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Effects of Ag and Pb metal accumulation on some biochemical parameters and anatomical characteristics of *Sesuvium portulacastrum* L. (Aizoaceae) plants

Widad M.T. Al-Asadi[1], Alla N. Al-Waheeb[2], Sahar A.A. Malik Al-Saadi[3], Sadeq S. Kareem Al-Taie[4*]

1. Department of Ecology, College of Sciences, University of Basrah, Basrah, Iraq
2. Department of Biology, College of Science, University of Thi-Qar, Iraq
3. Department of Biology, College of Science, University of Basrah, Iraq
4. Department of Biology, College of Science, University of Misan, Iraq

** Corresponding author's Email: sas_altti@uomisan.edu.iq*

ABSTRACT

The study reported the effect of contamination of *Sesuvium portulacastrum* L. after exposure to Ag and Pb for four weeks. The results showed that the total protein, chlorophyll, carotene, and biomass declined gradually by elevating the heavy metal concentrations. Elemental analyses of the leaves were performed using scanning electron microscopy with energy–dispersive- X-ray spectroscopy (SEM-EDS). The main mineral of control treatment contained 11 elements including carbon (57.50%) followed by oxygen (22.76%) and trace iron and lead. The minerals in the leaves treated with 100 mg L^{-1} Pb exhibited 12 elements with high levels of lead (52.03%). The leaves treated with 100 mg L^{-1} Ag showed 11 elements with high levels of silver (43.02%) followed by carbon (34.95%) and oxygen (11.18%). Anatomical study indicated that the Ag and Pb can accumulate in internal tissues and causes several alterations such as shape of leaves, stems and roots, as well as thickness and number of cortical parenchymal cells. In addition, unrecognized the endodermis, and exodermis, the root thickness was 663.21 µm in control group, while in Ag and Pb treatments were 498.32 µm and 375.61 µm respectively.

Keywords: *Sesuvium portulacastrum*, Chlorophyll, Protein, Biomass, Anatomy, Stem, Roots, SEM-EDS.
Article type: Research Article.

INTRODUCTION

Heavy metals are serious pollutants and can adversely affect the environment, especially agricultural soils (Azizi *et al.* 2020; Budovich 2021; Saleh Ibrahim *et al.* 2022; Naser *et al.* 2022). Plants can accumulate these elements and thus affect downstream organisms (Ali *et al.* 2013). *S. portulacastrum* (L.) also called sea purslane, belongs to the Aizoaceae family. It is a perennial succulent and facultative halophyte, grown in backshore zones of rocky shores, coastal beaches, estuaries, tidal flats, and margins of salt marshes (Lokhande *et al.* 2013). *S. portulacastrum* can accumulate and tolerate high concentrations of metals from contaminated sites via phytoremediation without involvement from thiol metabolism (Lokhande *et al.* 2011). In addition, *S. portulacastrum* can tolerate and accumulate salts; thus, they are better suitable to phytoextraction of heavy metals from salty soils (Zaier *et al.* 2010). The role of *S. portulacastrum* in accumulating pollution has been studied by many authors. Ghnaya *et al.* (2005) investigated the effects of accumulated cadmium, potassium, and calcium concentrations in tissues on the growth of *S. portulacastrum.* Ghnaya *et al*. (2007) found high toxicity of the Cd in the plants as a result of uptake of a large disturbance of the essential elements. Kalaikandhan *et al*. (2014)

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studied the effect of Cu and Zn on the pigment content such as carotene and chlorophyll A, B, as well as the total chlorophyll seen in *S. portulacastrum.* Ayyappan *et al*. (2016) studied *S. portulacastrum* grown in soil contaminated with heavy elements: The pollutants increased in the leaves versus the root and stem. Bioaccumulation and tolerance of cadmium and copper were also reported on seedlings of *S. portulacastrum* by Feng *et al.* (2018). The effects of Cu and Zn on the biochemical and ecophysiological contents of *S. portulacastrum* were examined by Kalaikandhan *et al.* (2018). SEM/EDS microanalysis is an important analytical method to study contamination (Miler & Gosar 2009). The SEM morphological changes and distribution of different elements in pollution of cell scan are evaluated using SEM-EDX techniques (Raize *et al.* 2004). This technique was employed to evaluate the toxic contamination in the *S. portulacastrum* leaves, roots, stems, fruits, soil, and water by some authors (Shakti & Sunita 2016). Our study evaluates the effects and accumulations of different concentrations of Ag and Pb on *S. portulacastrum*. SEM/EDX was used for qualitative and quantitative chemical analyses of the specimens.

MATERIALS AND METHODS

Plant material

Experiments were managed in Science College, Biology and Ecology Department at Basrah University. *S. portulacastrum* were collected after rhizogenesis, grown in the laboratory, and then were added a various concentrations of Ag and Pb for four weeks (control without heavy metals: 10, 25, 50, and 100 mg L^{-1} and the interaction between Ag and Pb = T_0, T_{10}, T_{25}, T_{50}, T_{100} and $T_{Ag\ \&\ Pb}$). After four weeks, 10 plants were harvested for analyses. The samples were taken, and their weights were stabilized to estimate the biomass. The physical and chemical properties were determined using standard methods.

Preparation of concentration

The standard solutions were made by taking a dilution of Ag and Pb standard solutions (1000 mg L^{-1}) form of nitrate Pb $(NO_3)_2$ and Ag (NO_3). Solutions were prepared and stored at 4°C. Analytical standard solutions (10, 25, 50 and 100 mg L^{-1}) were made from these stock solutions via serial dilutions according to the mitigation law.

$N1 \times V1 = N2 \times V2$

Estimation of photosynthetic pigments

The chlorophyll contents of fresh leaves were determined in 80% acetone via the literature (Arnon 1949). We prepared 10, 15, 25 and 100 mg L^{-1}. Then, 1 g of the sample was weighed via a Sartorius BL.210 balance and then grind with 20 mL of 80% acetone and filtered. The granules were re-extracted by 10 mL of 80% acetone until it become colourless. The chlorophyll in fresh leaves was determined using a spectrophotometer at 660 and 645 nm and reported in mg g^{-1} of leaves. The chlorophyll concentration was determined by the formulae below:

Total chlorophyll (mg L^{-1}) = [20.2 (D_{645}) + 8.02 (D_{663})] x V/1000 x W

Chlorophyll a (mg L^{-1}) = [12.7 (D_{663}) - 2.69 (D_{645})] x V/1000/W

Chlorophyll b (mg L^{-1}) = [22.9 (D_{645}) - 4.68 (D_{663})] x V/1000/W

where:

D = length of light path in the cell (1 cm)

V = volume (in mL) of the extract.

W = fresh weight (g) of sample.

Carotene estimation

The carotene contents were estimated by the method of Kirk & Allen (1965). The same chlorophyll extract was measured at 440 nm in a spectrophotometer to predict the carotene.

Carotene (mg L^{-1}) = [4.695 (D (C)) – 2.88 (D. (a)) + (D (b))] × V/1000/W

Nitrogen and protein estimation

The nitrogen in the plant samples was estimated according to the literature (Cresser & Parsons 1979). The biomass was estimated for fresh samples collected after 72 hours of exposure reporting in fresh weight m^{-2}.

Anatomical study of *S. portulacastrum*

Fresh material of 100 mg L^{-1} *S. portulacastrum* was collected after one week from treatment. For anatomy of samples, fresh material of leaves, stems and roots were fixed 24 hours in acetic acid (5 mL): formalin (5 mL):

alcohol solution (90 mL) preserved in 70% alcohol, then dehydrated in ethyl alcohol series. The samples were cut using a rotary microtome, and then stained in safranin and fast green followed by mounting via Canada balsam (Johansen 1940). The samples were observed by Olympus CH4 light microscope and photographed by digital camera DCE-2.

SEM and EDX microanalysis observation

The samples were treated to various concentrations of Pb and Ag for 30 days. Leaves of *S. portulacastrum* were collected and dried in an oven at 60 ^{o}C for 24 hours. Leaves over 10 cm in diameter were sampled. Plant samples were vacuum air-dried using an EDX Microanalyzer including. The 1-3 mm of leaves were used for the SEM analysis. We determined the Ag and Pb via SEM-EDX spectroscopy and reported atomic weight percent in *S. portulacastrum* as described (Islam *et al.* 2007).

Statistical analysis

Statistical package SPSS (Version 13.0) was used to determine the significance difference between treatments via least significant difference (LSD). Data were tested at a significance level of $p < 0.05$ by one-way analysis of variance (ANOVA). All values were calculated as the means of three independent replicates.

RESULTS AND DISCUSSION

Chlorophyll estimation

Table 1 summarizes the results of the Pb, Ag, and interaction between them on chlorophyll a, chlorophyll b and total chlorophyll. Total chlorophyll levels were reduced by elevating the Pb and Ag concentrations. At the last week of experiments, we compared the 100 ppm concentration with the control. The control group exhibited a chlorophyll a value of 1.087 mg L^{-1} at baseline. It decreased to 1.084, 1.074, 0.974, and 0.825 mg L^{-1} by T_{Pb10}, T_{Pb25}, T_{Pb50} and T_{Pb100}, respectively. The chlorophyll b was 0.674 mg L^{-1} at T_{Pb100} (Table 1), and the total chlorophyll of leaves reduced by rising Ag concentration: 0.973 and 0.874 mg L^{-1}, respectively, at T_{Ag100}. Total chlorophyll was 1.002 mg L^{-1} (Table 1). The $T_{Pb\ \&\ Ag}$ appeared to decrease in chlorophyll concentration: It was 0.794 and 0.605 mg L^{-1} at T_{100} for chlorophyll a and b, respectively. The Ag- and Pb-treated plants showed a gradually decrease in chlorophyll by elevating the heavy metal concentrations. The Ag and Pb toxicities inhibit many metabolic processes by inhibiting photosynthetic pigment synthesis in chlorophyll while also inhibiting enzyme activity (δ-aminolaevulinic acid dehydratase and protochlorophyllide). It also inhibits the Calvin cycle. Our results are in agreement with previous work (Kalaikandhan *et al.* 2014). The results signalled increased total chlorophyll and chlorophyll b content of *S. portulacastrum* at low concentration ($T_{Pb\ 25}$). They decreased at T_{Pb50} and T_{Pb100}. Higher levels show that *S. portulacastrum* plants salient depression in photosynthetic pigments (Kalaikandhan *et al.* 2014). The chlorophyll attribution (a/b) in Pb and Ag stress showed that chlorophyll b is sensitive to Ag and Pb. Heavy metal causes a reduction in electron transport and affects photosystem II. The leaves in polluted environmental conditions have irregular chloroplast shapes with pockets inside the organelles. This leads to larger surfaces of the chloroplasts leading to an increase in the total number of materials exchanged between the cytoplasm and chloroplasts.

Table 1. Effects of Ag and Pb concentration on total chlorophyll content (mg/l fresh weight) on *S. portulacastrum* as a function of exposure times (days).

Heavy metals	Photosynthesis pigments		Concentration (ppm)				
			0	10	25	50	100
Pb	Chlorophyll	a	1.087	1.084	1.074	0.974	0.825
		b	0.969	0.953	1.033	0.983	0.674
		total	1.981	1.054	1.066	1.028	0.882
	Carotene		0.708	0.674	0.702	0.704	0.564
Ag	Chlorophyll	a	1.087	1.093	1.089	1.009	0.973
		b	0.969	0.971	0.956	0.933	0.874
		total	1.981	1.893	1.755	1.082	1.002
	Carotene		0.708	0.677	0.673	0.577	0.377
Interaction between Pb and Ag	Chlorophyll	a	1.087	1.064	0.976	0.894	0.794
		b	0.969	0.944	0.994	0.967	0.605
		total	1.981	1.026	1.034	0.985	0.874
	Carotene		0.708	0.669	0.675	0.681	0.534

Carotenoid Estimation

The carotene concentration decreased in all treatments versus control: It was 0.377 mg L^{-1} in T_{Ag100} when treated with Ag (Table 1). The carotenoid content is inhibited in response to Ag and Pb toxicities and indicates a severe effect on the cell and its component parts. Decreases in carotenoid contents are in line with the literature (Mourato *et al.* 2015). Many authors have reported that carotenoids protect the composition of chlorophyll and cells against ROS under contamination stress due to replacement of peroxidation and demolition of chloroplast membrane (Keunen *et al.* 2013; Mourato *et al.* 2015). The results in Table 1 showed that Pb concentrations at 25 ppm and 50 ppm (T_{Pb25} and T_{Pb50}) exhibited higher negative effects than at 10 and 100 ppm (T_{Pb10} and T_{Pb100}). This is because carotenoid content increased to protect the cell against these contaminants. The levels also, increased to 0.704 mg L^{-1} at T_{Pb50}. The T_{Pb100} degraded carotenoid pigments (0.564 mg L^{-1}) by activating some mechanisms (Parmar & Singh 2015). The increase indicates the important role of carotene in detoxifying ROS. However, higher levels of metal and ion toxicity can affect photosynthesis leading to a destruction of the chloroplast ultrastructure (Keunen *et al.* 2013; Mourato *et al.* 2015). Chlorophyll and carotenoids were markedly affected. The hydrogen peroxide and malondialdeyde levels increased due to oxidative stress (Shakoor *et al.* 2014; Mourato *et al.* 2015).

Protein Estimation

Table 2 depicts Ag and pb on total protein of *S. portulacastrum* at 10, 25, 50 and 100 ppm versus control group. The total protein content decreased by elevating heavy metals. *S. portulacastrum* was more effective to Ag, and the total protein reached 6.57%. It was 8.99 % in T_{Ag100}, while in $T_{Pb\ \&\ Ag}$ was 7.83 % at 100 ppm.

Table 2. Effects of Ag and Pb on protein and accumulation content in *S. portulacastrum*.

	Concentrations	**Heavy metal**		
		Pb	**Ag**	**Pb + Ag**
Protein	0	13.07	13.07	13.07
content	10	11.02	16.22	11.87
	25	9.75	14.06	9.55
	50	9.06	11.19	9.76
	100	6.57	8.99	7.83
Accumulation (mg g^{-1})	0	0	0	0
	100	54.28	22.17	27.06

The decreased protein content in treated *S. portulacastrum* is similar to the literature (Guo *et al*. 2007; Zengin and Kirbag 2007: Al-Hakimi & Hamada 2011). Treatments with 10 and 25 ppm Ag (T_{Ag10} and T_{Ag25}) increased the total protein levels under contamination stress. This is due to the creation of stress proteins found in the Krebs cycle such as enzymes. There are many reasons for the decreased protein levels under contamination stress such as rises in protease activity and DNA-protein cross-links (Palma *et al.* 2002; Atesi *et al.* 2004) which decrease the synthetic protein degradation. The Ag and Pb might decrease protein contents by the reduced uptake of K and Mg. The binding of elements with sulfhydryl groups might be related to the Krebs cycle enzymes, phytochelation biosynthesis, glutathione, or their replacement with metalloproteins (Pal *et al.* 2006). A decrease in protein content may result in the upraised protease activity in various structural and fragmentation of proteins. This can occur in DNA-protein cross-links (Palma *et al.* 2002; Hall 2002; Atesi *et al.* 2004).

Accumulation of Pb and Ag

Accumulation of Pb and Ag in *S. portulacastrum* treated with 100 ppm of Pb or Ag resulted in a lower accumulation (22.17 µg g^{-1} in fresh weight for Ag). This was 54.28 µg g^{-1} in fresh weight for Pb (Table 2). The results indicated that *S. portulacastrum* can accumulate heavy metals. The metals enter roots and store in roots or translocate to shoots by the xylem. In addition, the metals can bind toxic elements at cell walls of leaves and roots or make up complexes with the organic acids or proteins. In other plants, the cells of root prevent translocation of heavy metal from the roots to above-ground tissues (Paivoke, 2002; Ghnaya *et al.*, 2005).

Biomass Estimation

Table 3 depicts alterations in the biomass as a result of heavy metal treatment. Biomass decreased by elevating in the Pb and Ag concentrations; the biomass in *Sesuvium* treated with Pb, Ag, or both at 100 ppm was 7.173, 6.401, and 5.776 g respectively. The highest values of Pb and Ag content caused the leaves to be oblong. The Pb and Ag treatments reduced the biomass production of *S. portulacastrum* versus the control similar to the literature (Zaier

et al. 2010). Lead decreased in root water absorption leading a significantly shorter shoot versus other treatments. Several groups have shown that heavy metals severely affect the water status of sensitive-metal species by osmotic potential of cell sap. This can affect transpiration and water content (Patra *et al.* 2004; Vernay *et al.* 2007).

Table 3. Effects of Ag, pb on biomass, total nitrogen of leaves and stem length in *S. portulacastrum*.

Factor	Concentration	Pb	Ag	Pb + Ag
Biomass	0	10.524	10.946	10.335
(g)	10	10.801	14.268	6.906
	25	9.938	10.881	6.763
	50	9.126	9.414	6.098
	100	7.173	6.401	5.776
Total Nitrogen	0	2.091	2.091	2.091
(mg g^{-1})	10	1.763	2.595	1,899
	25	1.65	2.249	1.528
	50	1.449	1.790	1.561
	100	1.051	1.438	1.252
Stem length	0	10	10	10
	10	15.76	22.35	12.45
	25	12.07	24.32	10.21
	50	9.55	12.99	9.66
	100	9.02	9.33	8.67

Nitrogen estimation

The results showed a reduced percentage of nitrogen with an upraise in the Ag and Pb levels. The results are reported in Table 3. The control treatment was 2.091 mg g^{-1}. The nitrogen levels were 1.051, 1.438 and 1.252 at 100 ppm of Pb, Ag or both. The nitrogen reduced at higher concentrations of heavy metals due to downregulation of denitrification enzymes in the cell membrane or periplasmic space (Dmitri & Maria 2008; Hamsa *et al.* 2017). Heavy metals inhibit nitrous oxide reductase (Hewson & Fuhrman 2006; Hamsa *et al.* 2017).

Anatomical studies

Transverse sections of mesophyll

Cross sections of mesophyll in control treatment exhibited that the epidermis is covered with a thin cuticle and trichomes, epidermis was uniseriate, cells are circular, semicircular, rectangular and square shaped. Mesophyll is isobilateral by found palisade layer on two sides of mesophyll; spongy tissue occurs between the palisade layers formed from 7 to 12 layers. The thickness of the palisade layer was between 35.77 µm in control and 21.60 µm in Ag treatment and 20.67 in Pb treatment, while the spongy tissue was 15.66 µm and 13.50 µm in the Ag and Pb treatments, compared to control (25.55 µm; Table 4). The mesophyll in plants exposed to Ag and Pb exhibited the reduced palisade layer, cells of palisade layer were sinuate and irregular in shape (Fig. 1). The shape of leaves in control treatments was regular, the upper and lower epidermal cells contained large cells circular or oval shaped, while in the treatments of leaves exposed to Ag and Pb were sinuate or irregular shaped, and the cells of upper and lower epidermis were small in size and irregular shaped (Fig. 1). The thickness of the leaf blade was between 133.52 µm in control treatment, however, it was 97.85 µm and 70.45 µm in Ag and Pb treatment (Table 4; Fig. 1). The vascular bundle midrib is solitary and surrounded by parenchymatic cells. This result is in agreement with Metcalfe & Chalk (1950). The vascular bundle thickness was 125.77 µm and 90.38 µm in in Ag and Pb treatments respectively (Table 4).

Table 4. Measurement of leaf blade in *S. portulacastrum* in micrometer (µm).

Treatment	Blade thickness	Upper epidermis thickness	Lower epidermis thickness	Palisade layer	Spongy layer	Midrib thickness
Control	(145.50-123.6) 133.52	(27.88-12.33) 18.60	(27.21-14.38) 19.85	(41.22-30.10) 35.77	(41.22-19.61) 25.55	(121.2-100.12) 115.33
Ag	(110.50-83.77) 97.85	(20.20-5.81) 10.65	(15.22-6.22) 11.47	(28.43-16.22) 21.60	(18.32-11.22) 15.66	(132.36-120.30) 125.77
Pb	(85.10-35.90) 70.45	(10.22-5.21 7.55	(12.20-8.22) 10.60	(31.21-15.55) 20.67	(15.32-9.80) 13.50	(105.54-75.11) 90.38

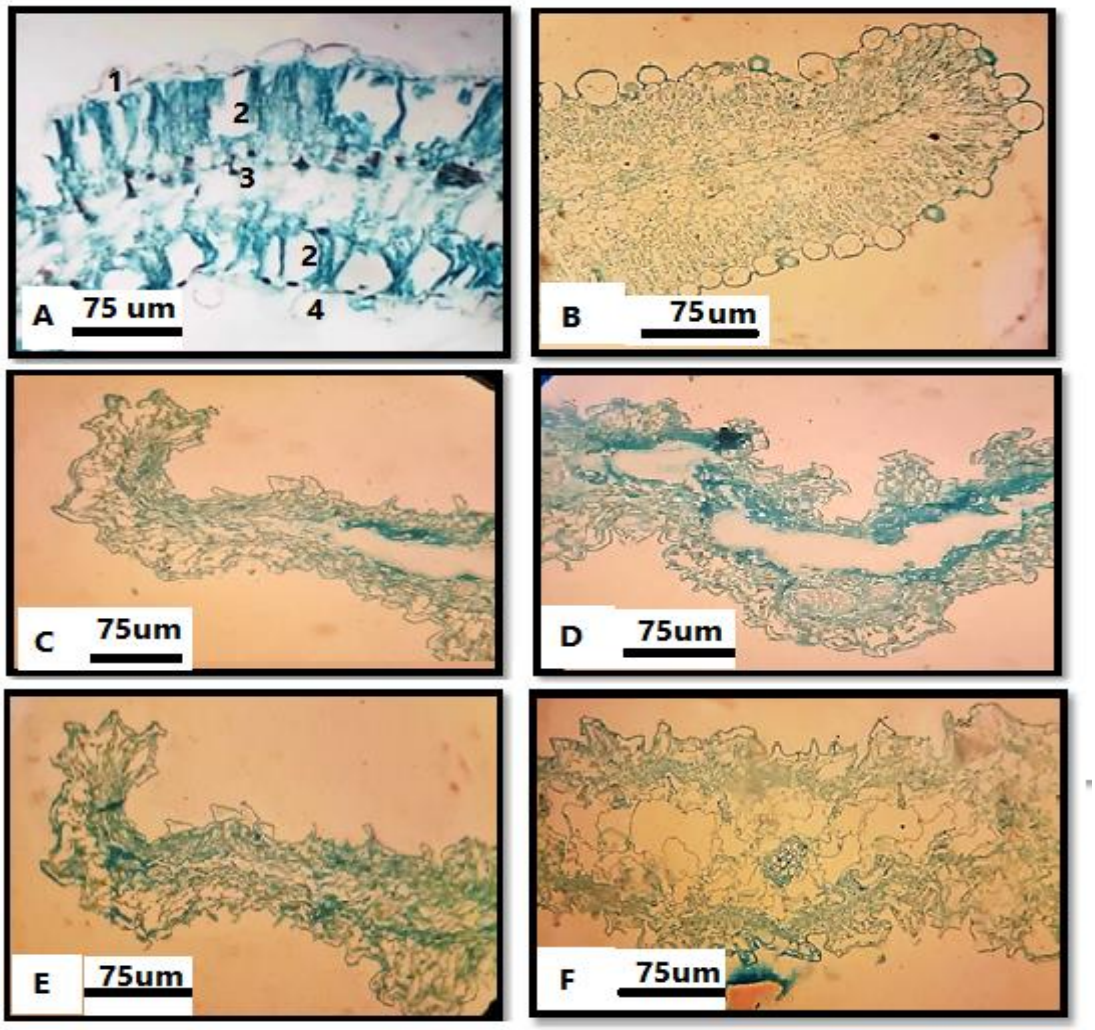

Fig. 1. Cross section of leaf blade of *S. portulacastrum*. (A-B) Control treatment; (C) Leaf exposure to Ag treatment; (D) midrib exposure to Ag treatment (E) Leaf exposure to Pb treatment; (F) Leaf of interaction between Ag and Pb.

Transverse sections of stems

Anatomical analyses of stem of *S. portulacastrum* exposed to elements showed several alterations in the stem compared with control treatment (Table 5; Fig. 2). Stems were undergone changes in shape, size, thickness and number of cortical parenchymal cells (Fig. 2). Transverse section of stem of *S. portulacastrum* in control treatment displayed that the stem is semicircular in shape. However, in Pb treatment was rectangle shaped, while it was irregular and more sinuate in Ag treatment. Stem thickness was 1210.23 µm in control group, while it was 850.43 µm and 910.20 µm in the Ag and Pb treatments. Plant exposed showed reduced in stem thickness (Table 5). Lower and upper epidermal cells were square-shaped or rectangular, uniseriate in control treatment, treated stems the epidermis was increased in size and irregular in shape. Thickness of epidermis were 20.33 and 21.66 µm respectively. The Ag and Pb treated plants observed alterations in stem structure compared to the control group (Table 5; Fig. 2). Many authors reported that the alteration in the plant shape and cells shape due to the ability of elements disrupt the hormonal balance (Gomes *et al.* 2011). In addition, some metals including Ag and Pb can acuminated in cell walls and intercellular space (Gomes *et al.* 2011; Al-Saadi *et al.* 2013). The cross-sectional view of *S. portulacastrum* stem (Fig. 2) showed reduction in conducting elements of the xylem and phloem as a result of heavy metal, which is not in agreement with Gupta *et al.* (2011). The thickness of vascular bundles reached 83.33 µm in Ag and 95.50 µm in Pb treatments compared to control plants (125.33 µm; Table 5). Pith in control group was found in the centre of stem created parenchymatous cells isodiametric or circular, thin layer cells with intercellular spaces. Pith diameters were between 500.16 µm in control and 750.22 µm in the Ag and 620.52 in Pb treatments (Table 5; Fig. 2).

Table 5. Measurement of stem tissues of *S. portulacastrum* in micro-meter (µm).

Treatment	Stem thickness	Epidermis thickness	Chlorenchyma thickness	Xylem thickness	Phloem thickness	Vascular bundle thickness	Pith thickness
Control	(1250-850) 1210.23	(22.30-10.25) 16.23	(18. -100.50) 150.21	(100.5-50.25) 80.69	(45.25-30.25) 35.91	(150.2-100.50) 125.33	(650-350) 500.16
Ag	(930-550) 850.43	(25.25-15.25) 20.33	(150.25-100) 121.67	(75.25-45.25) 60.43	(45.25-16.22) 23.23	(100.50-52.50) 83.33	(810-650) 750.22
Pb	(1110-650) 910.20	(25.25-16.25) 21.66	(65.5-25.2) 50.24	(10.50-52.50) 75.55	(25.25-14.50) 20.51	(125.12-75.35) 95.50	(750-450) 620.52

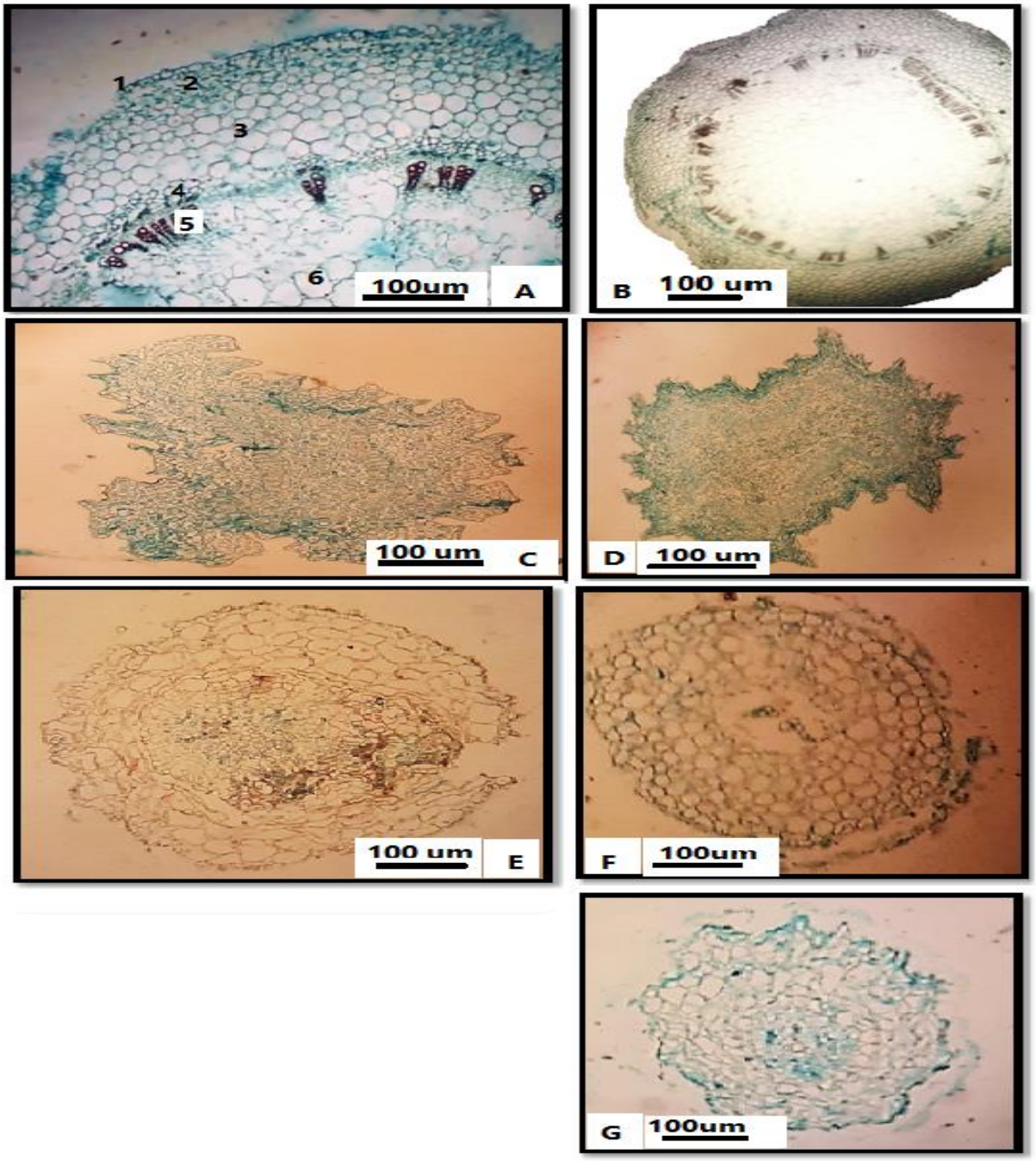

Fig. 2. Cross section of stem and root of *S. portulacastrum*. (A-B) Stem of control group; (C) Stem of exposure to Ag treatment; (D) Stem of exposure to Pb treatment; (E) Root of control group; (F) root of exposure to Ag treatment; (G) root in exposures to Ag and Pb treatments.

Anatomical root alterations in *S. portulacastrum*

Anatomical alterations between control group and the exposures to Ag and Pb were noticed (Table 6; Fig. 2). The *S. portulacastrum* roots showed several alterations in shape, size, and arrangement of cortical cells. The transverse section of roots exhibited uniseriate of upper and lower epidermal layers. It was square-shaped or rectangular. Its thickness was 24.44 µm, while in the roots exposed to the Ag and Pb decreased in thickness to reach 15.11 µm and 12.33 µm in Ag and Pb treatments respectively (Table 6). The results showed that reduction in root thickness because of acuminated of elements in the *S. portulacastrum* roots treated with Ag and Pb resulted in destroy some layers of epidermis and cortex. The root shape displayed more sinuate and the reduced root thickness in addition to the destroy or decreased vascular bundles. The results also showed unrecognized endodermis, and exodermis. It was reduced in the plants exposed to metals. The root thickness was 663.21 µm in plant of control group, while in Ag and Pb treatments were 498.32 µm and 375.61 µm respectively (Table 6). Cortex is composed of many (6-10) layers in control group, while in the Pb and Ag, most of layers of cortex were destroyed. However, the entrance between Ag and Pb showed minor alterations in shape of cells and number of layers of cortex (Table 6; Fig. 2). These alterations in the internal structure of tissues are due to the ability of motels to interrupt the hormonal balance of *S. portulacastrum*, or due to toxic effect of the elements, and that the metals can inhibit photosynthesis (Kabatapendias 2011; Bini *et al.* 2012). The effects of contamination on exodermis and endodermis are as a result of the movement of elements (Ederli *et al.* 2004; Wójcik *et al.* 2005; Najeeb 2017).

Table 6. Measurement of root tissues in *S. portulacastrum*in micrometer (µm).

Treatment	Root diameter	Epidermis thickness	Cortex thickness	Vascular bundle thickness	Xylem thickness	Number of xylem
Control	(800- 550.23) 663.21	(33.41-18.75) 24.44	(400-290.11) 340.32	(230.45- 150.45) 195.22	(58.4-58.33) 45.80	
Ag	(630.21-250.50) 498.32	(19.65- 10.11) 15.11	(250.25-35.32) 210.33	(140.3—105.25) 120.21	(40.22-25.50) 34.01	
Pb	(450.21-250.32) 375.61	(15.21-7.33) 12.33	(200.8-110.50) 175.10	(130.25-85.50) 100.50	(40.20-25.50) 32.45	

Mineralogical composition of leaves samples under heavy metal:

The results of *S. portulacastrum* leaves treated with 100 ppm Pb and Ag and control are shown in Table 4 and Figs. 1-4. The main mineral in the control contains 11 elements including carbon (57.50%) followed by oxygen (22.76%), iron, lead, and silver. The minerals in the leaves treated with 100 mg L^{-1} Pb showed 12 elements. The highest was lead (52.03%) followed by carbon (35.03%) and oxygen (5.47%). The sample lost iron and silver. The leaves treated with 100 ppm Ag, exhibited 11 elements. The highest was silver (43.02%) followed by carbon (34.95%) and oxygen (11.18%). The sample lost potassium, iron and lead. Interactions between Pb and Ag showed 13 elements. The leaves lost fluorine, while contained Pb (25.09%), Ag (16.57%) and iron (0.67%). The control samples without heavy metals (Pb and Ag) have no toxic substances. The plant was safe and contained C, O, K, Mg, S, Na, Ca, Cl and Si. Si maintained moisture in the leaves, however, the Si level increased upon interaction with Ag and Pb. It was 2.80 %, however, a high amount of silica can cause cancer. Pb toxicity inhibits the plant growth and development (Cobbett 2000). High concentrations of Pb causes toxicity to the plants including inhibition of enzyme activity and changes in physiological responses such as the membrane permeability imbalance and mineral nutrition distortion (Sharma & Dubey 2005). EDX with SEM gives information on chemical content including Ag and Pb. SEM can explore the distributions of elements in plant tissues (Patra *et al.* 2004).

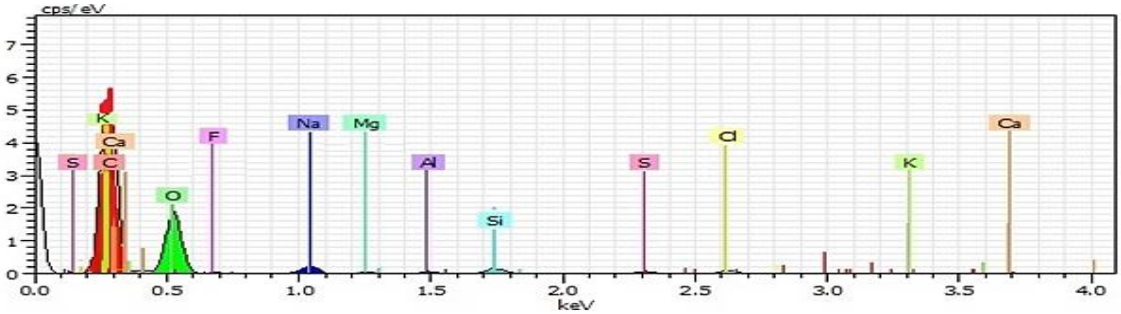

Fig. 3. EDS spectra of *S. portulacastrum* leaf control (100 mg L^{-1}).

Table 7. The Elemental analysis concentration in the *S. portulacastrum* leaves (control and treated with Ag and Pb by SEM-EDS methods.

Number of elements	Element	Control	Pb	Ag	Pb + Ag
1	Carbon	57.50	35.03	34.95	32.81
2	Oxygen	22.76	5.47	11.18	15.86
3	Sodium	3.36	1.64	0.43	1.25
4	Calcium	3.07	1.26	0.31	1.72
5	Fluorine	2.02	0.85	0.34	0
6	Sulphur	3.88	0.65	0.22	0.74
7	Magnesium	1.03	0.42	0.05	0.55
8	Aluminium	1.05	0.44	0.08	0.74
9	Silicon	1.77	1.45	0.22	2.80
10	Chlorine	2.04	1.13	0.19	0.60
11	Silver	0	0	43.02	16.57
12	Potassium	0.94	0.01	0	0.02
13	Iron	0	0	0	0.67
14	Lead	0	52.03	0	25.09

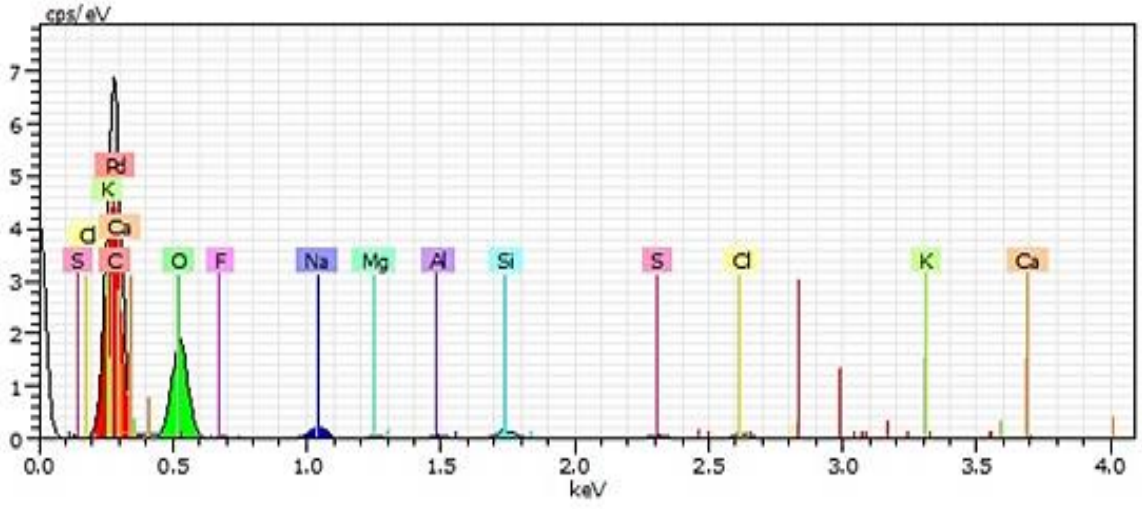

Fig. 4. EDS spectra of *S. portulacastrum* leaf treated with Pb (100 mg L^{-1}).

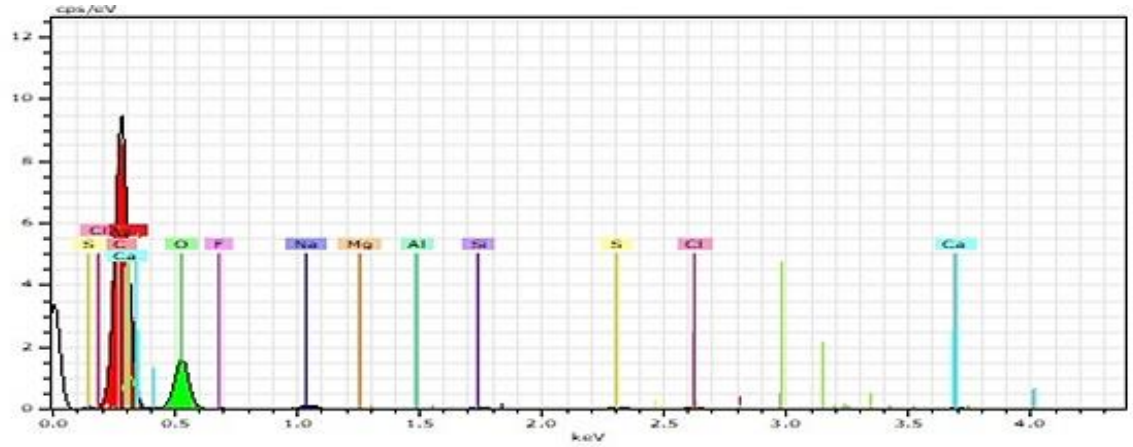

Fig. 5. EDS spectra of *Sesuvium portulacastrum* leaf treated with Ag (100 mg L^{-1}).

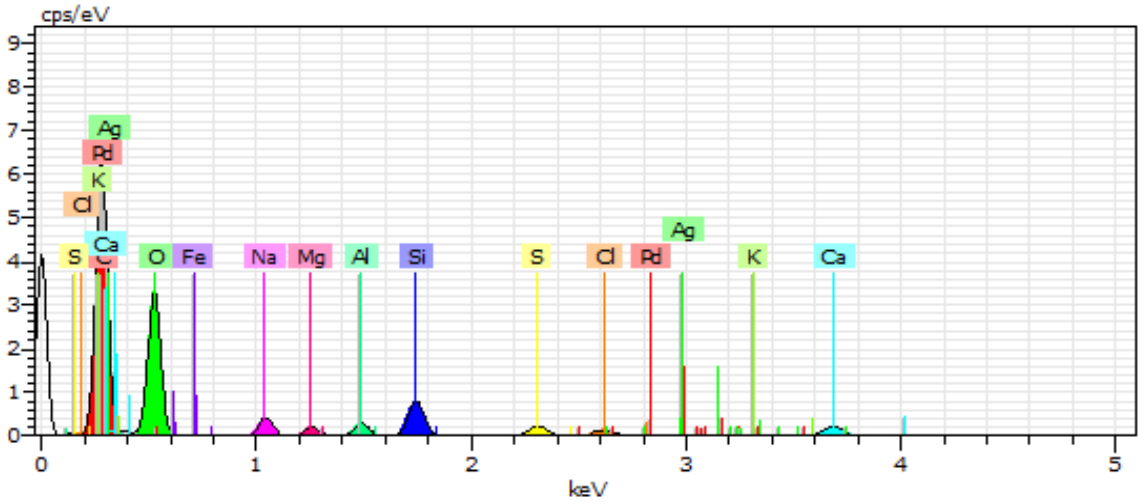

Fig. 6. EDS spectra of *S. portulacastrum* leaf represented interaction between Ag and Pb (100 mg L^{-1}).

CONCLUSION

EDS/SEM technology offers a new method for analyses of heavy metal composition in tissues and organs of plant. Twelve essential elements were detected by EDS including O, C, K, S, Mg, Cl, K. Fe, and Ca. Other non-essential elements were also observed such as Si, Pb, Ag and Al (Table 4). This technology can have broad applications to biological sciences (Qi *et al.* 2003). The presence of Pb and Ag in leaves indicated the heavy metal uptake via roots from polluted places. They were then accumulated in the leaves and stem. Our results indicated that *S. portulacastrum*is is suitable for remediation or phytoextraction. It has a strong tolerance and accumulation capacity for Pb and Ag. Anatomical study indicated that Ag and Pb can accumulate in internal tissues and causes several alterations such as shape of leaves, stems and roots, thickness and number of cortical parenchymal cells.

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