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Effect of Rice and Quinoa Straws on Reducing Lead Uptake in Lettuce (*Lactuca sativa)*

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Abstract: Two soil samples were obtained from a Farm on the roadway of Qalyub district (QF soil) and Farm of Faculty of Agriculture, Ain Shams Univ., Qalyubia Governorate (AS soil). Lead concentration was determined in the two soil samples. It was found that QF soil has more concentration of lead (300 mg Kg^{-1} soil) than AS soil (35.57 mg Kg^{-1} soil). In a pot experiment, lettuce seedlings were transplanted in two sources of lead contaminated soils during the seasons of 2013/2014 and 2014/2015 to study the effects of Pb on growth parameters, some biochemical constituents in lettuce and the efficiency of rice or quinoa straw in reducing Pb uptake from the contaminated soils. Increasing Pb level in soils caused a gradual decrease in plant fresh and dry weights, average leaves number, stem diameter and head circumference. Ascorbate peroxidase (ASP) activity and total soluble phenols, N, P, K and Ca concentrations were decreased. Whereas, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), glutathione (GSH), oxalic acid and Pb concentrations and the activities of guaicol peroxidase (POD) and polyphenol oxidase (PPO) were increased. Adding rice or quinoa straws as an agricultural residue to both Pb contaminated soils enhanced plants tolerance under lead stress by improving the growth vigor, increased uptake of N, P, K, Ca ions and soluble phenols concentration and reduced proline, H_2O_2, MDA, GSH, oxalic acid, Pb concentrations and activities of ASP, POD and PPO enzymes. The results concluded that rice straw is more effective than quinoa straw in most morphological and biochemical components.

Key words: *Lactuca sativa* (lettuce) · Rice straw · Quinoa straw · Lead · Glutathione · Oxalic acid · Antioxidant enzymes

INTRODUCTION

Our bodies are exposed to the heavy metals which present from the earth's crust. Some of these heavy metals are essential for human body's metabolism, but they become toxic substances at a rate greater than that the substance is lost through metabolism. These heavy metal pollutants can persist in the natural ecosystem for a long time and accumulate in successive concentrations in the biological chain causing acute and chronic diseases [1]. Lead (Pb) is considered one of the 16 toxic pollutants due to its carcinogenic effects for human bodies [2]. Recently, lead has been used in modern industries such as lead acid batteries, coloring ceramics, shielding from radiations, used in lead glass to reduce the transmission of radiation and change the glass optical characteristics, pesticides and other compounds [3, 4]. Lead is not biodegradable and considerably persistent in both soil and water. Most of Pb accumulates in the top 8cm of the soil surface as it has a very low mobility and it is so difficult to remove if it entered the soil matrix. The ability of the soil to adsorb Pb increases with high pH, cation exchange capacity of the soil as well as by root exudation, organic carbon content and presence of chelates specially phosphate and sulphur [5]. Lee *et al.* [6] reported that Pb absorption in the plant tissues increases with increasing pH between 3.0 to 8.5. Wierzbicka *et al.* [7] demonstrated that Pb accumulates in an insoluble form in roots, as well as, Sharma and Dubey [8] mentioned that significant amounts of Pb can translocate and accumulate in the leaves.

Lettuce (*Lactuca sativa* L.) is an annual plant of the family Asteraceae. Lettuce was cultivated by the ancient Egyptians as a weed which used to produce oil, then they turned it into a leafy vegetable plant which used as a fresh salad for its succulent leaves [9]. Also, lettuce provides

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the human about 20% of the daily value of vitamin A due to its high concentration of provitamin A compound and beta-carotene. It is rich in iron and folic acid. The potential transfer of soil lead into lettuce leaves is a concern when the soils are contaminated with this toxic compound. Leafy and root crops accumulate high levels of Pb from contaminated soils [10]. Lead toxicity causes a decrease in growth and yield of plants, disruption of macro and micro mineral nutrition, inhibition of photosynthesis, inhibition of enzymes activity, water imbalance, defects hormonal status and affects membrane permeability [11-16, 8, 12].

Some researchers were carried out experiments to reduce bioavailability of heavy metals from soil and waste water. It is usually achieved by chemical or physical processes such as precipitation, coagulation, reduction membrane procession change and adsorption [17, 18]. The chemical methods have proved to be less efficient and more expensive than the adsorption process. Adsorption has superiority over the other methods due to its simple design with a sludge free environment and can include low consumption in term of both initial cost and land required [19]. Using the inexpensive agricultural residues such as rice straw, saw dust, carrot residues, tobacco dust, orange residues, apple residues, coconut shell powder and castor leaves powder, as adsorbents have been used for lead and other metals removal from soil and waste water [20-23, 6, 24, 25]. Using agricultural residues avoid dramatic landscape disruption and preserve the ecosystem. Agricultural chaff comprises several functional groups that facilitates heavy metals absorption via it's containing of simple sugars, proteins, starch, hydrocarbons and ash [26]. Rice straw contains lignin, organic acids and tannins that help in the metal chelation process [27]. Quinoa is a highly nutritional food for both human beings and animals due to its higher value of minerals, proteins, vitamins, fatty acids, antioxidants and phytohormones [28, 29].

This work aims to reduce the adverse effect of lead on lettuce transplanting in lead contaminated soils using rice or quinoa straw which have the beneficial effect of adsorption and enhancing morphological parameters, biochemical constituents and the activity of antioxidant enzymes.

MATERIALS AND METHODS

Soils Collection: Two surface soil samples were collected from two different locations to investigate the presence of lead in these soils. The concentrations of lead (Pb) in the soil of the two different locations were determined.

The first soil sample was collected from a Farm closed to the roadway of Qalyub district (Qalyubia Governorate), as this way has heavy traffic and busy roadway. So, it may suspect to be lead contaminated soil. Other soil sample was obtained from the Experimental Farm of Faculty of Agriculture, Ain Shams University, Shoubra El Kheima, Qalyubia Governorate, Egypt. Surface soils samples (0-15 cm) were collected at August 2013 from the preselected locations. The properties of the two soil samples were analyzed at the Soil and Water Research Centre, ARC, Giza, Egypt. The two soils were classified as loamy texture. They have quiet similar physiochemical properties except for their total Pb concentrations. The measure properties of these soils were given in Table (1). Each type of soil was divided into 2 groups for the two cultured successive seasons during October 2013 and October 2014. Then, each group was divided into 3 subgroups with 3 replicates for each soil sample.

Preparation of Rice and Quinoa Straws: Rice and quinoa straws were obtained from Crop Agricultural Department Farm, Faculty of Agriculture, Ain Shams University and Agricultural Botany Department Farm, Faculty of Agriculture, Ain Shams University, respectively. Both rice and quinoa straws were chopped into small pieces, then were washed well under running tap water for 1 h with adding tween 20, followed by distilled water. The samples were dried in the oven at 40°C for 6 h, then left to dry at the room temperature for 1 week. The dried rice and quinoa straws were grinded separately by mechanical grinder to obtain homogenous grinding. Grinded straws were passed through fine sieves to obtain fine particles. The homogenous grinding of both straws types was divided equally and saved in dried containers for using in the two cultivated seasons.

Preparation of Pots: Rice and quinoa straws were added separately into each subgroup of the previous two samples of soils as 100 g 5 Kg^{-1} dry soil and mixed well together to obtain homogenous mixture of soil and straw. The third group of soil sample was left without adding rice or quinoa straw as a control for each soil sample.

Labelled plastic pots (30 cm in diameter and 28 cm in height) with bottom drainage holes were filled with 10 Kg pot^{-1} of previous subgroups of soil samples, with 3 replicates for each soil sample. Each replicate contains 3 pots distributed in a complete randomized block design. Nitrogen, phosphorus and potassium fertilizers were applied at the rate of 0.5g N/pot ammonium sulphate (N 20.5%), 0.63g P_2O_5/pot super phosphate (P18%) and 0.13g K/pot potassium sulphate (K 48%). Phosphorus was

Table 1: Mechanical and chemical analyses of collected soil samples from Farm of Faculty of Agriculture (AS soil) and Qalyub Farm (QF soil) without and with adding rice or quinoa straws.

		AS or QF soil		AS or QF soil with adding rice or quinoa straw			
Sources of soil		AS soil	QF soil	AS soil +rice straw	AS soil + quinoa straw	QF soil +rice straw	QF soil + quinoa straw
Soil properties					Values		
Practical size	Fine sand	12.00	9.80	11.11	11.30	10.20	11.90
distribution (%)	Rough sand	8.20	13.30	10.20	9.70	12.40	11.70
	Silt	29.8	28.70	27.80	27.90	26.90	29.30
	Clay	50.00	48.20	50.80	51.10	50.50	47.10
	Soil texture	Loamy	Loamy	Loamy	Loamy	Loamy	Loamy
EC (ds m^{-1})		1.02	1.09	1.04	1.06	2.01	1.09
pH		7.97	7.95	7.26	7.38	7.47	7.38
Soluble anions (meq L^{-1})	HCO_3^-	3.50	3.40	3.50	3.20	3.30	3.10
	Cl^-	2.50	2.30	2.50	2.40	2.50	2.40
	SO4-	5.10	5.40	5.40	4.20	4.90	4.70
Soluble cations (meq L^{-1})	Ca^{++}	4.50	4.20	5.20	5.40	5.30	5.40
	Mg^{++}	3.00	3.20	5.30	5.90	4.10	4.10
	Na^+	2.73	2.75	2.11	2.54	2.51	2.43

added to soil during preparation of the pots, while nitrogen and potassium fertilizers were applied in two equal instalments at 3 and 7 weeks after transplanting as recommended by Egyptian Ministry of Agriculture and Land Reclamation, Egypt. (Bulletin No. 1233 for the year 2011). The soils were watered to a field-moist state with tap water. A transparent plastic sheet (120 micron) was placed on the surface of each pot to protect soil against lead atmospheric fallouts and avoid transferring dust from each soil sample to others. Then all soil samples were left for 15 days to allowed equilibrium of each soil. Soil properties after adding rice and quinoa straws were analysed and the physiochemical properties of the soils were obtained in Table (1).

Lettuce (*Lactuca sativa* var. capitata L.) cv. Shanzy seedlings (5 weeks age), obtained from Arid Land Agriculture Research Institute, Faculty of Agriculture, Ain Shams University, were used in this study. Two holes were made in the plastic sheet and 2 seedlings were transplanting in the place of these holes on 1st of October 2013 and 2014 cultivated seasons. Pots were watered when the soil was visibly dry.

Vegetative Growth Characteristics: Three lettuce plants were taken from each replicate after 90 days from transplanting for recording plant growth measurements i.e. plant fresh and dry weights (g), average leaf fresh weight (g), average leaves number, stem diameter (cm) and head circumference (cm).

Biochemical Analyses: Randomly, three heads were taken at 60 days after transplanting from each treatment and washed carefully with tap water for 5 minutes and then in 2 paths of distilled water for another 2 minutes to eliminate the present particles of dust or lead on the leaf surface. Each head was cut into 4 quarters and one was randomly taken to determine the concentrations of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), total soluble phenols, proline, glutathione (GSH), oxalic acid and the activities of ascorbate peroxidase (ASP), guaicol peroxidase (POD) and polyphenol oxidase (PPO). Another quarter was taken randomly, excess water was dried in paper bags in the oven at 40°C and grinded to determine Pb, N, P, K and Ca concentrations.

Hydrogen peroxide was determined in leaf tissues as described by Zhou *et al.* [30]. H_2O_2 concentration was calculated as mg H_2O_2 100 g^{-1} f.wt. The level of lipid peroxidation was measured by determination of malondialdehyde (MDA) in leaf tissues as described by Heath and Packer [31]. MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol MDA g^{-1} f.wt. The colorimetric method of Folin-Ciocalteu as described by Shahidi and N aczk [32] was employed for the chemical determination of total soluble phenolic compounds. The soluble phenolic compounds concentration was expressed as mg gallic acid 100 g^{-1} f.wt. Proline concentration was determined using a ninhydrin colorimetric method of Bates *et al.* [33].

The proline concentration was expressed as mg proline $100g^{-1}$ f.wt. Reduced glutathione (GSH) on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) was estimated by the method of Moron *et al.* [34]. The concentrations were expressed as nmoles GSH g^{-1} f.wt. Oxalic acid was determined due to the oxidation of Bromosoluble phenols blue by potassium dichromate in presence of oxalic acid as assayed by Xu and Zhang [35]. The oxalic acid concentration was expressed as mg oxalic $100g^{-1}$ f.wt.

For measuring the concentrations of Pb, N, P, K and Ca, 0.5 g dry samples of leaves were digested for 12 h with (7.5 mL) 65% HNO_3 and (2.5 mL) 36% HCl at 25°C, then heated for 2 h at 105°C according to Cottenie *et al.* [36].

Total lead (Pb) concentration was determined by Inductively Coupled Plasma Spectrometry (ICP) (Ultima 2 JY Plasma) according to the procedure of "Environmental Protection Agency" [37]. Micro-Kjeldahl method was used for the determination of nitrogen (N) concentration according to the method described by A.O.A.C. [38]. Phosphorus (P) concentration was measured colorimetrically using UV-Vis spectrophotometer UV 9100 B, Lab Tech by ascorbic acid method according to Watanabe and Olsen [39]. The concentration of potassium (K) was determined using flame photometer (PEP 7 no 8923) as estimated by Eppendrof and Hing [40]. Total calcium (Ca) was determined according to Chapman and Pratt [41] by using atomic absorption spectrophotometer (AA.20). Pb was determined as mg Kg^{-1} d.wt. whereas, N, P, K and Ca concentrations were determined as g 100 g^{-1} d.wt.

Soluble protein concentration was estimated to calculate specific activity of enzymes. Protein concentration was quantified in the crude extract by the method of Bradford [42] using bovine serum albumin as a standard.

Enzymes Assay

Preparation of Enzymes Crude Extract: One-gram fresh leaves, already being frozen in liquid nitrogen to prevent proteolytic reactions, were homogenized with cold phosphate buffer (100 mM, pH=7.0) containing 0.1 mM EDTA and 1 % polyvinyl pyrrolidone (PVP) (W/V) at 4°C. The extraction ratio was 4 ml buffer for each one gram of plant materials. Homogenate was centrifuged at 15000 ×g for 15 min at 4°C. Supernatant was considered as enzyme crude extract and used to measure the activities of guaiacol peroxidase (POD) and polyphenol oxidase (PPO). For measuring ascorbate peroxidase (ASP) the leaf tissue was separately grinded in homogenizing medium containing 20mM ascorbic acid in addition to the previous ingredient.

Antioxidant Enzymes Assays: Ascorbate peroxidase (ASP) (E.C 1.11.1.11) was measured according to Nakano and Asada [43]. One unit of enzyme activity was defined as the amount of enzyme required for oxidation of 1 µmol of ascorbate per minute and caused a decrease in absorbance at 290 nm.

Guaicol peroxidase, POD (E.C 1.11.1.7) activity in enzyme crude extract was determined as described by Hammer Schmidt *et al*. [44]. The activity was calculated by measuring the absorbance changes at 470 nm per min. Unit of enzyme (IU) equal 0.01 ΔOD. min^{-1}.

Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was measured according to Benjamin and Montgomery [45]. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per min at 420 nm. All the aforementioned enzymes activities were measured by using spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). The enzymes specific activities were expressed as unit/ mg protein.

Statistical Analysis: Data of the two cultured seasons were arranged and statistically analyzed by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat software (version 6.4 CoHort software, USA). Significance between means was tested by Duncan's multiple range test according to the method described by Gomez and Gomez [46].

RESULTS

Table (2) indicated that all tested morphological characters of lettuce, i.e. whole plant fresh weight and dry weight, the average of leaf fresh weight, average leaves number, stem diameter and head circumference were decreased to the minimal values when lettuce grew in soil collected from Qalyub Farm (QF) with high lead concentration. Adding either rice or quinoa straws to soil achieved a significant increase of the plant vigour when comparing with control (soil collected from Faculty of Agricultural Farm; AS) in the two successive seasons.

The adverse effect of lead adsorption from lead contaminated soils on some plant biochemical components was studied in two successive experimental years. Data in Table (3) indicated that hydrogen peroxide (H_2O_2) concentration was significantly increased in plants grown in QF soil from 54.60 to 88.65 mg/100g f.wt. Malondialdehyde (MDA) concentration was increased in plants cultivated in QF soil to about two folds in comparing with AS soil. Adding straw of rice or quinoa to QF soil significantly decreased the concentration of H_2O_2 in lettuce plants to 68.17 and 65.95 mg/100g f.wt.,

Table 2: Effect of adding rice or quinoa straw to lead contaminated soils collected from Qalyub Farm (QF) and Experimental Field of Faculty of Agriculture (AS) on some lettuce growth parameters during 2013/2014 and 2014/2015 seasons

Seasons	Treatments	Plant f.wt. (g)	Plant d.wt. (g)	Average leaf f.wt. (g)	Leaves number	Stem diameter (cm)	Head circumference (cm)
1st season	AS	487.6b	23.2b	9.6c	27.3d	2.7c	38.7b
	QF	297.0c	16.2c	7.4d	13.0e	2.2d	28.3c
	AS + Rice straw	494.0b	28.1a	13.2a	44.3a	3.2a	54.3a
	AS + Quinoa straw	579.6a	30.2a	13.1a	41.3ab	3.1ab	55.0a
	QF + Rice straw	494.0b	27.0a	11.5ab	37.3bc	2.8bc	47.3a
	QF + Quinoa straw	487.6b	24.2b	10.6bc	34.7c	2.8bc	37.0b
2nd season	AS	506.6b	25.4bc	10.0b	27.7d	2.7c	40.3b
	QF	300.0c	15.6d	7.6c	12.3e	2.2d	32.0c
	AS + Rice straw	572.3a	32.7a	13.0a	47.0a	3.3a	51.7a
	AS + Quinoa straw	503.0b	29.2a	13.1a	41.7bc	3.2ab	51.0a
	QF + Rice straw	489.6b	26.7b	11.4b	43.3ab	2.9bc	51.7a
	QF + Quinoa straw	482.6b	26.3b	11.1b	37.7c	2.1d	50.3a

In each row, means of each season followed by different letters are significantly different at P = 0.05, Duncan's multiple range test

respectively comparing to soil sample collected from Qalyub. No significant differences were shown when AS soil amended with quinoa straw. Adding rice or quinoa straw to QF soil reduced the concentration of MDA to about the control level (AS soil). The same trend was shown in the cultivated second season.

Also, data in Table (3) referred that total soluble phenols concentration elevated inlead contaminated soils. Lettuce grew in low lead contaminated soil (AS soil; control) exhibited maximum total soluble phenols more than high lead contaminated soil (QF soil). Total soluble phenols in plants cultivated in QF soil was reduced to 114.4 mg/100g f.wt. comparing with control (148.49 mg/100g f.wt.). In addition, proline concentration was significantly increased in lettuce plants with 20.9% when cultivated in QF soil than AS soil.

Adding rice or quinoa straws enhanced the negative effect of lead through reducing total soluble phenols concentration in lettuce plants cultivated in both soil samples. The least amount of total soluble phenol was found in lettuce grown in QFsoil amended with quinoa straw than that cultivated in AS soil amended with quinoa straw. In the same time amending rice or quinoa straws to AS soil decreased the proline concentration in lettuce by about 65 and 68.25%, respectively, comparing with plants cultured in AS soil without any straws. Whereas, proline concentration was decreased by 71.29 and 77.82% in plants cultivated in QF soil amended with rice or quinoa straws, respectively when compared with plants cultivated in AS soil and by 76.21 and 81.65%, respectively, comparing with other plants cultivated in QF soil.

Table (4) indicated that glutathione (GSH) concentration in lettuce plants was significantly decreased when plants cultivated in AS soil previously amended with either rice or quinoa straws, respectively, comparing with control (AS soil). Whereas, culturing in QF soil led to increase the GSH concentration in plants to about 1.6 time than AS soil, but adding either rice or quinoa straw to QF soil decreased the GSH concentration in plants to about ¾ or ½ its concentration in comparing with culturing in QF soil without rice or quinoa straws in both seasons. Insignificant difference in GSH concentration was recorded with plants cultured in AS soil as compared with QF soil amended with rice straw in the second experimental season.

Also, it was shown that oxalic acid was significantly decreased when lettuce plants were grown in soils amended with rice or quinoa straws in comparing with control (AS soil) or QF soil in both seasons. Although, there is no significant difference in oxalic acid concentration was shown in plants grew in QF soil and AS soil in the first season and vice versa in the second season.

Data in Table (5) indicated that the difference between concentrations of lead (Pb) in lettuce leaves was highly significant in plants cultivated in QF and AS soils respectively during the both seasons. There is a high reduction in Pb concentration in plants cultured in both AS or QF soils mixing with rice or quinoa straws. These reduction in Pb concentration increased with adding rice straw more than quinoa straw.

High significant concentrations of N, P, K and Ca were shown in lettuce plants cultivated in soil amended with rice straw comparing with free AS soil. Plants cultivated in high lead contaminated soil (QF soil) were suffered from high concentration of lead and significant reduction in the concentrations of N, P, K and Ca. These concentrations significantly decreased in plants cultivated under QF soil. Treatments with either low lead

Table 3: Effect of adding rice or quinoa straw to lead contaminated soils collected from Qalyub Farm (QF) and Experimental Field of Faculty of Agriculture (AS) on H_2O_2, malondialdehyde (MDA), total soluble phenols and proline concentrations in lettuce leaves during 2013/2014 and 2014/2015 cultivated seasons

Seasons	Treatments	H_2O_2 mg 100g^{-1} f.wt.	MDA µmol g^{-1} f.wt.	Total soluble phenols mg100g^{-1} f.wt.	Proline mg100g^{-1} f.wt.
1st season	AS	54.60c	3.65x10^{-4}b	148.49a	83.52b
	QF	88.65a	7.20x10^{-4}a	114.46c	100.94a
	AS + Rice straw	59.26c	3.10x10^{-4}c	144.88a	29.06c
	AS + Quinoa straw	59.15c	3.77x10^{-4}b	138.13b	26.51cd
	QF + Rice straw	68.17b	3.77x10^{-4}b	107.13d	24.01d
	QF + Quinoa straw	65.95b	3.30x10^{-4}bc	105.18d	18.52e
2nd season	AS	45.61d	2.47x10^{-4}d	160.82a	63.47b
	QF	82.70a	5.30x10^{-4}a	125.46d	91.49a
	AS + Rice straw	50.68cd	2.23x10^{-4}d	152.89b	24.98c
	AS + Quinoa straw	53.52bc	2.60x10^{-4}c	140.13c	20.51d
	QF + Rice straw	55.80b	2.90x10^{-4}b	110.12f	20.47d
	QF + Quinoa straw	55.46bc	2.33x10^{-4}cd	115.07e	16.06e

In each row, means of each season followed by different letters are significantly different at P ≤ 0.05, Duncan's multiple range test

Table 4: Effect of adding rice or quinoa straw to lead contaminated soils collected from Qalyub Farm (QF) and Experimental Field of Faculty of Agriculture (AS) on glutathione (GSH) and oxalic acid concentrations in lettuce leaves during 2013/2014 and 2014/2015 seasons

	GSH nmol g^{-1} f.wt.		Oxalic acid mg 100 g^{-1} f.wt.	
Treatments	1st season	2nd season	1st season	2nd season
AS	1048.8c	1127.9b	655.26a	551.92b
QF	1616.4a	1769.6a	662.09a	582.77a
AS + Rice straw	666.66e	759.53e	605.36b	504.50c
AS + Quinoa straw	779.58d	826.39d	571.57c	478.22d
QF + Rice straw	1086.9b	1132.5b	562.27c	472.84d
QF + Quinoa straw	768.56d	864.95c	462.16d	448.16e

In each row, means followed by different letters are significantly different at P ≤ 0.05, Duncan's multiple range test

Table 5: Effect of adding rice or quinoa straw to lead contaminated soils collected from Qalyub Farm (QF) and Experimental Field of Faculty of Agriculture (AS) on the concentration of Pb, N, P, K and Ca lettuce leaves during 2013/2014 and 2014/2015 cultivated seasons

Seasons	Treatments	Pb mg Kg^{-1} d.wt.	N g 100g^{-1} d.wt.	P g 100g^{-1} d.wt.	K g 100g^{-1} d.wt.	Ca g 100g^{-1} d.wt.
1st season	AS	6.39b	3.14b	0.37b	2.404b	2.23b
	QF	9.45a	1.94e	0.26f	1.015f	1.00e
	AS + Rice straw	3.74e	3.38a	o.39a	2.663a	2.67a
	AS + Quinoa straw	4.44d	3.03b	0.33c	1.146c	2.23b
	QF + Rice straw	4.66d	2.40d	0.31d	2.052d	1.73c
	QF + Quinoa straw	5.39c	2.88c	0.29e	1.620e	1.53d
2nd season	AS	6.65b	3.46a	0.366b	2.403b	1.60c
	QF	9.32a	1.93c	0.260f	1.009c	0.93d
	AS + Rice straw	3.75d	3.83a	0.393a	2.865a	2.70a
	AS + Quinoa straw	4.58c	3.56a	0.334c	2.856a	2.60a
	QF + Rice straw	4.80c	3.46a	0.312d	2.374b	2.13b
	QF + Quinoa straw	5.09c	3.02b	0.286e	2.41b	1.43c

In each row, means of each season followed by different letters are significantly different at P ≤ 0.05, Duncan's multiple range test

scontaminated soil (AS soil) or high lead contaminated soil (QF soil) amended with rice or quinoa straw appeared high concentrations of N, P, K and Ca comparing with plants cultivated in QF soil separately.

Data in Table (6) indicated that ascorbate peroxidase activity (ASP) was decreased in all treatments comparing with cultivated in AS soil. Sowing lettuce in QF soil with high lead concentration showed significant decrease in the activity of ASP when compared with AS soil in both seasons. The least activity of ASP was observed in plants cultivated in QF soil mixing with quinoa in the two seasons. Whereas, cultured lettuce plants in QF soil increased the activity of both guaicol peroxidase (POD) and poly phenol oxidase (PPO) comparing with AS soil. The highest activity of POD was observed when plants cultivated in QF soil mixed with quinoa straw, these

Table 6: Effect of adding rice or quinoa straw to lead contaminated soils collected from Qalyub Farm (QF) and Experimental Field of Faculty of Agriculture (AS) on ascorbate peroxidase (ASP), guaicol peroxidase (POD) and poly phenol oxidase (PPO) activities in lettuce leaves during 2013/2014 and 2014/2015 cultivated seasons

	ASP unit/mg protein		POD unit/mg protein		PPO unit/mg protein	
Treatments	1st season	2ndseason	1st season	2nd season	1st season	2ndseason
AS	12.48a	19.90a	158.69d	204.26d	305.27b	344.16b
QF	8.75b	10.54c	235.84b	292.87b	337.43a	372.72a
AS + Rice straw	9.07b	12.29b	64.26e	44.00e	242.60c	262.91c
AS + Quinoa straw	9.49b	12.13b	194.64c	234.73c	224.89d	242.50d
QF + Rice straw	9.80b	11.88b	155.50d	203.61d	212.12e	227.66e
QF + Quinoa straw	6.23c	8.20d	479.39a	525.60a	214.84e	222.92e

In each row, means of each season followed by different letters are significantly different at $P \leq 0.05$, Duncan's multiple range test

increasing was about 2½ - 3 folds comparing with activity in plants growing on AS soil during the two successive seasons. Insignificant difference was found in POD activity with lettuce plants cultivated in QF soil amended with rice straw comparing with AS soil separately. Also, there is a high activity of poly soluble phenols oxidase activity (PPO) in lettuce plants cultivated in QF soil followed by AS soil (control).

The activity of PPO enzyme decreased gradually in other all treatments started from plants cultivated in AS soil amended with either rice or quinoa straws to plants cultivated in QF soil mixed with rice or quinoa straws comparing with either plants cultivated in AS or QF soil in the two growing seasons.

DISCUSSION

Atmospheric air pollution and industrial wastes include high concentrations of heavy metals that have disastrous effects on plant productivity and threatens human health [47]. The phytotoxicity of heavy metals can change the behaviour of plant and can be described by metabolic pathways at different levels of organization of the whole plant. Lead is one of these heavy metals. In the present study one soil sample was collected from a farm in front of the high way of Qalyub district (QF soil) to the possibility of presence a higher contamination with lead (Pb). On the other hand, another soil sample was collected from the Farm of Faculty of Agriculture (AS soil), both collected soils placed in pots and cultured with lettuce seedlings in the Experimental Farm of Faculty of Agriculture, Ain Shams University, Shoubra El Kheima. It was noted that both regions are in Qalyubia Governorate. As it was based on Chojnacha *et al.* [48] who reported that the most important source of lead contamination is atmospheric pollution from industrial or motor vehicle emission, which represent from a range between 73 to 95 % of the total lead in plants. The analysis of the two different sources of soils under study, Qalyub and Farm of Faculty of Agriculture, referred to presence of lead contamination in both collected soils under consideration. The result of the soil analysis indicated that lead concentration in soil collected from a farm closed to heavy traffic and busy roadway at Qalyub (QF soil) was higher than in AS soil by amount 300 mg Kg^{-1} dry soil comparing with AS soil which reached 35.57 mg Kg^{-1} dry soil (Table 1).

Environmental Protection Agency (EPA) classified the soil lead level into 5 groups according to the level of lead contamination and reported that when the soil lead level is less than 150 mg Kg^{-1} dry soil it considered as very low level of lead soil; as noted in AS soil but when the soil lead level ranging from 150 – 400 mg Kg^{-1} dry soil, it classified as intermediate level of lead contaminated soil; as noted in QF soil [49]. McBride *et al.* [50] mentioned that international health-based standards reported that leafy greens may exceed European Commission standard levels when plants grown in lead contaminated soils with more than 100-200 mg Kg^{-1}. Mangrich *et al.* [49] indicated that people exposed to this soil through direct contact with soil or from indirect contact with very fine soil particles carried into houses by air dust or clothes, shoes and pets. The children between 2-3 years of age are at high risk for lead exposure due to the apt to their mouth dirty toys and dirty fingers. Also, human may be exposed to lead danger because of eating lead contaminated plant tissue or contaminated dust may settle on edible leaves and fruits.

Pourrut *et al.* [51] mentioned that excessive lead contamination in plant cells impaired various morphological and biochemical functions in plant. They reported that lead causes inhibition of plant growth (Table 2), lipid peroxidation due to over production of reactive oxygen species (ROS) as H_2O_2 (Table 3), impaired uptake of essential elements (Table 5), deficient in CO_2 by stomatal closure due to destroy the chloroplast ultrastructure and inhibit Calvin cycle enzymes.

Present data pointed to a significant decrease in lettuce plant fresh weight, plant dry weight, average leaf fresh weight, average leaves number, stem diameter and head circumference under the two types of lead contaminated soils (Table 2). These decreasing reached to the minimal values when lettuce grew in soil obtained from Qalyub area which has the highest level of lead. These data were agreed with many researchers' results. Huang and Cunningham [52] reported that increasing Pb concentration in soil significantly decreased both shoot and root yield. Michalska and Asp [53] showed a yield reduction in lettuce under 0.5µM Pb solution. Kosobrukhov *et al.* [54] found a considerable decrease in dry weights of different plant parts under Pb treatment. Relative fresh mass of cowpea (*Vigna unguiculata*) decreased by 10% at a Pb activity of 0.2 µM for the shoots and at 0.06 µM for the roots [55]. Nagajyoti *et al.* [56] reported that the reduction in growth and biomass of lettuce and carrot under low amounts of lead (0.005 ppm) may possibly derive from negative effects on metabolic plant processes and impaired uptake of essential elements. Also, they mentioned that the primary cause of cell growth inhibition arises from a lead-induced simulation of indol-3 acetic acid (IAA) oxidation.

This study showed that hydrogen peroxide (H_2O_2) concentration was significantly increased in plants grew in lead contaminated soil obtained from Qalyub when compared with Faculty of Agriculture soil (Table 3). Also, Malondialdehyde (MDA) concentration was increased 2-fold times in plants cultivated in Qalyub soil (QF soil) when compared with plants cultivated in soil obtained from experimental field of Faculty of Agriculture (AS soil) as control (Table 3). These results are generally in line with those obtained with Liu *et al.* [57] who mentioned that after Pb treatment, *Ficus microcarpa* roots produced high concentrations of H_2O_2 along with an increase in O^{-2} accumulation. O^{-2} is produced by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the plasmamembrane and is converted to H_2O_2 through enzymatic and non-enzymatic pathways. H_2O_2 is known as the main regulator of plants' responses to stress factors resulting an extreme oxidative stress to biological molecules such as protein, lipids and DNA [58]. Bouziani and Yssaad [59] and Wu *et al.* [60] reported that there was a significant accumulation in MDA concentration under lead stress. Pourrut *et al.* [51] reported that lead reacting with active groups of various cellular enzymes involved in plant metabolism and replacing essential ions due to reacting with the phosphate groups of ADP and ATP which causing a phytotoxicity. Lead phytotoxicity causes lipid peroxidation by over production of ROS. Malondialdehyde (MDA) concentration used as an indicator in lipid peroxidation which led to membrane fluidity and increased membrane permeability [61].

Plants possess several defence strategies to detoxify lead accumulation and cope with lead toxicity, i.e. reduced lead uptake into the cell, binding lead by phytochelatins or glutathione to form a complex following by sequestration of these lead complexes into vacuoles [51]. Based on this comment, our results showed that there are significant accumulations in total soluble phenolic compounds and proline in plants cultivated in Qalyub soil (QF) as shown in (Table 3). Also, glutathione (GSH) and oxalic acid concentrations were increased in plants grown in QF soil (Table 4). In this connection, Sgherri *et al.* [62] mentioned that phenolic compounds are stress induced metabolites accumulate in plant under stress. These compounds involved in scavenging of reactive oxygen species (ROS) through the antioxidative enzymes which use polyphenols as substrates. Sharma and Dubey [8] found the same relationship between Pb and proline. They reported that proline is a one of non- specific defence system against lead toxicity through its ability to act as a metal chelator and as a protein stabilizer to detoxify metal toxicity. Yuan *et al*. [63] reported that plants adapt metabolically to heavy metals exposure by enhancing synthesis of glutathione (sulfur (S)-rich peptides) and proline (low-molecular-weight amino acids). They added that, Glutathione has an instrumental and effective role as an antioxidant compound in cellular defence against heavy metals oxidative stress resulting from increasing in H_2O_2 concentration. They reported that increasing cellular glutathione concentration under lead stress enhancing the negative effect of H_2O_2 and $O_2^{\cdot}$ levels and lipid peroxidation. Yang *et al.* [64] reported that the oxalate concentration in the root increased up on Pb treatment via the oxidation of glycolate into glyoxylate which oxidised into oxalic acid and they suggested that the role of oxalic acid in detoxification due to reducing the accessibility of Pb in presence of oxalate secreted from the roots which may constitute comprised Pb tolerance mechanism in the plant.

Regarding the effect of lead contaminated soil, the present study showed that leaves of lettuce plants exposed to high lead contamination (QF soil) contained a high concentration of Pb (Table 5). The European Union (EU) has set a standard at 0.3 mg Kg^{-1} f.wt. for lettuce [65]. Lead concentrations are recorded in the present study result section as mg Kg^{-1} dry weight. So, it should be converted mathematically and attributed to the fresh weight to compare our values with EU standard level. This conversion was based on McBride [10] who reported

that approximate water content in lettuce leaves is 94.9 %. He calculated the dry weight – based recommended limits for Pb in lettuce not exceeding 5.9 mg Kg^{-1} d.wt. This value was relatively equal to the Pb concentration recorded in plants cultivated in AS soil (6.39 mg Kg^{-1} d.wt.) as shown in (Table 5). The concentration of Pb in leaves of plants grown in soil most contaminated with lead (QF soil) increased to 9.45 mg Kg^{-1} d.wt. (Table 5), which represent 0.45 mg Kg^{-1} f.wt. This value seems to become higher than the critical limit allowed in lettuce leaves (Table 5). On the other hand, there were significant decreases in the concentrations of N, P, K and Ca in these leaves (Table 5). Capelo *et al.* [66] stablished that Pb phytotoxicity included a decrease in the uptake of K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, $Zn^{+2}$and Na^+. The restraint of mineral ions uptake appears to be a general result of exposure to lead. Malvi [67] mentioned that when potassium reduces, all of translocation of nitrates, phosphates, calcium, magnesium and aminoacids are depressed. They explained that based on phloem transport systems, the role of potassium in transport is often in conjunction with specific enzymes and plant growth hormones and this caused due to its role in enzymes activation, stomatal conductance, crop quality and in regulating the rate of photosynthesis. Besides, Lamhamdi *et al.* [12] noted that nitrogen, phosphorus and potassium have roles to play in photosynthesis and protein and carbohydrate metabolism. Brunet *et al.* [68] explained that roots exposed to lead contamination contained less Ca^{2+} than uncontaminated lead roots and showed that this reduction in Ca^{2+} concentration due to the replacement of Ca^{2+} ions by Pb^{2-} ions which bind with carboxyl groups of pectin cell wall. Also, Lamhamdi *et al.* [12] indicated that Pb^{2+} ions replaced to Ca^{2+} ions during specific physiological processes because of their ionic similarity.

In this sense, antioxidative enzymes mechanism have been regarded as their importance for the success of enhancing the adverse effect of lead accumulation in edible leaves. Imlay [69] reported that plants have strategies enhanced to Pb detoxification and developing tolerance to Pb due to increasing the activities of antioxidant enzymes. Likewise, high activities on guaicol peroxidase (POD) and poly phenol oxidase (PPO) were shown in both QF and AS soils (Table 6). These results are agreement with Ibrahim and Abdellatif [70] who mentioned that accumulation of proline and increasing the activities of peroxidase, catalase and ascorbate peroxidase can withstand the stress created in wheat plants. Ren *et al.* [71] added that ascorbate peroxidase involved in ascorbate - glutathione cycle.

Rice straw is an agriculture waste, which contains 32-47% cellulose, 19-27% hemicellulose and 5-24% lignin [72]. These several characteristics make it a potential adsorbent with binding sites capable to adsorb metals from aqueous solutions [73]. Murray *et al*. [74] carried some trials to reduce Pb solubility in the soil and plant uptake by adding composts, peat, calcium phosphate, ferric oxide and gypsum. They found that in effectiveness of these previous amendments due to the low initial lead solubility of the soils under investigation and the fact that the primary mechanism of lead transfer is physical contamination, so, rice straw is an effective and cheap adsorbent for reducing the bioavailability of Cd, Cu, Fe, Mn, Ni, Pb, Zn from soil and water [75, 76]. Bhargava *et al.* [28] and Antonio *et al.* [29] found that the chemical analysis of quinoa leaves is rich in minerals, fatty acids vitamins, phytohormones and antioxidants, in addition to its high value of proteins that can make complete organic complexes that have the ability to adsorb heavy metals. Adding rice or quinoa straw to both lead contaminated soils significantly decreased the adsorption of Pb from soils to the permissible limits for human health in the edible leaves during the two successive seasons. The main mechanism responsible for the metal chelating by rice and quinoa straw is ion exchange between the heavy metal ions and Na^+, K^+, Mg^{+2}, Ca^{+2} and functional groups (such as C=C, C-O, O-H and carboxylic acids) which form surface complexes [27]. On the other hand, Edi *et al.* [77] has concluded that surface complexation (chelation) was the main mechanism responsible for the uptake of Pb^{+2} and Cu^{+2} followed by ion exchange. In addition to ion exchange, chelation is an important mechanism for the removal of metal ions from aqueous solution [51]. Singh *et al*. [78] reported that Fourier Transform Infrared Spectroscopy (FTIR) analysis of rice straw showed appearance to functional groups free or H-bonded O-H groups, C=O that could be present in carboxylic acids on the surface of rice straw. Those groups probably act as proton donors that once get deprotonated, the hydroxyl group or the carboxyl group adsorbs the heavy metal ions. The presence of C=O bond may be due to carboxylic acids, aloxy groups or fiber carbonaceous that are presented in the structure of rice straw and composed of lignin and cellulose [77]. The present study mentioned that adding rice or quinoa straw led to decrease pH values and increase the N, P, K of the two collected soil samples (Table 1). McBride *et al.* [50] indicated that decreasing in the concentration of Pb with higher levels of organic matter, phosphorus and other nutrients is due to reducing the bioavailability of soil lead or a precipitation reaction of

Pb with phosphate (PO_4). Also, McBride [10] mentioned that increasing organic matter in the soil matrix decrease the soil pH, this decline resulted in reduce pb uptake from soil to edible leaves of plants.

It is obvious from our results that lead contaminated soils even at low or high concentrations induces large disturbances in ions concentrations by lettuce plants, which results in strong inhibition of plant growth and profound metabolic changes. On the other side, adding either rice or quinoa straw to lead contaminated soils increased the biomass of lettuce plants comparing with plants cultured in soil without straws, Also, soil amended with either rice or quinoa straws stimulated the uptake of N, P, K and Ca. Moreover, they reduced the total soluble phenols, proline, H_2O_2, MDA, GSH, oxalic acid concentrations which enhanced understress. POD and PPO activities decreased with rice or quinoa straws than under lead stress. Adding rice straw to soil contaminated with lead is more effective in lead adsorbance more quinoa straw.

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