



## Effect of herbicide (Pyroxsulam) on Nickle, Cadmium, Lead, and total Flavonoid levels of two Iraqi Wheat species

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### Abstract

**Background:** Since 2008, Pyroxsulam was registered by Dow Agro Sciences as efficient broad-spectrum herbicide for wheat control. Wheat is an important plant involved in the daily meal of human and it is a rich source for benefit contents. **Aim:** The present project aimed to study the effect of Pyroxsulam herbicide on the levels of some parameters in Iraqi wheat. **Materials and Methods:** We investigated the concentration of three metals (Ni, Cd, and Pb) and flavonoid in seeds of wheat plants collected from two Iraqi cultivates (Iba 99 and Abu Ghraib 3). Halve of the planted wheat was sprayed with Pyroxsulam herbicide (S group) while the other group was considered as a control group (C group). **Results:** The results showed that the levels of Ni (an essential cofactor of eight enzymes) and the antioxidant parameter (total flavonoids) were negatively affected as a result of treatment with Pyroxsulam. This effect was clearly apparent from the significant drop in most data of these elements, the same result was indicated for the (Cd, and Pb) concentrations which they passed to the soil. By comparison, Iba 99 cultivate is more crop with using the herbicide than Abu Ghraib 3 cultivate. **Conclusion:** According to the above results, wheat products after treatment with Pyroxsulam would have less in some antioxidant related contents and it would be useful to design an alternative dose or solution of this herbicide to get healthy products of wheat with high yield.

**Keywords:** Cadmium, Flavonoid, Lead, Nickle, Pyroxsulam, Wheat

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### INTRODUCTION

Pyroxsulam (N-(5,7-dimethoxy [1,2,4] triazolo[1,5-a] pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl) pyridine-3-sulfonamide) is a member of the triazolopyrimidine sulphonamides (Garcia 2012). It is a herbicide which is used in controlling many narrow and broad leaf weeds cereals such as wheat at early post-emergence in winter and spring (European Food Safety Authority 2013). It is considered to certain aquatic plant as toxic and algae organisms on an acute (single and high dose) basis. It is practically nontoxic to birds, fish, honeybees, earthworms, and aquatic vertebrates on an acute basis (Pyroxsulam 2008, Kadhum 2018; Mukumbaet al, 2016). For decades, wheat (*Triticum aestivum* L.) and cereal grains have been represented as an essential source for food worldwide. They are characterized as a food with high nutrient content that is rich in fat, carbohydrates, antioxidant, and other high energy compounds, such as Phenols and flavonoids as well as trace elements (Baublis 2000, Li 2015, Xu 2017). Antioxidants play important role in the protection against reactive oxygen species (ROS) that are usually

produced as a result of cell metabolism regulation (Fantke 2011). Also, these antioxidants action as anti-aging, anti-atherosclerosis, anti-inflammatory and anticancer have also been proved by several scholars (Tungmunnithum 2018, AL-haily 2018, Ali 2016). Nickel ions are enzyme cofactors including urease, nickel superoxide dismutase [NiFe]-hydrogenase, acetyl-CoA synthase, coenzyme M reduction, carbon monoxide dehydrogenase, lactate racemase, and nickel utilizing glyoxylase I (Shanying 2012) which have important roles in organisms from all kingdoms of life such as antioxidant activity of enzyme; this activity is important to avoid oxidative stress by fighting oxidants like reactive oxygen species (ROS). The biology of Ni involves many components in addition of the enzymes, such as different types of membrane transporters, metallochaperones, and regulators, which are important for preserving and distributing healthy levels of Ni (Ware 2013). Lead (Pb), is heaviest non-radioactive metals, it

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**Table 1.** Concentrations of Nickel, Cadmium, and Lead for both treated and untreated wheat (Abu Ghraib3 and Iba99 cultivars) with the pesticides

Cultivar	Treatment	Concentration of elements ( $\mu\text{g/ml}$ )		
		Pb	Cd	Ni
Abu Ghraib 3	C5	11.53 $\pm$ 0.20 <sup>A</sup>	2.40 $\pm$ 0.20 <sup>A</sup>	11.08 $\pm$ 0.25 <sup>A</sup>
	C90	8.73 $\pm$ 0.15 <sup>B</sup>	1.56 $\pm$ 0.15 <sup>B</sup>	10.76 $\pm$ 0.11 <sup>A</sup>
	S5	11.13 $\pm$ 0.23 <sup>A</sup>	2.23 $\pm$ 0.15 <sup>A</sup>	10.09 $\pm$ 0.15 <sup>B</sup>
	S90	7.46 $\pm$ 0.15 <sup>C</sup>	0.92 $\pm$ 0.01 <sup>C</sup>	5.16 $\pm$ 0.15 <sup>C</sup>
Iba 99	C5	10.10 $\pm$ 0.10 <sup>A</sup>	2.30 $\pm$ 0.10 <sup>A</sup>	8.56 $\pm$ 0.11 <sup>A</sup>
	C90	9.10 $\pm$ 0.10 <sup>B</sup>	2.20 $\pm$ 0.10 <sup>A</sup>	9.70 $\pm$ 0.10 <sup>B</sup>
	S5	7.53 $\pm$ 0.05 <sup>C</sup>	1.80 $\pm$ 0.10 <sup>B</sup>	6.80 $\pm$ 0.10 <sup>C</sup>
	S90	7.46 $\pm$ 0.15 <sup>C</sup>	1.10 $\pm$ 0.10 <sup>C</sup>	3.56 $\pm$ 0.15 <sup>D</sup>

C5= Wheat without spraying the herbicide collected at 5 days. S5 = Wheat after spraying the herbicide collected at 5 days. C90= Wheat seeds without spraying the herbicide collected at 90 days. S90= Wheat seeds after spraying the herbicide collected at 90 days. Differences in letters (A,B,C,D,E, and F) are significant ( $p < 0.05$ ) to comparison columns.

is the most abundant heavy metal contaminant that is released into the environment from diverse anthropogenic activities and induces serious harm to growth and productivity in plants, a wide range of adverse effects are observed on various physiological processes, including activation and inactivation of the enzymes, disturbed mineral nutrition, change in the hormonal status, alterations in the membrane permeability, water imbalance, and disruption of oxidative metabolism (Pourrut 2011, He 218). Cadmium (Cd) is a heavy metal that is considered as a toxic metal for environment, plant soils, and human, Cd enters the body, it is stored in the kidneys and liver, and then slowly secreted in urine (Marwat 2008, Das 2014, Caverzan 2019). Treatment by herbicides has been highly recommended as one of the main solutions to overcome the challenge of weeds growth, at the same time the use of herbicide can negatively affect wheat quality. Therefore, several scholars focused on the study of wheat content (nutrients and antioxidants) after exposure to herbicides. In this study the three elements and flavonoids were investigated in two Iraqi wheat species, Abu-Ghraib-3(AG) and Iba-99 (Iba), with and without treating with Pyroxsulam herbicide.

## PLANT SAMPLES AND METHODS

Two cultivars of Iraqi wheat (Abu-Ghraib-3, and Iba-99) seeds were planted in 4 lines [4 groups: sample group (sprayed with the herbicide) = S group, and Control group (without spraying) = C group]. After the wheat weeded at two to three leaves and reached jointing stage, 2 of the lines (sample groups; S group) were sprayed with the herbicide (125 mL/donum). Wheat plant of the 2 groups were randomly collected at 5, and 90 days after spraying the herbicide. The samples were dried and then stored in the dark (at  $-20^{\circ}\text{C}$ ) until further analysis. Digestion and analytical procedures A were used for Ni, Cd, and Pb concentration using Aflame Atomic Absorption Spectrometry (Bermudeza 2011). Content levels of flavonoids was evaluated according to Zhishen et al. (1999). Comparison Mean  $\pm$  SD of evaluated parameters for the studied groups was performed by ANOVA test using SPSS program (version 21).

## RESULTS

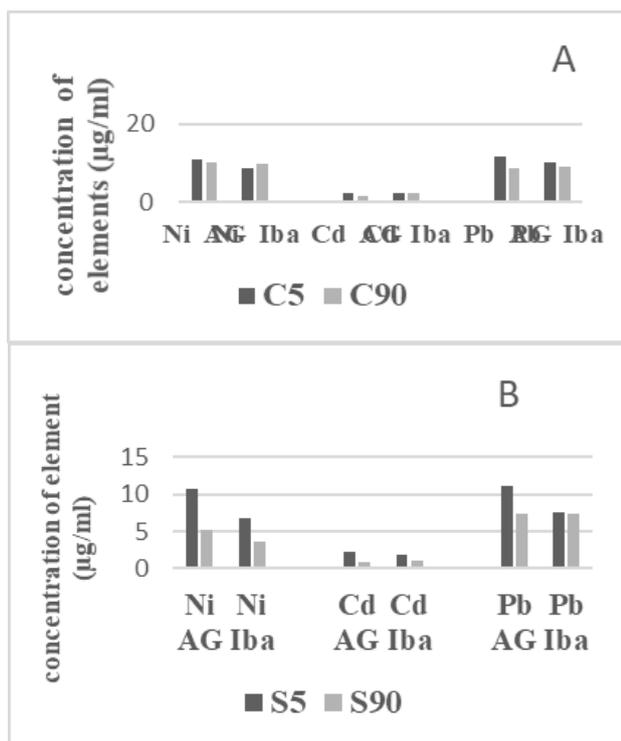
Pyroxsulam was chosen as one of common herbicides that are usually used to improve wheat grains cultivation. In this study, two types of Iraqi wheat Iba and AG-3 were treated with Pyroxsulam herbicides. This was followed by determination of Ni, Cd, Pb, and flavonoids levels after 5 days as well as 90 days and compared to untreated wheat (controls), see **Table 1** for results.

This research shows that levels of Ni in control seeds herbicide after 5 and 90 days of growing (C5, C90) had been higher than their levels in the treated samples with Pyroxsulam herbicides (S5, S90) in both types (AG and Iba). When it comes to Cd levels, non-significant difference was found in C5 group when compared with S5, while C90 > S90 showed significant decrease ( $P < 0.05$ ) for AG type. Meanwhile, for Iba type there were significant decreases ( $P < 0.05$ ) between (S5, S90) when compared with (C5, C90). For Pb levels, the result C5 was non-significant in comparison with S5 for AG type, in contrast, the result was significantly decreased ( $P < 0.05$ ) for Iba type, whereas C90 > S90 for AG and Iba types.

**Fig. 1** represents the comparison of (Ni, Cd, and Pb) levels between the two types of Iraqi wheat. Overall results for controls and treated samples indicated that the concentrations of the Ni, and Pb elements for AG type were higher than that of Iba type, whilst the Cd concentrations were approximate between both types.

Similarly, this comparison was carried out on total flavonoids levels. In **Table 2**, it is observed that they increased significantly with the increase of the growing time (90 days > 5 days) in controls and treated samples for both types.

Also, the total flavonoids contents for controls were higher than that of treated samples for AG and Iba types; the C90 of Iba type was the highest, as shown in **Fig. 2**.



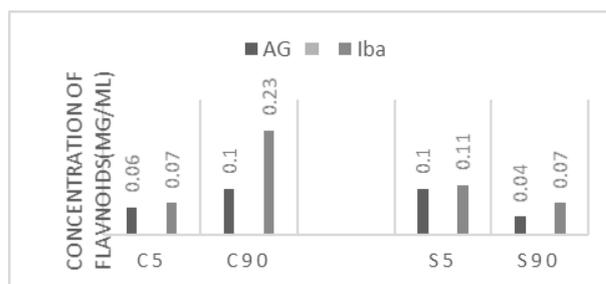
**Fig. 1.** Three studied elements levels of AG-3 type compared to Iba type for: A) untreated wheat (controls), and B) treated wheat with Pyroxulam herbicide cultivated after 5, and 90 days

**Table 2.** Concentrations of flavonoids for both treated and untreated wheat (Abu Ghraib3 and Iba99 cultivars) with the pesticides

Cultivar	Treatment	Flavonoids (mg/ml)
Abu Ghraib 3	C5	0.06 ± 0.01 <sup>A</sup>
	C90	0.10 ± 0.02 <sup>B</sup>
	S5	0.10 ± 0.03 <sup>B</sup>
	S90	0.04 ± 0.01 <sup>C</sup>
Iba 99	C5	0.07 ± 0.02 <sup>A</sup>
	C90	0.23 ± 0.03 <sup>B</sup>
	S5	0.11 ± 0.01 <sup>C</sup>
	S90	0.07 ± 0.01 <sup>A</sup>

## DISCUSSION

Due to the importance of results for wheat cultivation in Iraq, this study was designed to investigate the effect of using a selective winter and spring wheat herbicide (Pyroxulam) on an enzyme cofactor (Ni) and antioxidant compounds (flavonoids) in association with the contaminated heavy metals (Cd, and Pb). Under stress or pathological conditions, the ROS production increased in quantity that makes the antioxidant activity inefficient to scavenge ROS and this phenomenon is known as oxidative stress. Therefore, cells are regularly kept the ROS and antioxidants in an equilibrium status (Hussain 2012, Birben 2012). Nickel cofactor enzymes is redox active through the common oxidation states (Ni<sup>+1</sup>, Ni<sup>+2</sup>, and Ni<sup>+3</sup>), so, it can be used for challenging redox reactions in enzymes, like Ni superoxide



**Fig. 2.** Flavonoids concentrations of AG-3 type compared to Iba type for untreated wheat (controls), and treated wheat with Pyroxulam herbicide cultivated after 5, and 90 days

dismutase (the antioxidant enzymes, also, the nickel ion can act as a Lewis acid; during reactions, it stabilized anionic intermediates as well as the transition states (Per 2019). Our results indicated that Ni decreased by using the pesticide because (see **Table 1**). In this case, the cell lost its ability to detoxification of ROS and consequently ROS attacked cell contents such as DNA, proteins, lipids, and cell membrane. This mechanism leads to cell death through enhancing of cell apoptosis and stimulates wide range of damage (Pallavi 2012, Ali 2017). The other part of this study demonstrated that the treatment of wheat with Pyroxulam herbicides can cause a dramatic drop in the flavonoids (see **Table 2**) of most samples which were measured through this study. It is well-known that there is an inverse relationship between oxidants and antioxidants. Therefore, we suggest that when exposed to Pyroxulam herbicides, wheat plant undergoes stress and stimulates the oxidants production which is negatively affecting the antioxidant levels. Reduction in wheat production by weeds may reach to 80% depending on many factors such as weed type and density, wheat cultivar and density, timing of emergence, and environment and soil (Mhamdi 2018, Kaushik 2014). Heavy metal pollution (HMP) involving health risks have attracted global and research attention, especially their accumulation in soil-plant systems which compete the wheat in cultivation and this effects wheat production and quality (Zendeabad 2014). Cadmium can help the activities of antioxidant enzymes which act as electron donors that reacts with ROS to yield unharmed product, possibly by modulation of gene expression. But high metal loading may cause the antioxidants to be incapable of providing enough protection against oxidants. However, Cd stress causes a rapid change in plant physiology, its accumulation causes a growing problem for foodstuff and also for the environment; it has a negative influence on humans. Nonetheless, supplying Fe participates in the reduction of Cadmium toxicity in redox reaction pathways in mung bean plants (Krishnav 2019). Likewise, Malonaldehyde and antioxidant enzyme contents were believed to be a good indicator in determining Cd tolerance (Payandeh 2018, Muradoglu 2018). Statistical relationships between soil properties

and Cd, and Pb concentration in plant organs have already been studied (Baize 2009). The heavy metals produced adverse effect on population health and were especially hazardous for children (Xinpeng 2012). Our results indicated a decrease in the Cd, and Pb concentrations in treated samples when compared with control groups, that maybe because they were fixed in soil; Pb inhibited wheat root growth through an ROS-mediated oxidative damage caused ultrastructural modification in cell membrane, and also disruption of nuclear and mitochondrial integrity (Kaur 2013, Liu 2008).

## CONCLUSION

It can be concluded that using Pyroxsulam herbicides can improve the wheat cultivation and reduce the effect of weeds on wheat quality and production, yet, Pyroxsulam herbicides can also negatively affect the antioxidant contents of wheat. Consequently, the wheat products will lose their healthy value. Therefore, this study recommends finding alternative solutions to control weeds effect on wheat cultivation. It also suggests doing further study in order to overcome the difficulties that are associated with using Pyroxsulam herbicides.

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