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The Palliative Effect of Bio-Organic Fertilizer on Lead Pollution in *Lycopersicum esculentum* Plants

Mona M. Abdalla*,[1] and Nada El-Khoshiban[2]

[1]*Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt*

[2]*Biology Department, Faculty of Science and Arts, Qassim Uiversity, Qassim, Saudi Arabia*

Abstract: Lead is one of the hazardous heavy metal pollutants of the environment that originates from various sources. Soil contamination by lead reduces the quality of both soil and cultivated plants which often limits the production of some food products and animal feed. Thus, this study was undertaken to evaluate the effect of a bio-organic fertilizer, namely Acadian, a red algal extract, at recommended dose (RD) in alleviating the deteriorative effect of Pb at 0, 50, 100, 200 and 400 mg/l on tomato *(Lycopersicum esculentum)* plants. Accordingly, Pb-treated plants showed marked reductions in growth measurements as root and shoot length, fresh and dry weights of shoots,roots and fruits as well as number of leaves and fruits; in photosynthetic rates, stomatal conductance, net intercellular CO_2 rates (ΔCO_2) and in the contents of each of chlorophyll a, b and total chlorophyll.In addition, with the increase in level of Pb treatment in situ, total sugars, total nitrogen, catalase activity and major nutrient elements (P, K, Ca and Mg) were proportionally declined in both shoots and roots as well as proline of roots. At the other side, Pb treatment raised the levels of each of carotenoids, total soluble sugars, amino nitrogen, total soluble nitrogen, peroxidase, superoxide dismutase, phenols, lipid peroxidation, sodium, lead and iron in both roots and shoots of tomato plants as well as proline of shoots and transpiration rates. When tomato plants were supplemented with the recommended dose of Acadian solely or combined with Pb at all rates, significant increases in all measured growth parameters (shoot and root length, fresh and dry weights of shoots, roots and fruits, number of leaves and fruits), photosynthetic rates, stomatal conductance, ΔCO_2, the contents of each of chlorophyll a, b, total chlorophyll as well as the contents of total sugars, total soluble sugars, total nitrogen, total soluble nitrogen, amino-N, P, K, Ca and Mg in tomato shoots and roots were obtained. Conversely, Acadian fertilization negatively reduced the carotenoid values, the activity of antioxidant enzymes(catalase, peroxidase and superoxide dismutase), the amounts of phenol, Pb, Na, Fe and the level of lipid peroxidation in both shoots and roots of tomato plants, whereas, it positively affected transpiration rates. On the otherside, when Acadian where added to lead at different rates there were either synergistic increases in the activities of these antioxidant enzymes and the level of phenol and lipid peroxidation or decreases in the carotenoid, Na, Fe and Pb contents as well as transpiration rates. Thus, it is manifested that Acadian can be used to improve the safety, quality and productivity of lead polluted plants.

Keywords: Lead, bio-organic fertilizer, growth, gas exchange,metabolites, antioxidants, antioxidant enzymes.

1. INTRODUCTION

The global problem concerning the contamination of the environment as a consequence of anthropogenic activities is increasing. These include mining, metal smelting, electroplating, gas exhaust, energy and fuel production, down wash from power lines, intensive agriculture, municipal wastes, pesticides, pigments, spent batteries, sludge dumping, power transmission and military operations [1,2]. Most of the environmental contaminants are chemical by-products and heavy metals such as lead (Pb).Lead released into the environment makes its way into the air, soil and water [3]. Lead is a cumulative poison which when present at higher doses causes serious irreversible damage to the human brain, kidneys, nervous system and decline in mental, cognitive and physical health [4]. Lead absorption by plants is regulated by pH, cation exchange capacity of the soil, temperature, soil ions, organic matter content of the soil, lead concentration in soil and type of plants species [5]. Absorption by roots from the soil occurs *via* the plasma membrane, probably *via* calcium channels. Roots are capable of accumulating significant quantities of Pb and simultaneously restrict its translocation to the shoot through binding and deposition as lead carbonate in the cell wall or it can move in the root tissues *via* the apoplast and radially through the cortex to the endoderm where it accumulates [6]. Excess lead causes a variety of metabolic processes essential to plant growth and development which includes reduction in the rates of photosynthesis and transpiration, net CO_2 uptake, photosystem II efficiency, stomatal conductance, DNA synthesis and mitotic activity [7,6]. It also reduces plant growth, namely the length of roots and shoots, leaf area, the fresh and dry mass of both roots and shoots [6,8,9], plant survival rates [10], the number and size of leaves, tillers and inflorescence and finally plant yield [11,12]. Moreover, lead accumulation negatively affected the contents of each of chlorophyll a, b, a+b and carotene as a result of photosynthetic pigment degradation [12-15], the levels of total sugars, nitrogen, protein, nitrate and free amino

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*Address corresponding to this author at the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt; Tel: 002025083644; Fax: 009663851361; E-mail: messam_9156@hotmail.com

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acids in various tested plants [9,11,12] and the absorption of calcium, phosphorus, potassium and magnesium by polluted plants as it dramatically affects the permeability of plasma membranes to these elements resulting in nutrient imbalance [16-18]. Conversely, several studies showed that lead toxicity in plants stimulated the uptake and accumulation of several other heavy metals as copper, manganese, iron and cadmium [10,16]. Under normal circumstances, the concentrations of oxygen radicals (ROS) remains low because of the activity of protective enzymes, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase [19]. In stress conditions (biotic or abiotic stresses), different protective processes are enhanced such as accumulation of compatible solutes (proline and soluble sugars) which play an important role in osmoregulation, enzyme protection and scavenging of free radicals [19-21], increase in the activities of detoxifying enzymes and enhanced tissue lipid peroxidation. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage [22]. SOD is a metalloenzyme that catalyzes dismutation of superoxide anion into oxygen and hydrogen peroxide. Such enzyme provide a defense system for the survival of the aerobic organism [23].

In modern agriculture, special emphasis is placed on soil amendment, improved agricultural equipments, techniques and improved varieties of tolerant plants for increasing the quality and quantity of yield per hectare. However, full potential of the improved varieties can be realized only if essential inputs, particularly organic fertilizers are applied both in requisite quantities and in timely manner [24]. Organic farming is one of the fastest growing sectors of agriculture worldwide. Its main objective is to create a balance between the interreconnected systems of soil organisms, plants, animals and human. System to regulate the nutrition regime with organic forming is based on balanced crop rotations, application of compost, green or barnyard manures, bone meal or straw, leaves and sawdust mulches [25]. The target in the application of organic fertilizers should therefore two fold- first to obtain reasonable yields and second to increase soil fertility, water holding capacity to optimum levels [24]. Accordingly, a pure natural extract of a red algal seaweed *Ascophyllum nodosum* namely, Acadian agritech was applied as an amendment of soil metal toxicity. Addition of Acadian to soil improves the fertility by acting directly on its biological, physical and chemical properties which, in turn, activate the miocrobial biomass, improves soil structure, increase water holding capacity and aggregate stability. It ensures sustainable crop yield by creating favourable environment for plant growth, as it contains natural components including growth hormones, amino acids, chelating agents as manitol, laminarin and algnic acid and antioxidants as phytoalexins which resist stress and infers acquired resistance to plants (Agritech, Nova Scotia, Canada). Several studies showed that biofertilization with algal extracts highly significantly increased shoot length, fresh and dry weights of roots and shoots, total biomass, yield component, leaf number, photosynthetic pigments and growth promoting hormones [18,26,27], increased the functional activity of photosynthetic apparatus and leaf gas exchange [25], raised the contents of total carbohydrates, starch, amino acids and protein [28-30], enhanced the polyphenol content and antioxidant enzymes [31] and increased the uptake and accumulation of nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, iron, manganese, zinc and the ratio of K/Na, in addition to proline [32-35]. Reversibly, organic fertilizers positively reduced the levels of reducing and non-reducing sugars, nitrate, lead and cadmium in plants [18,28,29], as well as electric conductivity of soil but raised its pH [36]. Very recently, numerous studies were conducted using various bio-organic fertilizers (organic waste products, green manure or seaweed extracts), they claimed that organic fruits and vegetables produced had higher antioxidant activity (SOD, CAT, peroxidase (POD), phenols) and less lipid peroxidation level than conventional ones [37-39].

The goal of this study is to:(1) assess the capability of Acadian (a natural red algal extract, *Ascophyllum nodosum*) in a recommended dose (RD) to alleviate the phytotoxicity of lead, at ugraded rates, on the growth, gas exchange rates and metabolism of an important economic plant, which is tomato (*Lycopersicum esculentum*).

(2) investigate the possible interactive mechanism by which tomato plants fertilized by Acadian can tolerate lead pollution and thus attain the best growth, yield and chemical constituents.

2. MATERIALS AND METHODS

2.1. Soil Preparations, Plant Material and Treatments

The experimental soil used in this study was obtained from an agricultural research project at

Onizah-El-Qassim-Kingdom of Saudi Arabia. The soil was collected, dried, crushed and sieved through 2mm sieve. Some soil samples were used for physico-chemical analysis (Table **1**) while the remaining samples were filled in standard plastic pots (30cm in diameter) containing equal weights of soil (8kg/pot).To simulate soil pollution, the soil was amended with lead nitrate $Pb(NO_3)_2$ to produce Pb levels of 50, 100, 200 and 400 mg/l as well as water (control). The soil was watered to field capacity and incubated in green house for 10 days to allow the soil chemical reactions to equilibrate [40].

Table 1: Physico-Chemical Composition of Soil Samples. Values Listed are Expressed as mg/kg Soil. Values of EC are Expressed as ds/m

1060	**Na**
186	**K**
1378	**Ca**
140	**Mg**
34.2	**P**
8.4	**Fe**
19.2	**Zn**
15.8	**Mn**
0.26	**Cu**
0.26	**Cd**
44.1	**Pb**
2.6	**EC**
7.6	**PH**

The organic fertilizer, Acadian, was obtained from Agritech, Dartmout, Nova Scotia, Canada. It is 100% pure algal seaweed extract freshly gathered from the cold, pristine waters off the coast of Nova Scotia in the North Arctic and far from pollution sources. Its chemical analysis was presented in Table **2**. Acadian was added thrice at the recommended dose (8ml/10l H_2O), the first addition was two days before transplanting, the second time before flowering of tomato plants while the third after fruit set.

Seeds of tomato (*Lycopersicum esculentum*) were obtained from the Agricultural Research Center, (Onizah, El-Qassim, Kingdom of Saudi Arabia) and were sown (in green house conditions) in trays at average day/night temperature 35/30 ℃ ±2, relative humidity 18-25 % ±2. Five weeks later, uniform seedlings were transplanted (two seedlings/ pot) and irrigation was done following the common agricultural practice. The pots were arranged into 10 sets, 20 pots/set as follows:

Table 2: Chemical Composition of the Organic Fertilizer (Acadian)

Percentage of algal components	Components
100%	Algal extract
0.9% - 12%	Organic substances
0.7%- 8.0%	Carbohydrates
0.1%- 1.0%	Amino acids
0.3%- 0.6%	Nitrogen
0.2%- 0.5%	Phosphorus
0.5%- 0.7&	Potassium
0.3%- 0.6&	Sulphur
0.1%- 0.5%	Magnesium
0.1%- 0.2%	Calcium
Trace elements (ppm)	
20-50	Iron
0.1-0.5	Copper
0.5 15.0	Zinc
1.0-5.0	Manganese
20-30	Boron
8	pH

T1 Control (lead free, unfertilized plants), T2 (Acadian at recommended dose, RD), T3 (lead at 50mg/l), T5(lead at 100mg/l), T 7 (lead at 200mg/l), T9 (lead at 400mg/l), T4 (lead at 50mg/l + acadian, RD), T6 (lead at 100mg/l + Acadian, RD), T8 (lead at 200mg/l+Acadian, RD) and T10 (lead at 400mg/l+ acadian, RD).

The plants were harvested at 120 days after sowing (DAS) at fruit ripening at average day/night temperature 16/10 ℃ ±2, relative humidity 62-83 % ±2 and ambient light.

Ten plants were randomly selected for the measurement of growth criteria and gas exchange, another samples were taken (5 replicates/treatment) and either oven dried for determinations of carbohydrates, nitrogen, phenols and minerals or rapidly frozen for photosynthetic pigments, antioxidant enzyme, lipid peroxidation and proline estimations.

2.2. Measurements of Gas Exchange

Photosynthesis and transpiration rates and stomatal conductance were measured using an open gas portable photosynthesis system (LI-6400, LI_COR,Bio Sciences, USA). Measurements were performed on sunny days under natural light conditions and between 9.00h and 12.00h on the uppermost fully expanded leaves of 10 plants randomly chosen per treatment and expressed on a leaf area basis [41].

2.3. Chemical Analysis

Photosynthetic pigments (chl.a, chl.b and carotenoids) were determined spectrophotometrically [42]. Carbohydrate fractions were extracted, clarified and determined as total sugars (TS) and total soluble sugars (TSS) [43]. Total-N and total soluble –N were determined by micro-Kjeldahl, Tector model 1026 after digestion in sulphuric acid [44], while amino-N was determined photometrically, after deprotonization of the extract using ethanol / acetone mixture, as free amino acids with ninhydrin [45].

Free proline content was estimated photometrically in acidic ninhydrin assay according to the method adopted by Bates *et al.* [46]. Total phenols were determined in the ethanolic extract following the method described by Simons and Ross [47] using folin reagent.

For the assay of antioxidant enzymes (catalase, CAT, peroxidase, POD; superoxide dismutase, SOD), fresh material were extracted following the method of Guerrier and Strullu [48]. The activities of CAT and POD were determined according to Chance and Maehly [49]. CAT activity was determined by measuring the decomposition of H_2O_2, by following the decline in its absorbance at 240 nm for 3min. POD activity was assayed by measuring the oxidation of guaiacol and the increase in absorbance at 470nm was recorded in 3min. The activity was defined as OD/min/mg FW. SOD activity was assayed by the nitrobluetetrazolium (NBT) modified method from that described by Dhindsa *et al.* [50]. One unit of SOD was defined as that being contained in the volume of extract that caused a 50% inhibition of the SOD-inhibitable fraction of the NBT reduction. The level of lipid peroxidation in leaf tissues was measured by the determination of nmole of malonodialdehyde (MDA) formed using an extraction coefficient of 155 nmole $L^{-1}cm^{-1}$. MDA was determined using 20% trichloroacetic acid containing 0.5% thiobarbituric acid (TBA) reaction and the developed colour was extracted with 2ml n-butanol and the absorbance was measured at 532nm. The value for the non-specific absorption at 600nm was subtracted [51].

Mineral elements were extracted from tissues similar to that of Chapman and Pratt [52]. Phosphorus was determined following the method described by Humphries [53]. Sodium and potassium were estimated photometrically according to Williams and Twine [54]. Calcium, magnesium, iron and lead were determined by atomic absorption spectrophotometer according to A.O.A.C [55].

2.4. Statistical Analysis

Morphologic and gas exchange values are means ± standard error of 10 replicates while those of chemical analysis values are means ±standard error of 5 replicates. Significant differences were calculated using student's (t) test. SPSS version 16 was performed for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1. Growth Parameters

Data presented in Table **3** revealed an inverse relationship between all growth measurements of tomato plants and lead nitrate applied at different rates (0, 50, 100, 200 and 400 mg/l). Thus, the percentages of decreases were 31% and 50% for shoot and root length; 63%, 70% and 78% for fresh weight of shoot, root and fruit; 72.8%, 61.5% and 53% for dry weight of shoot, root and fruit and finally 27.7% and 60% for number of leaves and fruits respectively in response to the highest concentration applied of Pb (400 mg/l). These results are in conformity with the observations of LarbI *et al.* [16], Verma and Dubey [56], Romeiro *et al.* [6] and John *et al.* [57] using several experimental plants. Decreased growth vigour due to Pb phytotoxicity could possibly be attributed to the interference of Pb with the metabolic and biochemical processes (gas exchantge rate, chlorosis, water and nutritional status ….etc) associated with normal growth and development of tomato plants. Studies showed that Pb is unevenly distributed in roots, where different tissues act as barriers to apoplastic and symplastic Pb transport and hence Pb transport to shoot gets restricted [56]. Darkening of the root system and inhibition of root and shoot growth appears to result from Pb- induced inhibition of cell division of both root and shoot meristem. It inhibits photosynthesis, alters the mineral nutrition and water balance, modifies hormonal levels and affects the structure and permeability of the plasma membrane [6].

Reversibly, fertilization of tomato plants solely with Acadian or Acadian combined with Pb significantly increased all mentioned growth criteria. Noteworthy that the combined effect of both Acadian and lead at

Table 3: Impact of Organic Fertilizer, Lead and Mixture of both on the Growth Measurements of Tomato Plants

d.wt.of fruit/plant	f.wt.of fruit/plant	Fruit number/plant	Leaf number	root d.wt.	Shoot d.wt.	root f.wt.	shoot f.wt.	root length	shoot length	treatment
18.0±1.7	369.4±2.5	24±1.73	14.8±0.13	17.38±0.31	62.5±1.10	78.7±1.96	519.4±12.6	28.55±0.40	141.9±0.72	T1
22.3±1.3	378.0±2.31	29±1.60	15.5±0.17	19.71±0.55	72.8±1.31	91.3±3.76	686.1±6.68	30.87±0.50	150.3±1.09	T2
16.9±1.3	174.6±1.9	15±1.61	12.4±0.27	15.58±0.19	45.3±0.91	34.4±1.88	423.3±10.4	15.76±0.19	107.9±1.35	T3
17.9±1.3	322.0±1.7	20±1.63	16.6±0.22	18.15±0.55	52.8±1.88	53.08±1.6	586.6±11.1	30.80±0.42	145.3±1.17	T4
13.3±1.1	137.7±1.9	10±1.61	12.3±0.15	11.50±0.42	36.6±0.87	27.7±0.51	358.5±10.5	15.56±0.24	101.4±1.23	T5
20.8±1.4	318.3±2.5	16±1.63	15.7±0.26	16.50±0.30	33.3±0.98	34.5±1.35	543.3±8.2	29.05±0.38	140.7±0.37	T6
10.8±1.2	123.8±1.9	8±1.71	11.7±0.26	7.08±0.26	24.12±0.51	25.5±0.70	210.9±4.6	14.70±0.15	98.8±0.43	T7
15.03±0.6	233.3±1.3	12±1.72	15.2±0.13	14.60±0.41	32.9±0.78	35.4±0.80	448.1±10.5	27.20±0.24	130.9±1.37	T8
8.45±0.7	80.7±1.3	6±1.73	10.7±0.20	6.67±0.40	16.96±0.80	23.5±0.60	190.2±2.5	14.30±0.18	97.2±0.54	T9
9.50±1.2	181.6±1.9	11±1.68	12.7±0.26	11.6±0.44	23.49±0.63	31.4±0.63	374.7±6.25	24.90±0.27	122.6±0.76	T10

*The mean difference is significant at the.05 level.

various rates was synergistic as comparable to Acadian alone (Table **3**). Accordingly, the percentage of increases were 26% and 74% for shoot and root length; 97%, 33% and 125% for fresh weight of shoot, root and fruit; 37.6%, 73% and 124% for dry weight of shoot, root and fruit and lastly 18.7% and 50% for number of leaves and fruits / plant due to treatment with Acadian at RD and Pb at 400 mg/l. Similar results were indicated using either seaweeds or other sources of organic fertilizers in combination with heavy metal or salinity stresses [18,32,58].

It is known that slightly acidic soil(pH= 4.5-6.5) renders lead more soluble and available to plants and thus most hazard to plant growth, while higher pH (6.5-7.5) minimize lead uptake by plants which causes it to accumulate in soil away from plant roots and thus plants grow vigorously [59]. Organic fertilization by Acadian raised pH of soil to 8, thus Pb became unavailable to plants (Table **2**). The enhanced vegetative growth of tomato due to Acadian application might be due to the improvement of the soil structure through increasing the soil water holding capacity which, in turn, gave rise to better aeration and drainage, a situation that improves root growth, nutrient availability and absorption thus ameliorating lead toxicity. It is claimed that Acadian contains natural components as growth hormones, amino acids, chelating agents and antioxidants which creates favarouble environment for plant growth and yield. These suggestions corroborate with those of El-Meleigy [60] and Yassen *et al.* [18] using different types of organic fertilizers.

3.2. Gas Exchange Measurements and Photosynthetic Pigments Contents

Increasing lead concentrations up to 400mg/l were negatively correlated with photosynthetic rate, stomatal conductance, net intercellular CO_2 concentrations (ΔCO_2) as well as the content of each of chlorophyll a, b and total chlorophyll but positively correlated with transpiration rate and carotenoid levels of tomato plants (Table **4**). Thus results were in correspondence with those of Larbi *et al.* [16], Zengin and Munzuroglu [13] and John *et al.* [57] concerning the deteriorative effect of lead on chlorophyll a, b & total chlorophyll and its enhancing effect on the carotene/chlorophyll ratio. Our results also corroborated with those of Romerio *et al.* [6] and Ali and Al- Homaidan [15] concerning lead retardation of photosynthetic and transpiration rates, stomatal conductance and net CO_2 concentrations. Pb negatively affects many processes vital to the photosynthetic pathway as chlorophyll biosynthesis, as it inhibits the activity of delta aminolevulinic acid dehydratase enzyme responsible for the biosynthesis of heme pigments [61], alters the action of ribulose 1, 5- bisphosphate carboxylase- oxygenase, interfers with the dynamics of the thylakoid membrane, inhibits electron transport system in both photosystem I and II and finally reduces the metabolites of the carbon reduction cycle [8,15]. Any or all of these effects can explain the significant damage and death, or to even less extent, the reduction in growth, metabolism and yield of tomato plants, in the present study. Fertilizing tomato plants with the recommended dose of Acadian either alone or combined with lead at various rates highly elevated all of the above measurements (photosynthetic rate, stomatal conductance, ΔCO_2, chlorophyll a, b & total chlorophyll) but reduced carotene levels. Unexpectedly, the presence of Acadian combined with lead not only palliated the negative effect of this element but it has also a synergistic action in most measurements e.g. Addition of Acadian solely increased the photosynthetic ability and ΔCO_2 of tomato leaf by 7 % and 2 % as compared

Table 4: Impact of Organic Fertilizer, Lead and Mixture of both on the Gas Exchange Measurements and Photosynthetic Pigment Contents

Carotein	Chlorophyll (b)	Chlorophyll (a)	Trmmol mmol H2O m^{-2}s^{-1}	Stomatal cond. mol H2O m^{-2}s^{-1}	Photosynthesis µmol CO$_2$m^{-2}s^{-1}	Treatment
1.41±0.035	3.38±0.231	7.24±0.176	2.52±0.103	0.159±0.01	09.54±0.115	T1
1.17±0.015	3.74±0.015	8.19±0.211	3.10±0.115	0.179±0.03	10.22±0.110	T2
1.74±0.041	3.22±0.139	6.22±0.179	2.98±0.112	0.178±0.05	09.26±0.09	T3
0.93±0.048	4.17±0.012	7.04±0.471	1.98±0.106	0.216±0.02	11.70±0.08	T4
2.01±0.069	2.96±0.015	5.23±0.30	3.10±0.108	0.147±0.09	08.80±0.09	T5
1.29±0.045	3.40±0.133	6.65±0.465	2.28±0.113	0.198±0.008	11.10±0.10	T6
2.11±0.088	1.52±0.023	4.67±0.136	3.70±0.110	0.100±0.009	08.30±0.11	T7
1.42±0.012	2.77±0.049	4.82±0.003	2.57±0.111	0.124±0.010	10.94±0.09	T8
2.24±0.020	1.23±0.015	4.44±0.191	5.77±0.009	0.094±0.008	07.20±0.11	T9
1.55±0.036	2.16±0.012	4.35±0.012	2.92±0.112	0.106±0.012	10.48±0.09	T10

*The mean difference is significant at the.05 level.

to untreated plants while these criteria were raised 46% and 80% respectively when Acadian was added in a recommended dose to 400mg/l lead –treated plants. On the other side, Acadian fertilization increased the transpiration rate of tomato leaves but it reduced it when added to lead treated plants. These results were compatible with those of Amujoyegbe *et al.* [62], Karanatsidis and Berova [25] and Amaya-Carpio *et al.* [63] concerning gas exchange and those of Amujoyegbe *et al.* [62] and Noori *et al.* [64] concerning photosynthetic pigments.

Lead occurs in soils as soluble and insoluble salts.Solubility of lead is greatly reduced by increase of soil pH [5] and its mobility is hampered by organic particles and thus addition of different sources of organic fertilizers renders lead unavailable to plants [8]. Organic fertilization increased the photosynthetic ability due to various factors namely the efficiency of PS II, the stability of chloroplast ultrastructure, the rate of CO$_2$ absorption by leaf cells and its fixation [25,63].

3.3. Carbohydrate, Nitrogen and Proline Contents

It was prominent from Table **5** that Pb-treated tomato plants showed significant decreases in the values of total sugars (TS) & total nitrogen (TN) of both shoots and roots and in proline of roots in a dose dependent manner.

By contrast, this element raised the contents of each of total soluble sugars (TSS), total soluble nitrogen (TSN), amino nitrogen of both shoots and roots and proline of shoots as compared to untreated shoots and roots of tomato plants.

Table 5: Impact pf Organic Fertilizer, Lead and Mixture of both on the Carbohydrate, Nitrogen and Proline Contents of Shoots and Roots of Tomato Plants

Treatment	Total sugars mg/g.d.wt		Total soluble sugars mg/g.d.wt		Total nitrogen mg/g.d.wt		Total soluble nitrogen mg/g.d.wt		Amino-N mg/g.d.wt		Proline mg/g.f.wt	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots	shoots	roots	shoots	roots
T1	15.69±0.25	5.66±0.26	7.77±0.22	2.90±0.42	42.6±1.45	47.5±1.45	11.29±1.69	10.45±1.21	1.75±0.02	0.10±0.03	189.0±1.63	46.0±1.73
T2	19.22±1.13	8.33±0.24	8.49±0.56	5.26±0.25	58.6±0.65	55.6±0.97	16.27±0.53	13.53±1.22	1.96±0.01	0.11±0.02	200.0±1.73	83.0±2.31
T3	18.75±3.31	5.09±1.17	4.97±0.92	3.44±0.01	44.5±0.32	73.2±0.89	19.45±0.15	17.79±0.41	2.42±0.08	0.61±0.04	108.3±0.73	208.3±2.31
T4	19.47±1.01	9.02±0.55	5.11±0.13	3.50±0.29	54.3±0.97	84.8±4.77	22.04±1.62	18.21±1.05	2.68±0.01	1.09±0.07	153.7±0.33	298.0±3.46
T5	17.16±2.44	4.39±0.47	8.39±2.74	4.60±0.20	35.56±0.91	66.3±0.89	19.83±0.09	17.97±1.05	2.69±0.09	0.70±0.16	126.3±4.33	191.3±3.53
T6	18.10±0.47	8.89±0.34	9.61±0.09	5.92±0.96	44.2±0.74	72.3±0.65	23.89±0.65	18.87±0.09	2.68±0.11	1.25±0.09	161.3±0.33	231.3±0.89
T7	16.68±0.6	4.19±0.89	8.53±0.76	2.80±0.08	33.4±6.14	56.2±0.09	20.73±0.25	18.69±0.89	2.80±0.03	0.82±0.10	262.0±3.69	158.0±6.23
T8	17.34±0.19	7.67±0.28	11.29±1.15	3.76±0.10	42.7±5.01	60.97±0.56	24.56±0.16	21.93±0.09	3.17±0.04	1.34±0.02	274.0±2.31	120.0±1.73
T9	14.14±0.17	1.49±0.02	10.42±0.82	3.08±0.36	31.3±1.29	55.01±1.38	23.53±1.21	19.01±1.54	3.22±0.02	1.06±0.03	269.0±5.20	61.0±4.62
T10	16.69±0.8	1.43±0.03	11.58±0.30	4.27±0.04	42.0±0.16	60.33±0.25	26.76±1.45	22.88±0.65	3.38±0.07	1.44±0.02	280.3±9.62	113.0±2.31

*The mean difference is significant at 0.05 level.

Fertilization by Acadian solely or combined with Pb at different rates increased all the former measured criteria above either the untreated or Pb-treated shoots and roots.

Similarly, Chatterjee *et al.* [12] noticed reductions in the levels of total sugars and total nitrogen in lead treated rice plants. On the other hand, Pb exposure increased the contents of soluble sugars, glucose, soluble protein, ammonia, free amino acids and proline [12,21,57,65].

Applying organic fertilizers at the recommended rate, increased the contents of dry matter, total sugars, reducing and non-reducing sugars [28,29,35], the uptake and content of nitrogen [33,34,66] and the levels of some amino acids and proline [29,34].

Thus, organic manuring reduced lead phytotoxicity on plants by improving cation and anion exchange capacity and solubility. This situation, in turn, raises nutrient availability and balance to plants which was reflected on producing better growth parameters, photosynthetic effiency and sugar content [34]. Organic fertilization also increased proline accumulation under heavy metal stress. The accumulation of proline might result from increasing protein turnover and (or) due to enzyme stabilization and (or) osmoregulation at the cellular level in response to organic fertilization [34]. Moreover, proline acts as a major reservoir of energy and nitrogen, which can be used in resuming the growth under stress [57]. These suggestions can clearly explain the capability of Acadian fertilized tomato plants to endure lead toxicity and grow nearly normal (Table **3**).

3.4. Antioxidant Enzyme Activities, Phenols and Lipid Peroxidation Levels

With increasing levels of Pb applied, a concomitant increase in each of peroxidase (POD), superoxide dismutase (SOD), phenols and lipid peroxidation levels (measured as nmole of MDA formed) was observed in both shoots and roots of tomato plants (Table **6**). Reversibly, catalase activity registered progressive decline in both shoots and roots of tomato plants with the increase in the rates of $Pb(NO_3)_2$ applied (0,50,100 200,400mg/l). Data in Table **6** clearly manifested that tomato roots showed higher antioxidant enzyme activities (POD, SOD) and antioxidant compounds (phenols and MDA) more than shoots. Lead is a major hazard to the soil-plant system. It is readily absorbed mainly through the root system where it exerts its phytotoxicity symptoms and there after transported to various sites in the shoots. The effects of lead phytotoxicity include stunted growth, chlorosis, blackening of the root system, alteration in water and nutritional status of plants as well as various metabolic processes [19,56,67,68] which were indicated in the present study. These results are in conformity with the work of Verma and Dubey [56] and Olivares [67] who reported that Pb induced oxidative stress in rice and *Tithonia diversifolia* plants respectively. In many plant species, heavy metals have been reported to cause oxidative damage due to production of reactive oxygen species (ROS). To resist oxidative damage, the antioxidant enzymes (SOD and POD) and certain metabolites (lipid peroxides as MDA and phenols) were induced as a general strategy adopted by plants to overcome oxidative stress imposed by environmental stresses [56]. Lipid peroxidation is a biochemical marker for the free radical mediated injury. SOD is an essential component of antioxidative defense system in plants as it dismutates two superoxide radicals ($O_2^{\cdot -}$) to water and O_2, thus enabling plants to endure oxidative damage caused by Pb toxicity [19,56]. A decline in catalase (CAT) activity under Pb toxicity was observed in current work which suggests a possible delay in removal of H_2O_2 and toxic peroxides mediated by catalase and, in turn, an enhancement in the free radical mediated lipid peroxidation under Pb toxicity. Similar decline in catalase activity was reported under salinity, chilling, drought and hypoxia [56].

The enhancement in peroxidase activity observed in this study suggest that this enzyme serves as an intrinsic defense tool to resist Pb-induced oxidative damage in tomato plants. Induction in peroxidase activity has been documented under a variety of stressful conditions as water stress, chilling, salinity, γ-radiation and heavy metals [56].

Spraying tomato plants with the recommended dose of Acadian, significantly lowered the activities of the three antioxidant enzymes (SOD, POD and CAT), The phenol values and the level of lipid peroxidation in both shoots and roots as comparable to those of untreated plants. When Acadian was supplemented to tomato plants together with different concentrations of Pb, there were synergistic increases in the level of all antioxidant enzymes and phenols and decreases in lipid peroxidation in shoots and roots below and above the same organs of tomato plants treated with equivalent rates of lead only. These results corroborated with those of Carbonaro *et al.* [69] using

Table 6: Impact of Organic Fertilizer, Lead and Mixture of both on the Phenol and Lipid Peroxidation Levels and the Activities of SOD,PODand CAT in Shoots and Roots of Tomato Plants

CAT mg/g.F.wt/hr.		POD mg/g.F.wt/hr.		SOD units/mg protein/min		lipid peroxidation MDA/g.F.wt		Phenol mg/g.d.wt		Treatment
Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	
527±0.93	502±0.90	596±0.88	578±0.73	3.1±0.06	2.5±0.08	1.68±0.08	0.94±0.03	1.05±0.10	14.43±1.32	T1
539±0.84	519±0.81	611±0.86	593±0.68	3.8±0.04	2.7±0.07	1.14±0.06	0.72±0.02	6.45±0.35	57.07±2.68	T2
503±0.71	486±0.76	674±0.76	608±0.79	4.6±0.05	3.1±0.09	2.09±0.05	1.32±0.05	3.41±0.17	22.51±1.06	T3
518±0.63	496±0.74	685±0.66	619±0.84	5.0±0.08	3.9±0.05	1.71±0.09	1.09±0.09	9. 40±0.69	1.98±25.84	T4
491±0.66	472±0.66	693±0.65	653±0.63	5.8±0.07	4.6±0.10	2.78±0.07	1.89±0.08	5.61±0.70	34.63±0.45	T5
528±0.54	513±0.83	706±0.58	674±0.88	6.1±0.09	4.8±0.08	2.06±0.06	1.73±0.06	5.37±0.17	37.89±0.30	T6
478±0.68	451±0.69	713±0.58	687±0.91	6.9±0.10	5.3±0.09	3.97±0.09	2.16±0.09	7.37±0.23	66.16±0.16	T7
563±0.73	554±0.68	719±0.61	691±0.74	7.3±0.11	5.7±0.08	3.13±0.10	2.05±0.08	8.86±0.23	36.45±0.69	T8
451±0.94	423±0.74	726±0.83	706±0.65	8.1±0.12	6.2±0.09	4.62±0.11	3.96±0.07	8.16±0.11	38.89±0.41	T9
581±0.43	573±0.78	732±0.91	763±0.53	8.9±0.14	7.3±0.08	3.98±0.12	2.49±0.08	9.25±0.130	39.31±0.51	T10

*The mean difference is significant at the.05 level.

peach and pear, Montalba *et al.* [37] using blueberry and Wu *et al.* [38] using watermelon. These data indicated that the bio-organic fertilizer Acadian provided an improvement in the antioxidant defense system of the plant and in membrane integrity thus enabling plants to overcome the oxidative state and exert protection against tissue damage induced by lead toxicity. Currently available report supported this view [37,38,69].

3.5. Inorganic Nutrient Contents

It is evident from Tables (**7a** & **b**) that the shoot and root content of most major nutrient elements estimated in this study (P, K, Ca and Mg) decreased proportionally with the increase in Pb rates up to 400mg/l. By contrast, the values of each of Na, Pb and Fe increased progressively in both shoots and roots with the increase in the concentrations of Pb applied (0, 50, 100, 200, 400 mg/l). Addition of Acadian at the recommended dose either alone or combined with lead at various rates caused the accumulation of P, K, Ca and Mg but alternatively reduced the amounts of Na, Pb and Fe in both shoots and roots of tomato plants (Tables **7a** & **b**).

The above results were consistent with those of Lee *et al.* [65] who realized a reduction in the contents of Ca and P in Pb-treated soybean leaves. Similarly, Walker *et al.* [70] showed that Pb treatment reduced he uptake of each of K, Ca, Mg and P due to Pb toxicity of *Zea mays* and *Cucumis sativus.* Recent study showed that Pb treatment markedly decreased the contents of P, K and N in treated plants [18].

Accordingly, it is ascertained that Pb toxicity causes disorder in plant mineral nutrition, an alteration in membrane permeability and its physiological activity and lowering pH of soil thus rendering most nutritive elements in soil unavailable to plant roots.

However, when these elements were uptaken by plants, Pb induced an imbalance in their distribution and their concentrations in different plant tissues, thus, they became less beneficial to Pb-treated plants. This opinion was strengthed by the remarks of both Rolf and Bazzaz [71] and Olivares [67]. At the other side, the most hazardous effect of Pb was its ability to accumulate heavy metals as Mn, Zn, Cu and Fe in treated plant tissues to toxic levels [72]. Thus, plants under these circumstances show retardation in growth, yield and chemical disorders.

Regarding the effect of organic fertilizers, several comparable studies confirmed the current data. For instance, Murray and Anderson [73], Schuphan [29], El-Ashtar and El-Etreiby [33], Abou El-Magd *et al.* [34] and Noori *et al.* [64] observed increased accumulations of major nutritive elements as Mg, Ca, P, K as well as Fe in various tested plant organs whereas Na levels were greatly reduced in response to fertilization with organic fertilizers from different sources (plant and animal source).

Table 7a: Impact of Organic Fertilizer, Lead and Mixture of Both on the Mineral Contents of Tomato Shoots. Value Listed are Expressed as mg/g.d.wt while Lead Values were Expressed as µg/g.d.wt.

Sodium	Potassium	Phosphorus	Magnesium	Calcium	Iron	Lead	Treatment
13.93±0.000	7.613±1.204	2.413±0.199	4.443±0.193	1.45±0.214	0.948±0.036	15.33±0.33	T1
13.67±0.398	8.93±0.064	1.95±0.029	4.843±0.112	4.377±0.211	0.765±0.028	13.33±1.202	T2
16.173±0.032	6.40±0.231	2.473±0.450	4.897±0.095	3.31±0.682	0.555±0.147	17.20±0.577	T3
14.34±1.190	21.67±3.688	3.04±0.092	5.13±0.130	3.89±0.941	0.89±0.015	13.25±2.673	T4
17.70±0.548	5.91±0.179	2.133±0.499	4.08±0.029	2.71±0.436	0.62±0.064	19.67±0.333	T5
15.20±0.075	19.09±0.000	2.06±0.038	5.11±0.066	3.40±1.351	0.928±0.065	14.00±1.155	T6
18.51±0.393	5.22±0.118	1.88±0.052	3.09±0.066	2.35±0.092	0.72±0.079	20.00±2.309	T7
17.01±0.000	13.20±0.075	2.04±0.046	4.93±0.240	3.13±0.282	0.95±0.039	15.00±1.155	T8
19.42±0.355	4.99±0.101	1.08±0.121	2.99±0.162	2.11±0.724	0.79±0.010	25.00±2.309	T9
18.19±0.166	12.52±0.274	1.47±0.015	4.39±0.328	2.21±1.154	1.023±0.04	16.33±0.882	T10

*The mean difference is significant at the .05 level.

Table 7b: Impact of Organic Fertilizer, Lead and Mixture of both on the Mineral Element Contents of Tomato Roots. Values Listed are Expessed as mg/g.d.wt. while Lead Values were Expressed as µg/g.d.wt.

Sodium	Potassium	Phosphorus	Magnesium	Calcium	Iron	Lead	Treatment
5.47±0.205	6.39±0.799	1.80±0.058	4.85±0.078	0.46±0.190	0.46±0.138	21.67±1.453	T1
6.82±1.109	4.93±0.280	1.89±0.061	5.36±0.205	4.45±0.156	0.21±0.035	15.00±0.577	T2
7.10±0.987	4.38±0.032	2.43±0.090	4.58±0.282	2.32±0.980	0.15±0.004	15.33±0.333	T3
7.57±0.577	6.65±0.782	2.60±0.087	4.59±0.170	2.73±0.479	0.24±0.057	14.00±1.732	T4
7.55±0.277	3.95±0.201	1.85±0.079	3.99±0.531	0.77±0.352	0.15±0.003	17.00±1.732	T5
4.66±0.046	4.19±0.312	2.55±0.040	3.91±0.0658	1.60±0.084	0.34±0.097	48.00±31.0.21	T6
8.95±0.531	3.92±1.117	1.74±0.032	3.75±0.133	0.53±0.137	0.21±0.025	21.00±2.887	T7
4.38±0.460	4.13±0.381	1.96±0.078	3.86±0.080	1.33±0.087	0.34±0.033	17.00±0.000	T8
9.85±0.020	3.30±0.196	1.57±0.061	3.15±0.401	0.42±0.122	0.30±0.031	31.33±0.333	T9
3.35±0.017	2.70±0.217	1.90±0.058	3.27±0.398	0.89±0.020	0.45±0.011	19.67±1.202	T10

*The mean difference is significant at the .05 level.

4. CONCLUSION

Subsequently, it appears that the bio-organic fertilizer, Acadian can drastically inhibit the hazardous effect of Pb by exclusion, chelation, compartmentalization of lead and other toxic heavy metals in soil away from tomato roots or in the apoplastic tissues of root and hampered their translocation to shoots, and/ or by synthesizing metal detoxifying substances (phenols) or enzymes (antioxidant enzymes)or osmoprotectants (proline and soluble sugars), and/or by inducing nutrient balance which resulted from progressive uptake of beneficial elements. All these effects render tomato plants capable of enduring lead toxicity and restitute normal growth and productivity which were indicated in the present results.Eventually, the results of the present investigation clearly manifested that the addition of the recommended dose of the red algal extract, Acadian, at rate of 8ml/10l H_2O to *Lycopersicum esculentum* plants grown in soil contaminated by lead up to 400 mg/l, boosted plants to overcome or even reduce lead toxicity and thus obtained relatively better growth, better quality and yield, as well as better chemical composition.

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