INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0941/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1253
http://www.fspublishers.org

Full Length Article

Ultrastructural and Physiological Responses Induced by Nickel and Ozone Stress in Rice (*Oryza sativa* L.) Cultivars

Hanan Abou-Zeid[1*], Amel Tammam[1], Reem Badr[1], Aida Bader[2] and Yasser Ibrahim[3]
[1]*Botany and Microbiology Department, Faculty of Science, Alexandria University, Egypt*
[2]*Faculty of Science, Seventh of April University, Zawia, Lybia*
[3]*National Research Centre, Dokky, Egypt*
[*]For correspondence*:* hananmahmoud93@yahoo.com

Abstract

Two cultivars of rice (Sakha101 and Giza178) were grown in open-top chambers under O_3 (75 ppb/h) and/or treated with different concentrations of nickel sulphate (0, 10, 50 and 100 μM). The damaging effect of both stresses was more pronounced and manifested certain differences between the two varieties. Exposure of tolerant and sensitive cultivars to Ni^{+2} or fumigation with O_3 reduced shoots and roots dry weights, leaf relative water content, chlorophyll fluorescence and mineral content. Contrary, a significant accumulation of total phenolics, flavonoids and phenylalanine ammonia lyase (PAL) activity together with quercetin, catechin and gallic acid were recorded. Based on leaves scanning and transmission electron microscope, both stresses separately had negative effects on stomatal response, epicuticular waxy hairs and papillae. The effect of Ni^{+2} and O_3 on leaf ultrastructure was observed most conspicuously in the chloroplasts, which revealed alterations in shape and the construction of the thylakoid membranes. Anatomical study of roots displayed variations and disintegration of the exodermis, endodermis, cortex and vascular cylinder. SDS-PAGE protein profile of shoots from the two cultivars revealed qualitative and quantitative changes. Taken together, the interaction between Ni^{+2} and O_3 were, antagonistic on physiological, ultrastuctural alterations and up-down regulated proteins to counteract the negative effects of both pollutants. © 2020 Friends Science Publishers

Keywords: Cell ultrastructure; Chlorophyll fluorescence; Nickel; Ozone; Phenolics; Stomata

Introduction

With the development of urbanization, heavy metals and the concentration of tropospheric ozone (O_3) have been greatly augmented over recent years and their phytoxic effects have fascinated serious consideration as insidious infectivity problems (Gan-Wen and Li, 2014). Lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As) and nickel (Ni) are major heavy metals contaminants, chiefly in regions with high population pressure. At prominent levels, these metals are engrossed and stored in different parts of the plants, consequently disturbing their growth and metabolism. Lower concentrations of nickel deem as an essential element for higher plants, other than, high concentrations cause damage effects (Singh *et al*., 2012). The overflows from many industries are either inclined to water through surface-drains or allowable to increase near agricultural lands. As a consequence of soil pollution, a substantial amount of nickel can be taken up by the crops and penetrate the food chain and eventually human body (Iori *et al*., 2013).

Tropospheric ozone can be well conscious as an atmospheric pollutant with direct harmful effects on plant growth and development (Tausz *et al*., 2007). Intra-specific variations of ozone sensitivity of vascular plants were mainly noticed in crop species (Bergmann *et al*., 2017). Contingent on the sternness of the stress, some O_3 exposed plants display characteristic symptoms of fumigation such as, the reduction in carbon assimilation visible foliar damage, impaired stomatal conductance and increased leaf senescence (Joo *et al*., 2005).

The consequence of heavy metals on a plant's aptitude to contract other environmental stresses has received less interest (De Silva *et al*., 2012). Earlier study (Mitchel *et al*., 1978) reported that higher concentrations of cadmium (Cd) and zinc (Zn) emphasized ozone-induced visible leaf injury and the application of nickel (Ni) also enhanced ozone injuries which do not decrease growth. Moreover, Pietrini *et al*. (2010) showed that O_3 and Cd stresses confirmed variations at physiological and molecular levels. Combined treatment such as O_3 and drought stress identified a set of proteins that changed similarly, representing cross talk in the molecular response of plants exposed to these stresses (Bohler *et al*., 2013).

Rice (*Oryza sativa* L.) consider as one of the most important food crop for half of the world's population. With the rapid growth in population overwhelming rice and the declining soil and water quality, there is an imperative need

To cite this paper: Abou-Zeid, H., A. Tammam, R. Badr, A. Bader and Y. Ibrahim, 202x. Ultrastructural and physiological responses induced by nickel and ozone stress in rice (*Oryza sativa* L.) cultivars. *Intl. J. Agric. Biol*. 00: 000–000

to understand the rejoinder of this important crop towards these environmental stresses. This investigation aimed to study the impact of Ni^{+2} and ozone separately or in combination on growth, photosynthetic pigments, chlorophyll florescence, changes in anatomical and ultrastructural alterations, phenolics and flavonoids content, HPLC of individual phenolics and protein profile of two (*Oryza sativa* L.) cultivars.

Materials and Methods

Plant Material and Growth Conditions

Rice (*Oryza sativa* L.) seeds of, five cultivars (Giza 178, Giza 179, Sakha101, Sakha103 and Sakha106,) were achieved from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt as a pure variety. Seeds of all cultivars were tested for their viability; sterilized and applied for germination to study the effects of different Ni^{+2} concentrations (0, 10, 50 and 100 μM). To choose two extreme cultivars, germination percentage, seedlings parts lengths were measured daily along seven days. Prior to germination rice seeds (1) Sakha 101 and (2) Giza 178 were surface sterilized by soaking in 0.1% $HgCl_2$ for two minutes then washed several times with distilled water. After soaking, seeds were germinated in petri dishes (10x10 cm) on a distilled water saturated filter paper and incubated in complete darkness at 26°C for 24 h, then in plastic pots (40 cm height x 32 cm diameter) filled with sand and clay soil (1:1) twenty seeds were sown. The water holding capacity was kept at 80% during the whole experimental period using distilled water for one week, after; pots were divided into four groups. The first one was left as a control without any treatment and irrigated with half strength Hoagland solution (Hoagland and Arnon, 1950). The second was irrigated with half strength Hoagland solution supplemented with 10, 50 and 100 μM $NiSO_4(H_2O)_6$. The third group was similar to the first one and fumigating with ozone (75 ppb/h) generated from ozonator (Tonbridge, Kent, UK). The fourth group fumigating with ozone (75 ppb/h) and was irrigated with half strength Hoagland solution supplemented with 10, 50 and 100 μM $NiSO_4(H_2O)_6$. The pots were irrigated with the treatment solution every two-day interval throughout the whole experimental period. Ozone fumigation to plants under Open-Top Chambers (OTCs) in the botanical garden of Faculty of Science, Alexandria University, and the pots (in triplicates) were arranged (Treshow and Stewart, 1973). After 28 days, homologous plants were harvested, washed thoroughly from adhering soil particles, gently plotted, dissected to shoots and roots and quickly saved for estimating the various growth parameters and chemical analyses.

Estimation of Leaf Relative Water Content (RWC)

The leaf relative water content was determined according to Mata and Lamattina (2001) and evaluated from the equation:

RWC% = (fresh weight – dry weight)/(turgid weight – dry weight) × 100

Photosynthetic Pigments Analyses

Leaf chlorophyll extraction was done using 4 mL of N, N-dimethyl formamide (DMF) for 24 h at 4°C. Chlorophyll content was quantitatively determined using a spectrophotometer-double beam (T80 UV–Vis) at wave lengths of 646.8 and 663.8 nm (Porra, 2002). Total carotenoids content was calculated according to Moran (1982).

Measurement of Chlorophyll Fluorescence

The ratio of the maximum quantum efficiency of PSII (Fv/Fm) was performed by chlorophyll fluorimeter OS-30P pulse modulated (Opti-sciences, Hudson, USA). Before every measurement, leaves were dark- adapted for 30 min with leaf-clips (Branquinho *et al*., 1997).

Light and Transmission Electron Microscopy

The fragments of *Oryza sativa* L. (cv. (1) Sakha 101and (2) Giza 178) roots and leaves from both control and treated 100 μM Ni^{+2}, ozone (75 ppb/h) and 100 μM Ni^{+2}+ozone (75 ppb/h)) samples were fixed along with the method described by Spurr (1969). Semi-thin sections (1 μm) and ultrathin (90 nm) ones were observed with light microscope (Olympus- Japan) and the transmission electron microscope (JEOL-TEM 100 CX) respectively (Venable and Coggeshall, 1965).

Scanning Electron Microscopy

Small pieces of fresh samples of *Oryza sativa* L. (cv. (1) Sakha 101 and (2) Giza 178) leaves from both control and treated 100 μM Ni^{+2}, ozone (75 ppb/h) and 100 μM Ni^{+2} +ozone (75 ppb/h)) were fixed and observations of leaf morphology in the specimens were performed in a scanning electron microscope (Jeol JSM-5300) operated between 15 and 20 KeV.

Extraction and Estimation of Total Phenolics and Flavonoids Content

The powdered plant materials were individually extracted with 10 mL of 80% methanol kept on a rotary shaker for 24 h. Thereafter, it was centrifuged at 5000 xg for 15 min. The supernatant was kept at 4°C in air tight bottles for further use (Tawaha *et al*., 2007). Total phenolic contents were determined following the method described by Demiray *et al*. (2009). Absorbance of samples and standard were measured at 765 nm using a spectrophotometer-double beam (T80 UV–Vis). Flavonoids content was estimated by the aluminum chloride, colorimetric assay method (Potter *et al*., 2010).

Analysis of Individual Phenolics

Individual phenolic compounds isolation and quantification were obtained using a reversed-phase high performance liquid chromatography (HPLC) method described by Padda and Picha (2007). Identification and peak assignment of the compound were based on comparison of its retention time with corresponding authentic samples.

Phenylalanine Ammonia Lyase (PAL) Activity

The activity of PAL was determined following the method explained by Jiang and Joyce (2003). Fresh plant material was homogenized in borate buffer (0.1 M, pH 8.0) containing 5 mM β-mercaptoethanol and 2 m*M* EDTA, centrifuged for 20 min and supernatant was collected for enzyme assay. For determining PAL activity, 0.5 mL of supernatant was incubated for 1 h at 30°C in 2 mL of 0.1 M borate buffer (pH 8.0) containing 1 mL of L-phenylalanine (0.1 M).

Elemental Analysis

Following the method previously described by Chapman and Pratt (1961), using atomic absorption spectrophotometer (Perkin-Elmer, 2380), elements content (Ni^{+2}, K^{+}, and Ca^{+2}) were measured in shoots and roots of both (*Oryza sativa* L.) cultivars.

Protein Extraction and Gel Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were carried out using the discontinuous buffer system as described by Laemmli (1970) and modified by Hames and Rickwood (1990). Isolated proteins were applied to determine the molecular masses using total lab.110 software nonlinear dynamics Newcastle upon tyne, UK to analyze banding pattern, molecular mass and band percentage.

Statistical Analysis

Results analysis was carried out according to Duncan's multiple range tests using SPSS-20. Following the method of Sokal and Rohlf (1995), data were subjected to one-way ANOVA. Differences between treatment-means were considered statistically significant at $p \leq 0.05$.

Results

Dry Weights and Relative Water Content

Among the two (*Oryza sativa* L.) cultivars, Sakha 101 is more susceptible for nickel effluent toxicity than Giza 178. Data showed that Ni^{+2} and O_3 treatments significantly inhibited the shoots and roots dry weights (Table 1). At 100 μMNi^{+2} the reduction in shoots reached 57% and 66% for Sakha 101 and Giza 178, the corresponding values for roots were 48% and 56% respectively, with respect to their controls (Table 1). Antagonistic effect of O_3 and Ni^{+2} treatments was documented by the declines of the shoots and roots dry weights; the inhibition was shifted to some extent in both tested cultivars. RWC of leaves was significantly decreased in response to increasing Ni^{+2} concentrations and O_3 fumigation. RWC of leaves of 100 μM Ni^{+2}-stressed Sakha 101 and Giza 178 plants were 60% and 48%, respectively, compared to controls. Ozone exposure led to RWC values of 65% and 54%. Rice plants were ameliorated by the interaction between the two stresses (100 μM Ni^{+2} + O_3), the RWC corresponding values were 81% and 62% for Sakha 101 and Giza 178, respectively (Table 1).

Photosynthetic Pigments Content

The levels of photosynthetic pigments decreased as the concentrations of Ni^{+2} increased in the nutrient solution. The decrease was relatively greater in Giza 178 than in Sakha 101 cultivar. Chlorophyll a content decreased by about 18% and 33% in leaves of Sakha 101 and Giza 178 exposed to O_3, with more notable reductions to about 41% and 62% in 100 μM of Ni^{+2}-stressed plants and the reduction reached about 33% and 51% under the combined interaction between O_3 and 100 μM Ni^{+2}. Carotenoids showed a reduction pattern similar to chlorophylls (Table 2).

Chlorophyll Fluorescence

The ratio of the maximum quantum efficiency of PSII (Fv/Fm), decreased significantly when the concentration of Ni^{+2} increased in the nutrient solution and in the O_3-treated rice plants. At 100 μM Ni^{+2}, the reduction was 26% in Sakha 101 and 42% in Giza 178, the corresponding values for O_3-treated plants were 23% and 37% respectively, compared to controls. Combined treatment of O_3 and Ni^{+2} showed antagonistic effects on Ni^{+2}-induced toxicity to the maximum quantum efficiency (Table 2).

Scanning Electron Microscope (SEM)

The abaxial leaf surface of control plants showed that, the structure looked like typical rice stomata (Fig. 1A). The stomata were defective and abnormal when exposed to 100 μMNi^{+2} or O_3, a wide range of differences were detected in stomatal size, formation of epicuticular waxy hairs and papillae. In leaf treated with 100 μM Ni^{+2} stomata seemed to be slightly sunken and guard cells were covered by numerous waxy hairs, these observations were much noticed in Giza 178 than in Sakha 101. The interaction of both stresses (100 μM Ni^{+2} and O_3) revealed that Sakha 101 showed larger, slightly opened aperture than Giza 178 (Fig. 1D).

Table 1: The influence of different concentrations of Ni^{+2}, O_3 (75 ppb/h) and their combinations on dry weights of the roots and shoots and relative water content of 28-day-old leaves of two *Oryza sativa* cultivars

Treatment	Root DW (μg plant $^{-1}$)		Shoot DW (μg plant $^{-1}$)		Leaf RWC (%)	
	Sakha 101	Giza 178	Sakha 101	Giza 178	Sakha 101	Giza 178
Control	4.83±0.43^{a}	4.5±0.40^{a}	23.33±2.08^{a}	17.33±1.54^{a}	85±7.57^{a}	81±7.21^{a}
10 µM Ni^{+2}	3.83±0.30^{b}	4.1±0.32^{a}	15.50±1.21^{b}	8.50±0.66^{b}	75±5.84^{b}	64±4.98^{b}
50 µM Ni^{+2}	3.50±0.30^{bc}	3.5±0.30^{b}	13.33±1.15^{b}	7.66±0.66^{b}	69±5.93^{bc}	53±4.56^{c}
100 µM Ni^{+2}	2.50±0.22^{c}	2.00±0.18^{c}	10.00±0.89^{c}	5.83±0.52^{b}	60±5.34^{c}	48±4.27^{c}
Ozone	3.16±0.23^{bc}	2.16±0.16^{c}	22.16±1.63^{a}	12.50±0.92^{c}	65±4.79^{c}	54±3.98^{c}
10 µM Ni^{+2}+Ozone	3.50±0.29^{b}	2.80±0.23^{cb}	16.66±1.36^{b}	8.33±0.68^{b}	73±5.94^{b}	58±4.72^{bc}
50 µM Ni^{+2}+Ozone	3.66±0.26^{b}	2.85±0.21^{cb}	16.66±1.20^{b}	13.33±0.96^{c}	81±5.84^{a}	59±4.25^{bc}
100 µM Ni^{+2}+Ozone	3.82±0.32^{b}	2.90±0.25^{cb}	18.66±1.58^{b}	13.33±1.13^{c}	81±6.88^{a}	62±5.26^{b}
P	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.001**

Values are means ± SD based on triplicate independent determinations, and different letters means significant difference as evaluated by Duncan's multiple comparison test

Table 2: The influence of different concentrations of Ni^{+2}, O_3 (75 ppb/h) and their combinations on photosynthetic pigments content and chlorophyll fluorescence in the leaves of 28-day-old plant of two *Oryza sativa* cultivars

Treatment	Chl. a		Chl. b		Chl.a/Chl.b		Carot.		Fv/Fm	
	Sakha 101	Giza 178	Sakha 101	Giza 178	Sakha 101	Giza 178	Sakha 101	Giza 178	Sakha 101	Giza 178
Control	1.704±0.15^{a}	1.498±0.13^{a}	1.01±0.09^{a}	0.877±0.08^{a}	1.687±0.15^{a}	1.708±0.15^{a}	0.452±0.04^{a}	0.389±0.03^{a}	0.826±0.07^{a}	0.794±0.07^{a}
10 μM Ni^{+2}	1.332±0.10^{b}	1.126±0.09^{b}	0.861±0.07^{a}	0.743±0.06^{ab}	1.547±0.12^{ab}	1.515±0.12^{ab}	0.401±0.03^{a}	0.338±0.03^{a}	0.764±0.06^{a}	0.632±0.05^{b}
50 μM Ni^{+2}	1.114±0.10^{cb}	0.875±0.08^{c}	0.784±0.07^{b}	0.615±0.05^{ab}	1.420±0.12^{b}	1.422±0.12^{b}	0.305±0.03^{ab}	0.203±0.02^{ab}	0.680±0.06^{b}	0.537±0.05^{bc}
100 μM Ni^{+2}	1.019±0.09^{c}	0.569±0.05^{d}	0.659±0.06^{b}	0.574±0.05^{b}	1.546±0.14^{ab}	0.991±0.09^{bc}	0.248±0.02^{b}	0.185±0.02^{b}	0.613±0.05^{b}	0.464±0.04^{c}
Ozone	1.394±0.12^{ab}	1.011±0.07^{bc}	0.894±0.07^{a}	0.733±0.06^{a}	1.559±0.12^{a}	1.379±0.09^{bc}	0.400±0.03^{a}	0.332±0.02^{a}	0.634±0.05^{ab}	0.500±0.04^{bc}
10μM Ni^{+2}+Ozone	1.249±0.10^{b}	0.938±0.08^{bc}	0.841±0.07^{a}	0.675±0.05^{ab}	1.485±0.12^{b}	1.389±0.11^{b}	0.306±0.02^{ab}	0.259±0.02^{ab}	0.677±0.06^{b}	0.627±0.05^{b}
50μM Ni^{+2}+Ozone	1.313±0.09^{b}	0.940±0.07^{bc}	0.805±0.06^{ab}	0.689±0.05^{ab}	1.631±0.12^{a}	1.364±0.10^{b}	0.268±0.02^{b}	0.229±0.02^{ab}	0.666±0.05^{b}	0.624±0.04^{b}
100 μM Ni^{+2}+Ozone	1.138±0.10^{cb}	0.732±0.06^{c}	0.769±0.07^{b}	0.640±0.05^{ab}	1.479±0.13^{b}	1.143±0.10^{bc}	0.213±0.02^{b}	0.161±0.01^{b}	0.648±0.06^{b}	0.510±0.05^{bc}
P	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*

Values are means ± SD based on triplicate independent determinations and different letters means significant difference as evaluated by Duncan's multiple comparison test

Light Microscope of Root Cross Sections

Well-developed root structure and fully developed aerenchyma was observed in untreated roots in both cultivars (Fig. 2A). Cross-sections of roots exposed to each treatment individually were observed as more disordered (Fig. 2B and C) than control roots, some alterations in the epidermal cells, cortical cells and vascular cylinder were noticed. The exodermis were highly disordered, comparable to the endodermis and vascular cylinder in both cultivars but the response was palpable in Giza 178 than in Sakha 101.

Roots of rice plants treated with O_3 (75 ppb/h), revealed that the reduction in vascular cylinder was more apparent in Giza 178 than in Sakha 101. The prominent changes due to the interaction between Ni^{+2} and O_3 showed an improvement in the performance of the cortical tissues and endodermis differentiation, to be in the whole appearance, near to the relevant control of the both cultivars (Fig. 2D).

Transmission Electron Microscope (TEM)

The ultramorphological study showed that the control leaves of both cultivars exhibited typical ellipsoid chloroplasts with well-defined chloroplast membrane, and an archetypal arrangement of the grana and stroma (Fig. 3A). Well-developed mitochondria and clear nucleus surrounded with nuclear membrane containing a dense chromatin material. Foremost and visible alterations in both cultivars treated with 100 μM Ni^{+2} like the subcellular structure was distorted, the cell wall seemed to be more thicker in Giza178 leaf than Sakha 101, the chloroplast structure showed a dilation of grana as well as chloroplast membranes. Additionally, most of chloroplast become more or less rounded or curved and not normally distributed as the control (Fig. 3B).

The negative impact of O_3 showed that some of chloroplasts become more elongated with reduced system of stacks and stromal thylakoids in Sakha 101 while in Giza178 the dilation of grana stacks was more evidently compared to their corresponding controls. The nucleus performed elongated unusual form and may be inter current tightly stacked to the plasma membrane (Fig. 3C). Combined stress resulted in an overall change of chloroplast shape, arrangement to the cell wall and number of chloroplasts, compared to nickel or ozone stressed plants, but not as compared to the control.

Elemental Analysis

Results indicated that in Sakha 101 rice seedlings, the accumulated Ni^{+2} level was lower than the Giza 178 ones. At 100 μM Ni^{+2}, for Sakha 101 the values were 17.5 and 66.0 in shoots and roots, respectively, the corresponding values were 29.5 and 72.0 for Giza 178 (Fig. 4). The metal accumulation showed a step decrease in Ni^{+2}-treated samples and did not further increase at the highest Ni^{+2} treatments in O_3-treated rice plants.

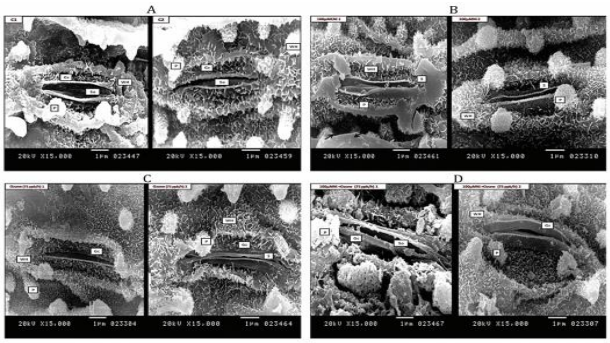

Fig. 1: Scanning electron microscopy (SEM) of abaxial leaf surface from two *Oryza sativa* L. cultivars [(1): Sakha 101 and (2): Giza 178] of 28- day old untreated and treated with Ni^{+2} at (100 μM), O_3 (75 ppb/h) and their combination. (S) stomata, (Gc) guard cell, (WH) waxy hairs, (P) papillae and (So) stomatal opening. A: control, B: 100 μMNi^{+2}, C: O_3 (75 ppb/h) and D:100 μM Ni^{+2} + O_3 (75 ppb/h)

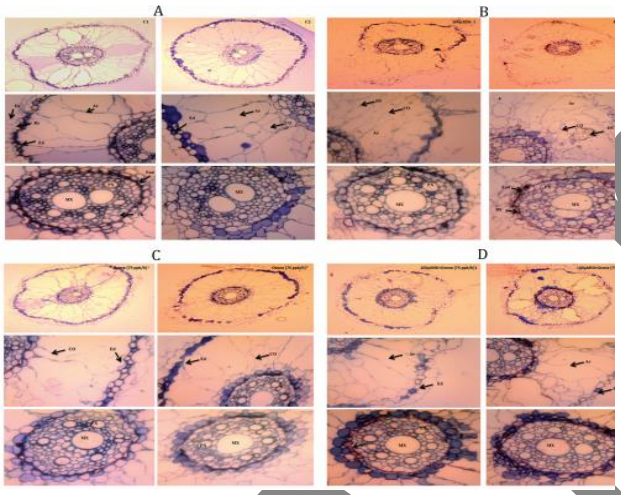

Fig. 2: Light microscope photography showing transverse sections of roots from two *Oryza sativa* L. cultivars [(1): Sakha 101 and (2): Giza 178] of 28-day-old untreated and treated with Ni^{+2} at (100 μM), O_3 (75 ppb/h) and their combination. (Ep)Epidermis, (Hy) Hypodermis, (Ed) Exodermis, (End) Endodermis, (Co) Cortex, (Ae) Aerenchyma, (MX) Metaxylem, (PX) Protoxylem. A: control, B: 100 μM Ni^{+2}, C: O_3 (75 ppb/h) and D: 100 μM Ni^{+2} + O_3 (75 ppb/h)

As Ni^{+2} level increased in nutrient solution an enduring decline in the concentrations of K^+ and Ca^{+2} in both cultivars was recorded but the reduction was more obvious in Giza 178 than Sakha 101 (Fig. 4). Mutual-treated samples in comparison to the corresponding singly-treated ones commonly showed a higher ability to accumulate K^+ and Ca^{+2} in shoots and roots.

Total Phenolics and Flavonoids Content

With increasing Ni^{+2} concentrations, shoots and roots exhibited a significant accumulation of total phenolics and flavonoids. The accumulation of phenolics in Giza

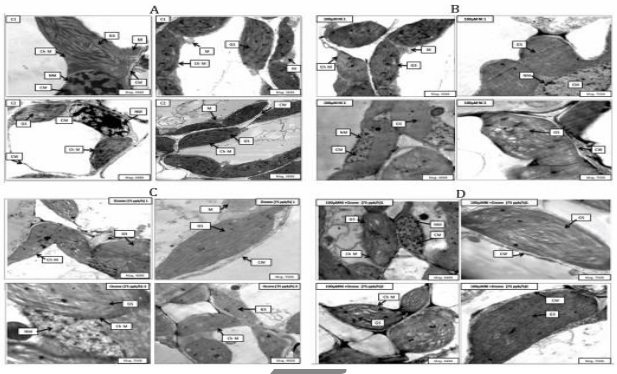

Fig. 3: Transmission electron micrographs of leaf mesophyll cells from two *Oryza sative* L. cultivars [(1): Sakha 101 and (2): Giza 178] 28–day-old Untreated (control) and treated with Ni^{+2} (100 μM), O_3 (75 ppb/h) and their combination. (CW) cell wall, (NM) nuclear membrane, (CM) chromatin material, (GS) grana stacks, (Chl M) chloroplast membrane, (M) mitochondria. The magnification panel is 4000X and 7500X. A: control, B:100 μMNi^{+2}, C: O_3 (75 ppb/h) and D: 100 μMNi^{+2} +75 ppb/h

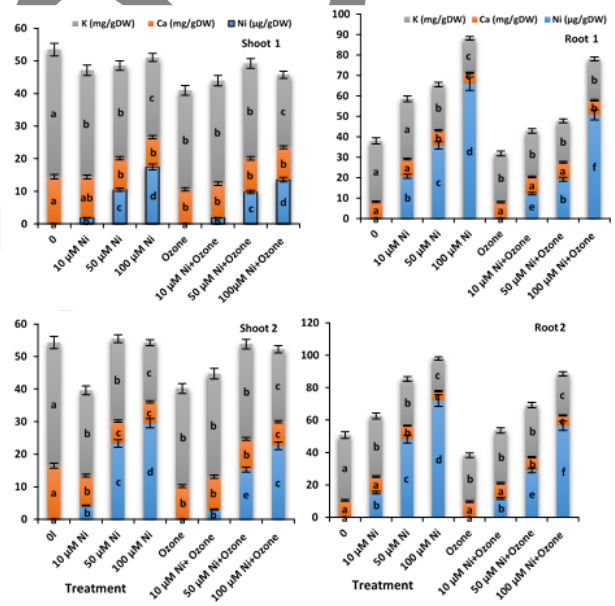

Fig. 4: The influence of different concentrations of Ni^{+2}, O_3 (75 ppb/h) and their combinations on some elements content in the shoots and roots of 28-day-old plants of two *Oryza sativa* cultivars (1): Sakha 101 and (2): Giza 178

Values are means ± SD based on triplicate independent determinations, and different letters means significant difference as evaluated by Duncan's multiple comparison test

178 cultivar was greater than in Sakha 101 plants and in shoots more than roots (Fig. 5A and B**)**. In O_3-exposed Sakha 101 plants shoots and roots, phenolics accumulation reached 3.4- and 1.5-fold respectively; the analogous values for Giza 178 were 4.5- and 1.6-fold with respect to their corresponding controls. The additive effects of O_3 and Ni^{+2} together in both cultivars were observed on the noticeable increase in the production of phenolics in comparison with the controls.

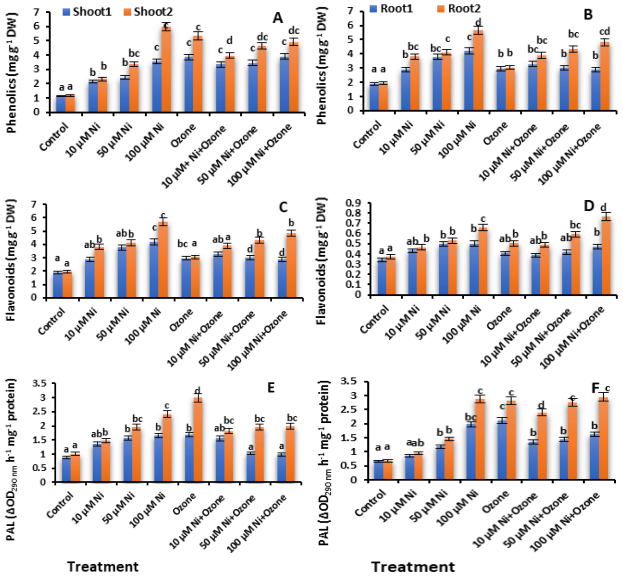

Fig. 5: The influence of different concentrations of Ni^{+2}, O_3 (75 ppb/h) and their combinations on (A and B) phenolics, (C and D) flavonoids content and (E and F) phenylalanine ammonia lyase enzyme (PAL) in the shoots and roots of 28-day-old plants of two *Oryza sativa* cultivars (1): Sakha 101 and (2): Giza 178

Values are means ± SD based on triplicate independent determinations, and different letters means significant difference as evaluated by Duncan's multiple comparison test

Ozone stress caused significant increase in the content of flavonoids in shoots and roots of both cultivars. Enhancement in flavonoids content was more pronounced in the Giza 178 roots which attained 2-fold of the control value. Combined treatment of Ni^{+2} and O_3 treatment, increased flavonoids contents, the concentration values was nearly similar to each of the stresses alone (Fig. 5C and D).

Phenylalanine Ammonia Lyase Activity (PAL)

Increasing Ni^{+2} concentrations resulted in a significant increase of PAL activity in shoots and roots (Fig. 5E and F). The values of 100 μM Ni^{+2}-stressed Sakha 101 plants reached about 1.9-and 3-fold of the control, the corresponding values in Giza 178 plants were 2.4-and 4.3-fold, respectively. Similar trend of stimulation in the activity of PAL was observed in the O_3-stressed plants of both rice cultivars and the maximum increase was noticed in Giza 178. The interaction between 100 μM Ni^{+2} and O_3 treatment showed a slight reduction in PAL activity for shoots in comparison with 100 μM Ni^{+2}-stressed alone, but statistically significant increase for PAL activity was recorded with respect to the control.

High Performance Liquid Chromatography (HPLC) Analysis for Individual Phenolics

The HPLC data of shoots of both rice cultivars (Table 3) in comparison with 14 reference standard phenolic compounds showed that cinnamic acid was detected in both cultivars in untreated shoots. All of the treatments exhibited an increase in quercetin and catechin that detected in both rice cultivars in shoots treated with 100 μM Ni^{+2} and /or O_3 as compared to their corresponding controls. Gallic acid was detected in plants treated with 100 μM Ni^{+2} and O_3 for Sakha 101; furthermore it was detected in shoots of Giza 178 grown under combination of both stresses. Chlorogenic acid was detected in untreated shoots of Sakha 101 while in Giza 178 it was detected in control, 100 μM Ni^{+2} and in shoots treated with both stresses.

Changes in Protein Profiles

SDS-PAGE analysis of control shoots showed the presence of twenty-four peptides ranging from 152 to 27.5 kDa molecular masses in Sakha 101 and twenty-five peptides ranging from 141 to 26.1 kDa molecular masses in Giza 178. Preliminary analyses by 1-DE of the effect of Ni^{+2} stress on shoot soluble protein profiles of Sakha 101 induced the synthesis of peptides with molecular masses of 143, 130, 119, 115, 109, 105, 103, 97, 94, 88, 85, 83, 73, 66, 60, 57, 53, 47, 43 and 31 kDa in response to 100 μM Ni^{+2}, while peptides with 152, 128, 102, 59, 30.8 and 27.5 kDa disappeared. Peptides with molecular masses 133, 122, 114, 109, 108, 104, 87, 81, 70, 65, 57, 52, 41, 38 and 17kDa were synthesized in response to 100 μM Ni^{+2}, down regulation of the Ni-induced 141, 124, 110, 107, 99, 89, 86, 84, 56 and 26kDa were lesser extent in Giza 178 (Fig. 6).

Exposure to O_3 (75 ppb/h) revealed that peptides with molecular masses 136,121, 111, 108. 107, 92, 88, 85, 80, 67, 65, 53, 47 kDa and 43KDa were synthesized in cultivar Sakha 101, while peptides with 152, 128, 59, 56, 52, 48, 44 and 30 kDa disappeared. Those of 135, 123, 117, 115, 114, 112, 103, 95, 88, 83, 79, 65, 54,47, 40, 38 and 29 kDa were synthesized, while peptides with 141, 124, 116,113, 102, 99, 92, 86, 84, 63, 56, 32 and 26 kDa disappeared from control in rice cultivar Giza 178 in response to O_3 fumigation.

The interaction between 100 μM Ni^{+2} and O_3 up regulated peptides with molecular masses 124, 121, 116, 112, 108, 100, 94.7, 87.9, 80.2, 73.2, 65.8, 63.4, 62.3, 57.3, 55.1, 46.9, 36.2 and 32.3 were synthesized in Sakha 101, , while, peptides with 152, 128, 38.1, 30.8 and 27.5 disappeared from the control samples (Fig. 6). Concerning cultivar Giza 178, peptides with molecular masses 122, 119, 117, 115, 108, 104, 95, 76, 70, 69, 67, 66, 64, 62, 60, 54, 38 and 35 were synthesized after the exposure to 75 ppb/h ozone and 100 μM Ni^{+2}, while, peptides with 141, 124, 116, 102, 92, 84, 78, 63, 45, 36, 32 and 26 were disappeared.

Discussion

In response to elevated Ni^{+2} treatments particularly with high concentration (100 μM Ni^{+2}), the growth of (*Oryza sativa* L.) cultivars was significantly abridged as evidenced by the reduction in dry weights, RWC, photosynthetic pigments and chlorophyll fluorescence (Fv/Fm). The reduction was higher in cultivar Giza 178 than in Sakha 101.

Table 3: HPLC analyses of phenolic constituents of methanolic extracts of 28-day-old shoots of *Oryza sativa* cultivars treated with 100 µM Ni^{+2} and O_3 (75 ppb/h) separately and in combination

Peak Name	Retention Time (RT min)	Control	100 μMNi	Ozone	Ozone + 100 μMNi	Control	100 μMNi	Ozone	Ozone + 100 μMNi
		Sakha 101				Giza 178			
Gallic acid	2.22	-	3.25	72.69	-	-	5.96	26.13	8.94
Chlorogenic acid	2.34	17.72	-	-	-	3.623	4.15	-	6.29
Caffeic acid	2.89	-	58.88	-	27.78	-	-	41.63	15.80
3,4- Dicaffeoylquinic acid	2.99	-	-	-	6.16	-	-	-	27.06
2,5-dihydroxy Benzoic acid	3.24	-	-	7.23	-	-	-	-	4.082
3,5- Dicaffeoylquinic acid	3.43	-	-	-	1.42	-	-	-	2.86
4,5-Dicaffeoyl quinic acid	3.77	3.25	3.09	22.92	11.58	2.75	2.53	-	-
Catechin	3.95	71.19	71.18	81.85	66.79	35.82	41.86	100.84	61.03
Rutin	4.66	9.24	-	-	-	2.69	3.19	-	9.47
Phloridzin	5.20	-	3.57	-	-	-	1.02	-	-
Tannic acid	5.40	-	-	-	-	-	-	-	-
Geraniol	7.66	-	-	1.02	-	-	1.02	-	-
Quercetin	9.06	5.01	7.81	8.74	7.244	6.88	8.04	8.91	6.685
Cinnamic acid	13.21	5.20	-	-	-	12.35	-	-	-

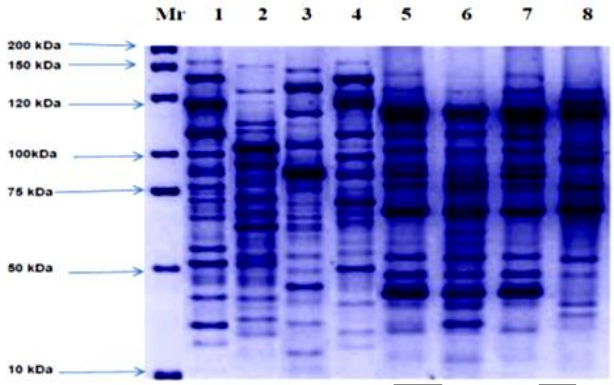

Fig. 6: SDS-PAGE for scanning the up-down regulated proteins of 28 –day-old *Oryza sativa* cultivars [Sakha 101 and Giza 178] treated with 100 μM Ni^{+2} and / or O_3 (75 ppb/h) and combinations between Ni^{+2} (100 μM) and O_3 (75 ppb/h). Columns (1-4) Sakha 101 cultivar,1: Control, 2:100 μM Ni^{+2}, 3: O_3 (75 ppb/h) and 4: 100 μM Ni^{+2} + O_3 (75 ppb/h). Columns (5-8) Giza 178 cultivar, 5: Control, 6:100 μMNi^{+2}, 7: O_3 (75 ppb/h) and 8:100 μM Ni^{+2} + O_3 (75 ppb/h)

This diminution may be the result of the reduction in the water uptake as evident with reduced RWC or enhanced water loss, and subsequent damages to the membrane (Latif, 2010). Furthermore, growth inhibition by Ni^{+2} toxicity results from metabolic disorders altered the process of photosynthesis that diminishes the release of carbohydrates or proteins and hense decline the growth of the tested plant. Disturbance in gas exchange through the stomata also altered photosynthesis as shown by SEM results, Ni^{+2} stimulate stomata to close as, it created disarray in water balance and translocation. Moreover, the waxy layer that acts as a barrier against threats from the external environment (Hussain *et al*., 2013; Kotapati *et al*., 2017). In fact, the inhibition of chlorophyll biosynthesis or its degradation has been recommended as being accountable for the inhibition of light reactions and photosynthesis and growth reduction due to this metal in other plant species (Parlak, 2016).

Ozone -fumigated rice cultivars reduced total biomass particularly roots dry weight and relative water content. This reduction was created due to the decline in transpiration and respiration owing to stomatal closure (Ainsworth *et al*., 2012); additionally, stomatal responses were altered owing to ozone and a variety of environmental stimuli (Dumont *et al*., 2013).

In the current study, it is difficult to separate the effect of O_3 on growth from Ni^{+2} stress as the latter alone can produce severe osmotic stress and growth reductions. Nevertheless, it can be speculated that mechanism by which O_3 stress increases plant tolerance to Ni^{+2} may be due to the effect of O_3 on Ni^{+2} uptake and accumulation. In confirmatory to this, there is a diminution in stomatal conductance (Fig. 1). Induction of growth stimulation in the present study by the interaction between Ni^{+2} and O_3 may be due to increase in shoots and roots dry weights, RWC and photosynthetic pigments content. The effects of interactions of O_3 and environmental factors are quite complicated, and mostly depend on plant species, O_3 concentration, time, ozone uptake into leaves; plant sensitivity to these environmental factors, the specific climate damage depends on the O_3 uptake into leaves which managed by stomatal conductance and the detoxification capability of oxygen radicals (Gan-Wen and Li, 2014; Pandey *et al*., 2017). Contrary, Rebouças *et al.* (2017) reported that ozone did not modify the stomatal response to drought under the combined stresses, and the whole plant physiological attributes were similar to the drought stress alone.

Under the prevailing experimental condition, the decline in quantum yield of PSII (Fv/Fm) in *Oryza sativa* by Ni^{+2} and/or O_3 treatment has been suggested to be associated with damages to the structural and function of PSII and augment in the inactivation of PSII reaction centers (Oukarroum *et al*., 2015). The results of TEM observed that in Ni^{+2}-treated Sakha 101 the chloroplast ultrastructure was quite tolerant to Ni^{+2} stress. 100 μM Ni^{+2}-treated Giza 178 rice cultivar showed a disruption of the chloroplast membrane and thylakoid membranes indicated that the chloroplast was relatively sensitive to Ni^{+2}-stress (Fig. 3B).

According to these observations, it can be concluded that the cellular ultrastructure revealed the extent of injure caused by nickel as observed by Zhou *et al*. (2018).

Little anatomical modifications were recorded for Sakha 101 than Giza 178 (Fig. 2), which indicated that the former cultivar was able to limit entrance to some extent of Ni^{+2} in root tissues. In previous studies, it was suggested that plants themselves possess homeostatic mechanisms to minimize damage from heavy metal toxicity. In the current study rice plants exposed to O_3 (75 ppb/h) showed a reduction in size of vascular cylinder as protoxylem pipes more obvious in Giza 178 than in Sakha101. Reduction in the number of conducting pipes has been reported in literature as being an adaptive assesses to protect water flow (Baas *et al*., 1983). The interaction of both stresses (100 μM Ni^{+2} and O_3) made the rice plant get recovered to better performance. Stetsenko *et al*. (2017) reported that the combined treatment of *Atropa belladonna* plants with Ni^{+2} and moderate salinity was found to decrease the toxic impact of Ni^{+2}, which was partly due to the diminished Ni^{+2} content in roots and shoots. This attenuation may result from the retarded rate of Ni^{+2} absorption as well as from binding of Ni^{+2} in the rhizoderm and cortex cells to vacuolar chelators with the consequent restriction of Ni^{+2} transport to the central cylinder. Additionally, the adsorption of pollutants on polyphenols may be the potential of immobilization of them in the plant roots (Jiang *et al.*, 2017).

Nickel exposure resulted in various unfavorable changes in the macronutrient content together with increase of Ni^{+2} accumulations in the rice cultivars. The effect of ozone on uptake and accumulation of nickel could be the basis by which O_3 stress increases plant tolerance to Ni^{+2} (Dumont *et al*., 2013). High concentrations of Ni^{+2} decreased significantly the K^+ concentration, suggesting that Ni^{+2} could influence the impasse of K^+ channels and implicated in a rapid inhibitory effect of plant growth. In the present study, Ca^{+2} decreased significantly by Ni^{+2} stress in the lower and upper parts of both cultivars and as a result a decrease in stress tolerance because Ca^{+2} is firmly implicated in stress tolerance and signal transduction (Knight, 2000). Several studies have focused on the inhibition of respiration by Ni^{+2} and may retard the release of energy required for active absorption of elements by plants (Bhalerao *et al*., 2015). Combined treatment between 100 μM Ni^{+2} and 75 ppb/h ozone revealed that concentrations of Ca^{+2} and K^+ of rice cultivars increased significantly compared to their Ni^{+2} -treated ones, the higher content of K^+ regulate stomatal opening. Such interaction caused efficient membrane stability and function (Tammam *et al*., 2019). Hedrich (2012) suggestions were that stomatal pore size controls the transport of molecules across the tonoplast and the plasma membrane through the regulation of activity of guard cell ion channels and transporters.

In the present study, it was found that induction of phenolics biosynthesis in Ni^{+2} or O_3-treated rice cultivars significantly depended on Ni^{+2} concentrations in culture medium, which may verify their role in cell protection and detoxification mechanisms. Phenolic compounds can scavenge free radicals or react directly with ozone (Alothman *et al*., 2010). Our findings revealed that flavonoids content increased under all the stress factors where the sensitive plants (Giza 178) accumulated more total phenolics and flavonoids contents than the tolerant one (Sakha 101) as membranes' integrity preserved by flavonoids help preventing the access of harmful molecules that induce oxidative damage to the membrane components (Arora *et al.*, 2000).

Results showed a connection between growths at Ni^{+2} or elevated O_3 increased the antioxidant ability of phenolics and flavonoids to avoid the oxidative damage in rice cultivars, while growth improved under Ni^{+2} and O_3 interaction decreased or induced similar response as individual stress on the antioxidant capacity of phenolics and flavonoids. This might be due to over active of ozone and Ni^{+2} possibly will contribute to the inhibition of peroxidase and polyphenol oxidase, which often causes oxidation of phenolic compounds (Chauhan *et al.*, 2011).

HPLC analysis in the present study revealed that the hydroxy cinnamic acid derivates, chlorogenic acid was the most abundant compounds in rice cultivars in Ni^{+2}-stressed and combined stress of Ni^{+2} plus O_3 in Giza 178 rice cultivar which has antioxidative assets that are associated with its structure, chlorogenic acid accumulated as a response to abiotic stresses such as heavy metal (Kováčik *et al*., 2011) and ozone (Peltonen *et al*., 2010). The activity of phenylalanine ammonia-lyase and the enhancement of soluble sugar elevations caused stimulation of polyphenols. Jiang *et al*. (2017) findings were that heavy metals increase the activity of PAL in plant tissues. Thus, PAL plays an essential role in amendment the resistance against the nickel toxicity in rice cultivars. Srinandhinidevi *et al*. (2015) revealed that increase in the ozone concentration enhances PAL activity in *Cicer arietinum* and *Trigonella foenum*. The interaction between the two stresses in the shoots-PAL activity showed a statistically significant increase as compared to control and a slight reduction in comparison with 100 μM Ni^{+2}-stressed alone. Thus, the ability of rice plant to regulate PAL activity which could be due to the existence of multiple PAL-encoding genes within the cultivars of the same species (Macdonald and D'cunda, 2007).

Nickel treatment resulted in up and down regulated proteins in rice shoots with differential expression between control and Ni-treated samples. In all Ni^{+2}-treatments, there were more up regulated than down regulated proteins in the examined rice cultivars, these polypeptides may be stress proteins produced to overcome the toxic effect of Ni^{+2} and may have significant role in Ni^{+2}-binding capacity and resistant of rice cultivars to Ni^{+2}-stress. On the other hand, the disappearance of some protein bands in both cultivars of rice could be due to lowered protein synthesis and/or the

diminution of reserve protein to conquer the anxiety originated by nickel.

Mishra *et al.* (2006) recorded that the increase in soluble protein under heavy metal stress may be related to the induced synthesis of stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins. Latif (2010) confirmed that two peptides with molecular mass 183 and 69 kDa are appeared at 100 ppm Ni^{+2} in two cultivar of radish. Exposure to 75 ppb/h ozone alone and subsequent SDS-PAGE analysis showed the appearance of a number of peptides with different molecular masses. Kerner *et al.* (2014) recorded that nine proteins related to the Calvin cycle showed decreased amounts in the twice ambient O_3-treated juvenile beech trees compared to the ambient O_3-fumigated group. The interaction between individual stresses revealed that up and down regulation of several peptides, that appear to have a role in the mechanism of tolerance which allows making biochemical and structural adjustments that enable the rice plant to cope with stress conditions.

Conclusion

The combinations of two stresses, with some common toxicity pathways, persuade plant responses not always expected on the basis of the known effects induced by the single stress. The antagonistic effect of both pollutants on growth parameters, photosynthetic machinery and antioxidant content improves mechanisms of growth reduction in rice plant exposed to combination of Ni^{+2} and O_3. Additionally, Sakha 101 cultivar was found to be resistant than Giza 178 as it depicts comparatively strong defense system causing minimum biomass loss.

References

Ainsworth, E.A., C.R. Yendrek, S. Sitch, W.J. Collins and L.D. Emberson, 2012. The effects of tropospheric ozone on net primary productivity and implications for climate change. *Annu. Rev. Plant Biol.*, 63: 637–661

Alothman, M., B. Kaur, A. Fazilah, R. Bhat and A.A. Karim, 2010. Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Sci. Emerg. Technol.*, 11: 666–671

Arora, A., T.M. Byrem, M.G. Nair and G.M. Strasburg, 2000. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Arch. Biochem. Biophys.*, 373: 102–109

Baas, P., E. Werker and A. Fahn, 1983. Some ecological trends in vessel characters. *IAWA J.*, 4: 141–159

Bergmann, E., J. Bender and H.J. Weigel, 2017. Impact of tropospheric ozone on terrestrial biodiversity: A. literature analysis to identify ozone sensitive taxa. *J. App. Bot. Food Qual.*, 90: 83–105

Bhalerao, S.A., A.S. Sharma and A.C. Poojari, 2015. Toxicity of nickel in plants. *Inter. J. Pure App. Biosci.*, 3: 345–355

Bohler, S., K. Sergeant, Y. Jolivet, L. Hoffmann, J.F. Hausman, P. Dizengremel and J. Renau, 2013. A physiological and proteomic study of poplar leaves during Ozone exposure combined with mild drought. *Proteomics*, 13: 1737–1754

Branquinho, C., D.H. Brown and F. Catarino, 1997. The cellular location of Cu in lichens and its effect on membrane integrity and chlorophyll fluorescence. *Environ. Exp. Bot.*, 38: 165–179

Chapman, H.D. and P.F. Pratt, 1961. *Methods of Analysis for Soils, Plants and Waters*. Berkeley: University of California

Chauhan, O.P., P.S. Raju, N. Ravi, A. Singh and A.S. Bawa, 2011. Effectiveness of ozone in combination with controlled atmosphere on quality characteristics including lignification of carrot sticks. *J. Food Eng.*, 102: 43–48

De Silva, N.D.G., E. Cholewa and P. Ryser, 2012. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple *(Acer rubrum* L.). *J. Exp. Bot.*, 63: 5957–596

Demiray, S., M.E. Pintado and P.M.L. Castro, 2009. Evaluation of phenolic profiles and anti-oxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves, and *Polygonum bistorta* roots. *WASET*, 54: 312–317

Dumont, J., F. Spicher, P. Montpied, P. Dizengremel, Y. Jolivet and D. Le Thiec, 2013. Effects of ozone on stomatal responses to environmental parameters (blue light, red light, CO_2 and vapour pressure deficit) in three *Populus deltoids*, *Populus nigra* genotypes. *Environ. Pollut.*, 173: 85–96

Gan-wen, L. and X. Li, 2014. Interactions of ozone stress and other environmental factors on plants. *Chin. J. Ecol.*, 33: 1678–1687

Hames, B.D. and D. Rickwood, 1990. An introduction to poly acrylamide gel electrophoresis. *In: Electrophoresis of Proteins: A Practical Approach*, pp: 34–48. Hames, B.D. and D. Rickwood (eds.). England publishing Co. TRL, Lond

Hedrich, R., 2012. Ion channels in plants. *Physiol. Rev.*, 92: 1777–1811

Hoagland, R. and D.I. Arnon, 1950. The water culture method for growing plants without soil. *California Agric.*, 347: 1–32

Hussain, M.B., S. Ali, A. Azam, S. Hina, M.S. Farooq, B. Ali, S.A. Bharwana and M.B. Gill, 2013. Morphological, physiological and biochemical responses of plants to nickel stress: A review. *Afr. J. Agric. Res.*, 8: 1596–1602

Iori, V., F. PIetrini, A. Cheremisina, N.I. Shevyakova, N. Radyukina, V.V. Kuznetsov and M. Zacchini, 2013. Growth responses, metal accumulation, and phytoremoval capability in *Amaranthus* plants exposed to nickel under hydroponics. *Water Air Soil Pollut.*, 224: 1450–1459

Jiang, S., B. Weng, T. Liu, Y. Su, J. Liu, H. Lu and C. Yan, 2017. Response of phenolic metabolism to cadmium and phenanthrene and its influence on pollutant translocations in the mangrove plant *Aegiceras corniculatum* (L.) *Blanco* (Ac). *Ecotoxicol. Environ. Safe.*, 141: 290–297

Jiang, Y. and D.C. Joyce, 2003. ABA effects on ethylene production, PAL activity, anthocyanin, and phenolic contents of strawberry fruit. *Plant Growth Reg.*, 39: 39–343

Joo, J.H., S. Wang, J.G. Chen, A.M. Jones and N.V. Fedoroff, 2005. Different signalling and cell death roles of heterotrimeric G protein alpha and beta subunits in the *Arabidopsis* oxidative stress response to ozone. *Plant Cell*, 17: 957–970

Kerner, R., E. Delgado-Ecker, D. Ernst, J.W. Dupuy, T.E. Grams, E. Barbro, J. Winkler, C. Lindermayr and G. Müller-Starck, 2014. Large-scale protein analysis of European beech trees following four vegetation periods of twice ambient ozone exposure. *Proteomics*, 109: 417–435

Knight, H., 2000. Calcium signalling during abiotic stress in plants. *Intl. Rev. Cytol.*, 195: 269–325

Kotapati, K.V., B.K. Palaka and D.R. Ampasala, 2017. Alleviation of nickel toxicity in finger millet (*Eleusine coracana* L.) germinating seedlings by exogenous application of salicylic acid and nitric oxide. *Crop J.*, 5: 240–250

Kováčik, J., B. Klejdus, J. Hedbavny and J. Zoń, 2011. Significance of phenols in cadmium and nickel uptake. *J. Plant Phys.*, 168: 576–584

Laemmli, M.R., 1970. Cleavage of structural protein during assembly of the head bacteriophage T4. *Nature*, 277: 680–685

Latif, H.H., 2010. The influence of nickel sulphate on some physiological aspects of two cultivars of *Raphanus sativus* L. *Arch. Biol. Sci.*, 62: 683–691

MacDonald, M.J. and G.B. D'Cunda, 2007. A modern view of phenylalanine ammonia lyase. *Bioch. Cell Biol.*, 85: 273–282

Mata, C.G. and L. Lamattina, 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiol*., 126: 1196–1204

Mishra, S., S. Srivastava, R.D. Tripathi, R. Kumar, C.S. Seth and D.K. Gupta, 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, 65: 1027–1039

Mitchel, G.A., F.T. Bingham and A.L. Page, 1978. Yield and metal composition of lettuce and wheat grown in soils amended with sewage sludge enriched with cadmium, copper, nickel and zinc. *J. Environ. Qual*., 7: 165–171

Moran, R., 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. *Plant Physiol*., 69: 1376–1381

Oukarroum, A., L. Barhoumi, M. Samadani and D. Dewez, 2015. Toxic effects of nickel oxide bulk and nanoparticles on the aquatic plant *Lemna gibba* L. *Bio. Med. Res. Inter*., Article ID: 501326

Padda, M.S. and D.H. Picha, 2007. Methodology optimization for quantification of total phenolics and individual phenolic acids in sweet potato (*Ipomoea batatas* L.) roots. *J. Food Sci*., 72: 412–416

Pandey, P., V. Irulappan, M.V. Bagavathiannan and M. Senthil-Kumar, 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. *Front. Plant Sci*., 8: 1–15

Parlak, K.U., 2016. Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings. *Wagenien. J. Life Sci.*, 76: 1–5

Peltonen, P., R. Julkunen-Tiitto, E. Vapaavuori, J. Heinonen and J. Holopainen, 2010. Do elevated atmospheric CO_2 and O_3 affect food quality and performance of folivorous insects on silver birch? *Global Chang. Biol*., 16: 918–935

Pietrini, F., M. Zacchini, V. Iori, L. Pietrosanti, M. Ferretti and A. Massacci, 2010. Spatial distribution of cadmium in leaves and its impact on photosynthesis: examples of different strategies in willow and poplar clones. *Plant Biol*., 12: 355–363

Porra, R.J., 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynth. Res*., 73: 149–156

Potter, S.M., A.J. Mitchell, W.B. Cowden, L.A. Sanni, M. Dinauer, J.B. De Haan, K.L. Krishna, K. Mruthunjaya and J.A. Patel, 2010. Antioxidant and hepatoprotective potential of stem methanolic extract of *Justicia Gendarussa* Burm. *Intl. J. Pharmacol*., 6: 72–80

Rebouças, D.M., Y.M. De Sousa, M. Bagard, J.H. Costa, Y. Jolivet, D.F. De Melo and A. Repellin, 2017. Combined effects of ozone and drought on the physiology and membrane lipids of two cowpea (*Vigna unguiculata L.*) walp cultivars. *Plants*, 6: 1–18

Singh, S., M. Zacharias, S. Kalpana and S. Mishra, 2012. Heavy metals accumulation and distribution pattern in different vegetable crops. *J. Environ. Chem. Ecotoxicol*., 4: 170–177

Sokal, R.R. and F.J. Rohlf, 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*, (3rd edition). New York: W.H. Freeman Publishers

Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastructure Res.*, 26: 31–43

Srinandhinidevi, K.M., M. Khopade and K.D. Pangsatabam, 2015. A preliminary study on the effects of ozone on induction of resistance in *Cicer arietinum* and *Trigonella foenum* against acute ozone exposure. *J. Biotech. Biochem.*, 1: 6–14

Stetsenko, L.A., A.D. Kozhevnikova and A.V. Kartashov, 2017. Salinity attenuates nickel-accumulating capacity of *Atropa belladonna* L. plants. *Russ. J. Plant Physiol*., 64: 486–496

Tammam, A., R. Badr, H. Abou-Zeid, Y. Hassan and A. Bader, 2019. Nickel and ozone stresses induce differential growth, antioxidant activity and mRNA transcription in *Oryza sativa* cultivars. *J. Plant Interact.*, 14:87–101

Tausz, M., N.E. Grulke and G. Wieser, 2007. Defense and avoidance of ozone under global change. *Environ. Pollut*., 147: 525–531

Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem*., 104: 1372–1378

Treshow, M. and D. Stewart, 1973. Ozone sensitivity of plants in natural communities. *Biol. Conserv*., 5: 209–214

Venable, J.H. and R. Coggeshall, 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol*., 25: 407–408

Zhou, J., Z. Zhang, Y. Zhang, Y. Wei and Z. Jiang, 2018. Effects of lead stress on the growth, physiology, and cellular structure of privet seedlings. *PLoS One*, 13: e0191139

(Received 05 June 2019; Accepted 27 August 2019)