way to study the mechanism of metal toxicity on plants [32,33,70]. In the present study, increasing Cu, Ni and As levels had different effects on root elongation of barley and wheat seedlings which reflected by a difference of effects on tolerance index and root toxicity index (Fig. 3 and Fig. 4). Tolerance Index decreased significantly with increasing Cu and As concentrations, suggesting that these elements inhibited root elongation of barley and wheat. The same results were observed for wheat in presence of Ni while only the highest concentration of Ni (200 mg L^{-1}) that reduced significantly the TI of barley. Results indicated that high values of TI were recorded in presence of 10 mg L^{-1}of Ni treatment (barley: 111.46%; wheat 102.1%) followed by Cu at the same concentration (barley: 110.85%; wheat: 101.26%). At the highest concentration (200 mg/L), tolerance degree of different elements was varied showing that the lowest TI value was recorded for As (22.18 % and 29.17 % for barley and wheat respectively) followed by Cu (41.26 % for barley and 33.79% for wheat). Ni was tolerated at all concentrations applied (at 200 mg L^{-1}, 81.10% and 54.47 % for barley and wheat respectively). In general, results suggest that barley seedlings were more tolerant to increasing concentrations of Ni than Cu and As. Mahmood et al. (2007) reported that increasing level of metals such as Cu induced a negative effect on tolerance index of barley. Likewise, other elements such as Cr, Cd, Pb, Mn, B and Zn were found to decrease the TI of wheat [26,56,61,63,64].

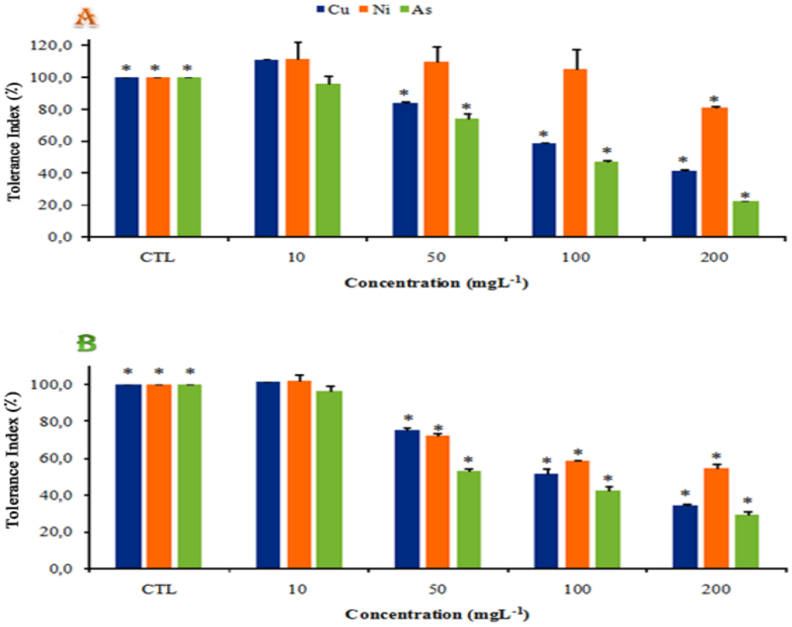

Fig.3. Effect of different concentrations of Cu, Ni and As on the Tolerance Index (%) computed for of barely (A) and wheat (B) grown in metal (loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by "star *". Statistical differences were tested at $p<0.05$.

Toxicity of As was higher than Cu and Ni toxicity with the increase of elements level. Higher values of RTI were recorded when As treatments were applied reaching almost 80% in the highest concentration (200 mg L^{-1}). Higher values of RTI indicated a higher toxicity of the substrate tested [42]. The results obtained indicated that As caused the most pronounced effect on root elongation than Cu for both species. However, increase of Ni treatments showed negative values of RTI at 10, 50 and 100 mg L^{-1}, while 19 % was recorded at 200 mg L^{-1} (Fig. 4). For wheat, only 10 mg L^{-1} of both Cu and Ni that induced a negative value of RTI while increased concentrations of the three elements tested showed an increase in RTI values. These results indicated low toxicity of Ni on barley seedlings even at high concentrations, followed by Cu than As in increasing tendency. Barley seedlings showed a high sensitivity to As stress than Cu or Ni.

3.4. Seeds vigor index

Figure 5 showed the results obtained for seeds vigor index of barley and wheat seeds in presence of increasing concentration of Cu, Ni and As. SVI was significantly reduced when As and Cu concentrations were increased starting from 10 mg L^{-1} for barley and 50 mg L^{-1} for wheat. Similarly, Ni reduced SVI value of wheat from 50 mg

L^{-1} however, only the highest concentration of Ni (200 mg L^{-1}) that reduced significantly the SVI of barley. As compared to the control, As treatments induced the most pronounced effect on SVI leading to an extreme decrease at 200 mg L^{-1}. Arsenic was found to affect seedling development of barley [20,57], wheat [54,62] as well as other plant species such as mung bean [71] and sunflower [67]. Other metals such as Cr, Pb, B, Cu and Zn induced a decrease of seeds vigor index of other plant species (wheat, fenugreek, been and tomato) [56,64,72–75]. As reported by Menon et al. (2016), the decrease of the SVI of barley might be due to decline in germination percentage and plant high under metals stress.

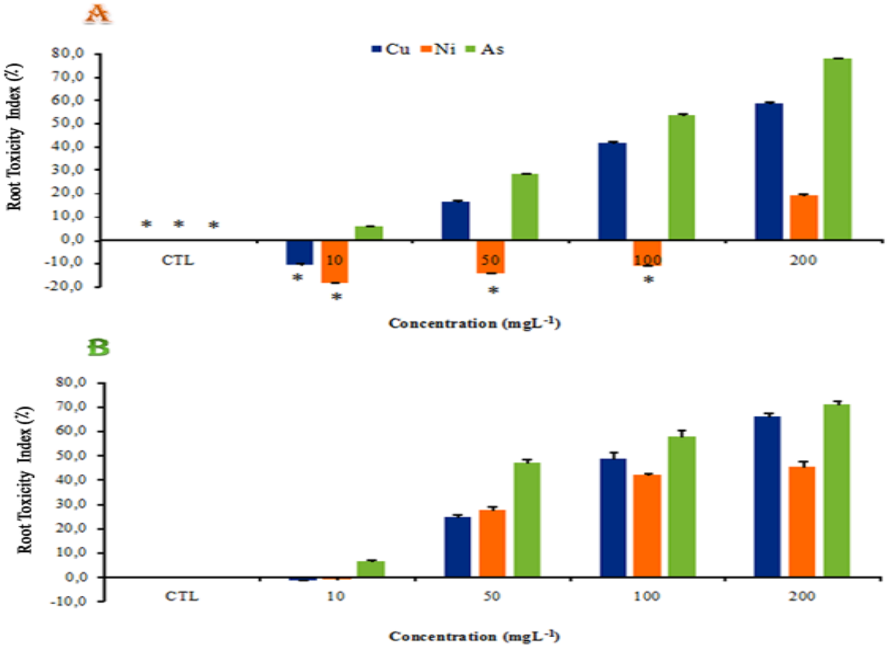

Fig.4. Effect of different concentrations of Cu, Ni and As on Root Toxicity Index computed for of barely (A) and wheat (B) grown in metal (loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by “star *”. Statistical differences were tested at $p<0.05$.

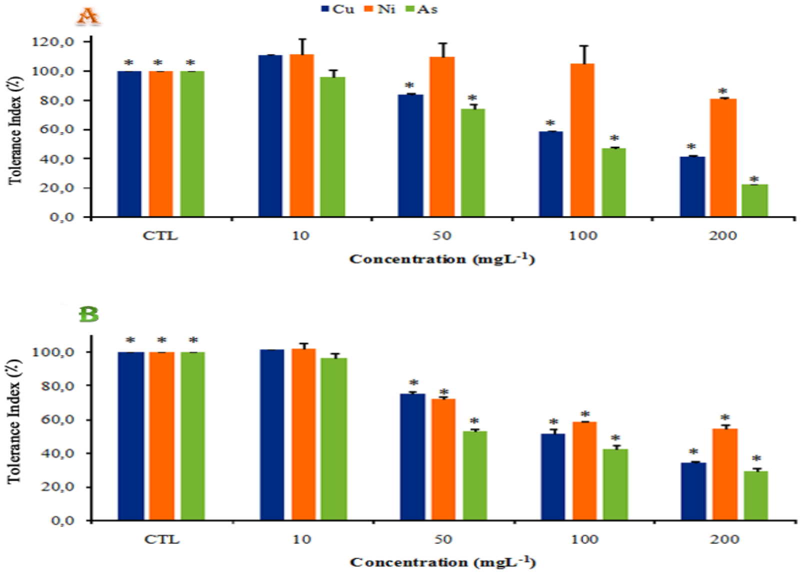

Fig.5. Effect of different concentrations of Cu, Ni and As on Seeds Vigor Index computed for of barely (A) and wheat (B) grown in metal (loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by “star *”. Statistical differences were tested at $p<0.05$.

3.5. Dry biomass

In the present work, dry biomass of barley and wheat seedlings under different levels of Cu, Ni and As were higher than that found in the control in presence of all the concentrations tested (figure 6). Moreover, an increase of dry biomass was recorded when the concentrations of all metals were increased. Comparing to the control (barley: 22.3 %), the highest dry biomass values were recorded at 200 mg L^{-1} of Cu (46.3 %) and As (34.6 %) respectively. In contrast, As induced the highest value of dry biomass in wheat (39.66 %) followed by Ni (33.5 %) comparing to the control (20.55 %). Trace elements were found to modify several plant structure such as cell well by affecting its porosity and plasticity and affecting the translocation of trace elements within xylem[17,76,77]. In addition, exposure of plants to Cu and As was found to enhance the biosynthesis of several organic compounds such cellulose, hemicellulose, pectin and lignin [76].The cell wall rigidity and production of these compounds might be the reason of increasing plant biomass. Plants used this process as an adaptation strategy and mechanism of tolerance against numerous kind of stress such as metals stress [17,76,78,79].

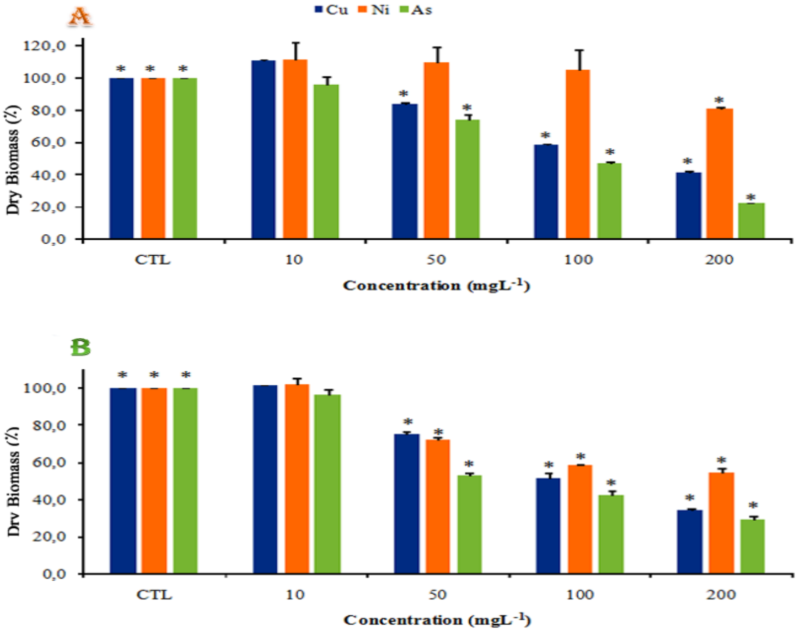

Fig.6. Effect of different concentrations of Cu, Ni and As on Dry Biomass computed for of barely (A) and wheat (B) grown in metal(loid)-spiked filter paper. Error bars represent standard deviation. Significant differences between test and reference (< reference) are indicated by “star *”. Statistical differences were tested at $p<0.05$.

4. Implications

In the present study, the response of barley and wheat to trace elements stress (Cu, Ni and As) during germination and early seedling growth was investigated. The results obtained revealed that the effect of these elements is extremely linked to the element itself and its concentration. Barley seeds were highly sensitive to increasing concentrations of As during seedling growth period. This has been reflected by a clear decrease of root and shoot length. Arsenic is easily taken up by root plants and translocated to aerial parts [80] using the same transporters as phosphate [68,81]. Once in plants, arsenic may generate reactive oxygen species which cause several biochemical and physiological damages in plants [19,80] as well as reduction of the plant morphological traits which is indicated by the present study.

Our results suggest that barley seeds showed a clear tolerance to increasing concentrations of Ni. The applied doses ranged from 10 to 200 mg L^{-1} of Ni had almost no effect or reduced effect in the highest concentration on germination, root and shoot length, tolerance index, root toxicity index and dry biomass of barley seedling comparing to wheat. Ni have been reported as an essential elements [16,18] playing a major in plant life since it is a component of plant enzymes such as urease [16,82] and its deficiency may interrupt the plant life cycle [83]. The deficiency of Ni might affect the period of seeds maturation, earlier opening of seeds heads and several morphological symptoms (plants were less green, smaller) [83,84]. In this study, Cu was found to reduce the discussed parameters (except dry biomass) for both plant species. Even that Cu is an essential element to plant life [15–17], but in excess it can be highly toxic and may induce several abnormalities in plants [15,75,85]. In general, the present results revealed that barley and wheat plant species showed a high sensibility to increasing levels of As. Furthermore, the general decreasing order effect of the tested elements on barley and wheat seedlings was As > Cu > Ni.

In this study, several questions were solved; nevertheless, others still remain un-discussed. We focused on early stage of seedling growth and studied the effect of single elements on germination and early seedling growth of the tested species. Further studies needed to be done in order to investigate the behavior of barley and wheat seeds in presence of a mixture of the tested elements since in soil a mixture of trace elements is always found. Moreover, this study should be extended for a long growing period in order to obtain enough plant biomass to analyze biochemical, physiological biomarkers such as MDA content, proline accumulations, enzymes activities, trace element translocation and accumulation in plant tissues. In addition, a molecular study is suitable to better understand the molecular response of both species to trace elements toxicity. The suggested analysis might help to increase our knowledge regarding trace element toxicity and better understand the mechanism of toxicity of Cu, Ni and As and plant strategies of tolerance/sensibility to excess of these elements.

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