The effect of soil properties and plant species on the absorption of heavy metals in industrial sewage contaminated soil: A case study of Eshtehard Industrial Park

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In recent years, the rapid development of industries in developing countries has led to the excessive contamination of the soil in these areas. In Eshtehard Industrial Park, Iran, untreated industrial and domestic sewage containing heavy metals has entered the soil along the northern edges of the area. The present study has sought to measure the contamination of this particular area of soil with heavy metals including Cr, Zn, Cd, Pb and Ni, normally present in untreated sewage. The study also measured the absorption rate of heavy metals by local plants. The physicochemical properties of the soil were also examined through using standard methods. The results of the Kruskal-Wallis test showed that the species of the plant and its particular organs have an effect on the absorption of heavy metals. The Pearson test was used to assess the correlation between the physicochemical properties of the soil and the absorption of its heavy metal content by plants. The present study examined identified *Alhagi pseudalhagi* (M.B.) Desf., *Salvia aristata Aucher* ex Benth, *Stipa barbata* Desf., *Salsola kali* and *Peganum harmala* L., which have been examined for phytoremediation, for the first time, and identified them as hyperaccumulators according to the EPA definition. Planting the seeds of hyperaccumulating plants and adding DTPA to the soil helps absorb the heavy metal contaminants of the soil and constitutes a measure that can be used to purify the soil; the plants used can then be harvested, collected and disposed of as regular just like industrial sewage.

Keywords: Soil properties, phytoremediation, heavy metals, hyperaccumulating plants, Eshtehard Industrial Park

INTRODUCTION

Sanitary and industrial sewage have become massive sources of soil and water contamination in developing countries and the presence of heavy metals in raw industrial sewage constitutes a major health problem. Heavy metals are nonbiodegradable types of metal that cause dangerous diseases. Through the proper treatment and careful observation of sewer standards [1], sanitary and industrial sewage can be used for agricultural irrigation, and the resulting sewage sludge [2] containing organic compounds and nutrients required for plants can be used as an inexpensive fertilizer if treated by standard criteria. The sanitary and industrial sewage exiting Eshtehard Industrial Park is currently contaminating the soil in the northern edges of the area, caused by miscalculations in the treatment plant's capacity given the number of industrial units built in the park and also the plant's frequent break-downs. This untreated sewage contains heavy metals originated from various industrial processes such as plating, and is discharged into the soil in the northern part of the industrial park. The present study was conducted to investigate the contamination of soil with heavy metals including Cr, Zn, Cd, Pb and Ni. According to the standards set bv Kabata-Pendias and Pendias, soil contamination is considered critical when the concentration of heavy metals in the soil falls in these ranges: 75 to 100 mg kg⁻¹ for Chromium, 70 to 400 for Zinc, 3 to 8 for Cadmium, 100 to 400 for Lead and 100 for Nickel; any concentration below considered these ranges is normal [3]. Phytoremediation is a bioremediation technique used for the phytoextraction and remediation of contaminated soil. This method is inexpensive and can be used on a large scale. Phytoaccumulation is one of the techniques used for phytoremediation, in which the removal of heavy metals from the soil depends on the plants' natural metal absorption capacity. Using hyperaccumulating plants therefore provides the best option available for the removal of heavy metals from the soil.

According to the EPA definition, а hyperaccumulating plant is a plant that accumulates metals in its shoot and root at the following total concentrations: 100 mg kg⁻¹ for Cadmium, 1000 mg kg⁻¹ for Chromium and Lead and over 10,000 mg kg⁻¹ for Zinc and Nickel [4]. The present study examines hyperaccumulating plants that can accumulate Cr, Zn, Cd, Pb and Ni. Ziarati used the hyperaccumulating plant Amaranthus sp. for the phytoremediation of soils contaminated with the heavy metals present in Tehran's municipal sewage [5]. The phytoremediation of heavy metal contaminated soils should be carried out with a full knowledge of the complex effects of plant-soil parameters. An important condition for the storage and accumulation of metal in a plant is to increase the metal's available concentration for the plant. In other words, a soluble form of the metal should remain near the root membrane of the plant in the soil for a set period of time. In geographical terms, factors affecting the solubility of the metal and its available form in the soil are vastly different and include the concentration and chemical form of the metal present in the soil, the physicochemical and biological properties of the soil (including pH, EC, OC, CCE, available phosphorus and soil texture) and additives such as DTPA. Hung conducted a study in Taiwan to examine the effects of the physicochemical properties of soil and the addition of DTPA on the absorption of heavy metals by plants [6]. Among the plants examined, some hyperaccumulators were discovered for the first time, and others were found to absorb high concentrations of heavy metals due to the changes made in the physicochemical and biological properties of the soil caused by its contamination with untreated sewage and were thus identified as hyperaccumulators for the first time.

EXPERIMENTAL

Study Site: The present study was conducted in areas contaminated with untreated sewage in Eshtehard Industrial Park, located at the longitude of $35^{\circ} 42' 18''$ N and the latitude of $50^{\circ} 18' 22''$ E and the mean altitude of 1200 m above sea level with a relatively dry climate and an average annual rainfall of 220 mm [7].

Sampling: Samples were collected between 2011 and 2013 during the period from early June to mid-September from mature plants in the contaminated areas and the control areas by the full removal of their shoot and root from the soil. Given the sparseness of vegetation in the contaminated 212

areas, sampling was conducted using systematic random sampling, i.e. without a frame and merely through observation [8]. The plant samples were then transferred to the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran, where they were identified by name. Soil samples were collected separately from each plant's sampling area from the 0 to 20 cm depth of the rhizosphere soil. The soil samples were packed separately in polyethylene bags, and after being numbered and having their location and date detailed, they were sent to the environmental laboratory of Islamic Azad University, Science and Research Branch, Tehran, Iran.

Measuring Cr, Zn, Cd, Pb and Ni concentrations in the samples:

The researchers used the acid digestion method to determine the overall concentration of heavy metals in the soil samples [9]. They poured two grams of each soil sample into an Erlenmeyer flask with stopper and then added in 15 ml of 4 normal nitric acid. They then placed the flasks in a water bath at 80 °C for 12 hours. After passing the samples through a Whatman filter paper 42, the researchers recorded the samples' heavy metal concentrations using a Varian Spectraa 200 atomic absorption spectrophotometer made in Australia.

To measure the soil samples' concentrations of plant-available heavy metals, the researchers used Lindsay & Norval's [10] method with a DTPA extractor. To prepare the DTPA solution (Diethylene Triamine Penta-acetic Acid), they weighed 0.005 mol of DTPA salt (1.96 grams) and transferred it to a 1-liter flask containing 950 ml of distilled water. They then placed the flask on a shaker for the salt to dissolve, and then added in 1.4 grams of dehydrated calcium chloride and 13.3 ml of Triamine N(CH2CH2OH)₃, and raised the volume to 1000 ml by adding in distilled water and carefully stirring it. They then added. 20ml of the produced extract to 2 grams of the soil sample and placed it for 2 hours on a shaker at 20-25 °C for 2 hours. They filtered the soil extract through a Whatman filter paper 42 and read the heavy metal concentrations using a Varian Spectraa 200 spectrophotometer.

To determine the exchangeable concentrations, the researchers used the Tessier extraction method [11]. They transferred 2 grams of each soil sample to an Erlenmeyer flask with stopper and added in 20 ml of 1 mol ammonium acetate (pH=7) and then placed the flask on a shaker at 20-25 °C for 30 minutes. They then filtered the soil extract through a Whatman filter paper 42 and used a Varian Spectraa 200 spectrophotometer to read the heavy metal concentrations.

The researchers then carefully examined the physicochemical properties of the soils, including their pH, EC, OC, CCE, available phosphorus and soil texture, using standard methods [12].

To determine the total concentration of heavy metals in the plant samples, the researchers used the Dry Ash extraction method [13]. They transferred 2 grams of each plant sample to a crucible and placed it in an oven at 550 °C for 2 hours. They then added 5 ml of hydrochloric acid 2N to the samples and passed them through a filter paper. They used an atomic absorption spectrophotometer to read the heavy metal concentrations.

RESULTS AND DISCUSSION

The identified plants are presented in Table 1. As seen in Table 1, the plants are coded.

The physicochemical properties of the soils were measured over 3 years in the contaminated

 Table 1. The name of identified plants

and control areas. Table 2 presents the mean values obtained for these properties in the sewage contaminated area.

As shown by table 2, the entry of sewage has changed the physicochemical properties of soil in the contaminated area compared to in the control area.

The concentration of heavy metals in the soil was measured in the contaminated and control areas. The control area was selected based on the physicochemical similarities of its soil to the contaminated soil (table 2).

Figure 1 shows the total, exchangeable, and extractable concentrations of heavy metals using DTPA extractor with a mean of 3 replicates in the area contaminated with sewage. Furthermore, to facilitate locating the sampled soil, each area was coded with the number of the plant grown in that area (as in Table 1). For example, soil No.1 was sampled from the area that *Launaea acanthodes* (Boiss.) O.Kuntze grew.

Plant	Plant species	Plant	Plant species	Plant	Plant species	
No	r funt species	No	i fuit species	No	Thank species	
1	<i>Launaea acanthodes</i> (Boiss.) O.Kuntze	10	Salsola kali	19	Bromus tectorum L.	
2	Thevenotia persica DC	11	Ceratocarpus arenarius L.	20	<i>Phragmites australis</i> (Cav) Trin. ex Steud	
3	Carthamus oxyacantha M.B	12	Salsola persica Bunge ex Boiss.	21	<i>Echinochloa crus-galli</i> (L.) P.Beauv.	
4	Artemisia herba-alba Asso.	13	Alhagi persarum Boiss. & Buhse	22	Hordeum vulgare L.	
5	Amaranthus graecizans L.	14	Alhagi pseudalhagi (M.B.) Desf.	23	Stipa barbata Desf.	
6	Nonnea persica Boiss.	15	Astragalus aureus Wild	24	Papaver piptostigma Bienert ex Fedde	
7	Salsola nitraria Pall.	16	Alhagi camelerum Fisch.	25	Polygonum patulum M.B.	
8	Atriplex aucheri Moq.	17	<i>Erodium Cicutarrium</i> (Jusl) L'Her.exaition	26	Pteropyrum aucheri Jaub. & Spach	
9	Kochia scoparia (L.) Shrad	18	<i>Salvia aristata</i> Aucher ex Benth	27	Peganum harmala L.	

Table 2. The mean and standard deviation of	the physicochemical	properties of the sewage	contaminated soils
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The name of Area	Clay	Sand	Silt	Soil texture	pН	EC	OC	CCE	Available phosphorus
	%	%	%			dS m ⁻¹	%	%	mg kg-1
Contaminated area	18.7±6.6	44.4±3.4	36.8±9.9	Sandy Clay Loam and Loam	6.5±0.2	1.8±0.6	0.6±0.1	5.7±1.2	24.3±4.2
Control area	19.8±7.1	45±2.5	35.1±10.1	Sandy Clay Loam and Loam	6.9±0.1	0.6±0.2	0.3±0.1	7.9±1.3	16.3±4.3



Fig. 1. The mean of concentrations of Cr (A), Zn (B), Cd (D), Pb (Pb) and Ni (Ni) in 3 replicates of soil samples contaminated with raw sewage (mg kg⁻¹ of soil).





Fig. 2. Min of metal concentration of Cr (A), Zn (B), Cd (C), Pb (D) and Ni (E) in plants (shoots + roots) collected from raw sewage polluted area of Eshtehard industrial park (three replicates).

As shown in Figure 1, a decreasing trend can be observed for the total, DTPA-extractable and exchangeable concentrations of Cr, Zn, Cd, Pb and Ni. Some soil samples contained higher concentrations of heavy metals. The concentration of heavy metals was sampled and measured for all the plants in 3 replicates.

Figure 2 shows the total heavy metal concentration values in the shoot and root for identifying hyperaccumulating plants based on the EPA definition. For an easy identification of the minimum hyperaccumulation threshold in the plants based on the EPA definition, a horizontal line has been drawn on the diagrams.

As shown in figure 2, some of the plants absorb heavy metals beyond the minimum hyperaccumulation threshold.

The significance of the effect of the plant species and plant organs on the absorption of heavy metals was analyzed in SPSS using the Kruskal-Wallis test and is presented in tables 3 and 4.

As shown in tables 3 and 4, the correlations are significant.

The correlation between the physicochemical properties of the soil and the plants' heavy metal absorption rates in the study site was analyzed in SPSS using the Pearson correlation test and is shown in table 5. **Table 3.** Kruskal-Wallis test results showing the effect of the plant species on the absorption of heavy metals in the sewage contaminated area.

	Heavy metal concentration in the plant									
	Cr Zn Cd Pb N									
Chi-Square	48.30*	46.05*	48.72*	47.33*	49.24*					
df	26	26	26	26	26					
Asymp. Sig	0.00	0.00	0.00	0.00	0.00					

*Differences of 0.05 and less considered significant

Table 4. Kruskal-Wallis results test showing the effect of the plant organs on the absorption of heavy metals in the sewage contaminated area

	Heavy motel concentration in the plant									
	C	'n	7	neavy ille		nuanon n 'd	i ule pian	l Ph	N	li
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Chi- Square	48.21*	48.01*	46.01*	35.13*	48.43*	48.81*	47.21*	47.34*	49.18*	48.20*
df	26	26	26	26	26	26	26	26	26	26
Asymp. Sig	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Differences of 0.05 and less considered significant

Table 5. The Pearson correlation coefficients between the concentrations of heavy metals absorbed by the plants examined and the physicochemical properties of the sewage contaminated soil

Hearry				physico	chemical	properties	of the soil			
metal concentrati	Clay	Silt	Sand	Availabl e Phospho rus	OC	рН	EC	CCE	Heavy metal concentration	
on in the plant									Total	DTPA Extractabl e
Cr	ns	+0.61**	ns	+0.58**	+0.71*	Ns	ns	- 0.58*	ns	+0.44*
Zn	-0.51*	+0.66**	ns	+0.49*	Ns	- 0.63**	ns	ns	+0.79**	+0.55**
Cd	ns	ns	ns	ns	-0.41*	-0.44*	+0.50**	-0.49*	ns	+0.64 **
Pb	-0.55*	ns	-0.78**	ns	Ns	-0.59**	+0.60**	-0.42*	+0.61**	ns
Ni	ns	+0.59**	-0.71**	+0.68**	+0.89**	Ns	ns	-0.58*	ns	ns

* Correlation at the 0.05 level; ** Correlation at the 0.01 level; NS No correlation

As shown in table 5, the correlation between the physicochemical properties of the soil and the concentration of heavy metals can be either positive or negative, and NS indicates cases in which there are no significant correlations.

Table 2 shows that raw sewage has reduced the soil pH in the contaminated area. Heavy metal concentrations have also increased with the raw sewage, resulting in higher soil magnetism and consequently a higher electrical conductivity. Hung [6] found similar results in his study. As raw sewage increases, so does organic carbon (OC), which is an organic source of plant-available phosphorus. Calcium Carbonate Equivalent (CCE) indicates the soil's calcareous properties; the soil in the contaminated area was already slightly calcareous, but this property further increased with the entry of sewage. There was a slightly higher amount of silt in the contaminated area compared to in the control area due to the entry of sewage

sludge, which resulted in better conditions for plant growth.

As shown in Figure 1 and based on the Kabata-Pendias standards, the total concentration of heavy metals in the area was found to fall in the critical range, indicating the contamination of the soil. The control area was not contaminated, indicating that the entry of sewage has been fact the source of contamination. Figure 1 shows both the concentration of heavy metals that can be absorbed with a DTPA extractor by the plants and the naturally-exchangeable concentration of heavy metals and can thus be of use for the assessment of the correlation between them. As suggested by the Figure, the concentration of heavy metals that can be absorbed with a DTPA extractor by the plants is higher than the exchangeable concentration of heavy metals. DTPA can therefore make greater amounts of heavy metals available to the plants.

Figure 2 shows the heavy metal absorption rate of the whole plant (the shoot and root) separately for each plant examined. According to the EPA definition, heavy metal hyperaccumulating plants include:

Alhagi persarum Boiss. & Buhse absorbed 3418.23 mg kg⁻¹ of lead and *Pteropyrum Aucheri* Jaub. & Spach accumulated 11585.56 mg kg⁻¹ of zinc and 3408.47 mg kg⁻¹ of Lead and were therefore considered hyperaccumulators of these two metals. Ghaderian et al. [14] found similar results for Alhagi persarum Boiss. & Buhse; however, despite its high levels of zinc and lead accumulation, Pteropyrum Aucheri Jaub. & Spach was not considered a hyperaccumulating plant in this study. Alhagi pseudalhagi (M.B.) Desf. and Salvia aristata Aucher ex Benth absorbed 2959.01 mg kg⁻¹ and 1376.6 mg kg⁻¹ of lead and were therefore considered hyperaccumulators. Salsola kali absorbed 6948.97 mg kg-1 of Chromium and 1651.4 mg kg⁻¹ of lead and was also considered a hyperaccumulator of these two metals. No studies have yet been conducted on phytoremediation in these three plants. Astragalus aureus Wild absorbed 7343.68 mg kg⁻¹ of lead and is considered a hyperaccumulating plant. Salari et al. [15] did not find this plant to be a hyperaccumulator despite its high accumulation of metals. Accumulating 1676.4 mg kg⁻¹ of lead, Bromus Tectorum L. was also found to be a hyperaccumulator. Parsadoost et al. also found similar results [16]. Phragmites australis (Cav) Trin. Ex Steud absorbed 140.23 mg kg⁻¹ of cadmium and 3703.3 mg kg⁻¹ of lead and is thus a hyperaccumulator of these two metals. Al-Taisan and Deng studied this plant's cadmium and lead absorption, respectively however, despite their high levels of metal accumulation, the plants were not considered hyperaccumulators [17. 18]. Echinochloa crus-galli (L.) P. Beauv absorbed 158.21 mg kg⁻¹ of cadmium through all of its organs and is therefore considered а hyperaccumulating plant. Messou also proposed this plant to be a hyperaccumulator of cadmium [19]. Hordeum vulgare L. accumulated 1032.37 mg kg⁻¹ of Chromium, 18628.83 mg kg⁻¹ of zinc, 325.83 mg kg⁻¹ of cadmium and 3930.7 mg kg⁻¹ of lead and is therefore considered a hyperaccumulator of these metals [20]. Stipa barbata Desf. absorbed 3316.4 mg kg⁻¹ of lead and is also a hyperaccumulating plant. Lorestani et al. [21] discovered the manganese stabilization of this plant, but the present study showed its lead hyperaccumulation capacity for the first time. Peganum harmala L. absorbed 10948.67 mg kg⁻¹ of nickel and 1274.37 mg kg-1 of lead and is identified as a hyperaccumulator of these two metals. Zamani *et al.* achieved similar results for lead, but no studies have yet been conducted to investigate Nickel accumulation [22].

Tables 3 and 4 and the Kruskal-Wallis test results, showing the significant effect of plant species and plant organs on the absorption of heavy metals in sewage contaminated areas, support the present study's hypothesis regarding plant species' and plant organs effects on the absorption of heavy metals.

Table 5 presents the study findings on the correlation between the physicochemical properties of the soil and the concentration of heavy metals in the plants and their underlying reasons.

A significant negative correlation was observed between the soil pH and the concentration of zinc, cadmium and lead in the plants, which is due to the sewage's reduction of the soil pH to about 6.5, turning insoluble metals to soluble ones and leading to their greater absorption by the plants. Ziarati and Alaedini achieved similar results in his study [5].

There was a significant positive correlation between soil EC and the concentration of cadmium and lead. The increased EC is due to the increased heavy metal concentrations caused by the entry of sewage into the area, resulting in a higher electrical conductivity in the soil. The increased heavy metal concentrations in the soil lead to an increased heavy metal concentration absorbed by the plants. Hung also found similar results [6].

A significant positive correlation was observed between organic matters in the soil and Chromium and nickel concentrations in plants; however, the correlation between organic matters in the soil and cadmium concentrations was found to be significant and negative. The reason for the negative correlation might be that organic matters present in soils have a potential for stabilizing heavy metals in an unavailable concentration, and the positive correlation is perhaps because organic matters can act as a chelate, increasing the usability and mobility of trace elements. Furthermore, the ligand bond formed between the metal and the organic matter affects its availability, as the ligand bond is capable of easily turning into an available form through the organic acids excreted by the plant roots and thus be absorbed by the plant. According to Nakatsu *et al.* the ligand may become available to the plants through certain soil-borne microorganism activities [23].

A significant positive correlation was found between the available phosphorus in the soil and Chromium, zinc and nickel concentrations in the plants. The positive correlation was due to the high

CONCLUSION

amounts of phosphorus present in sewage (phosphorus acts as a fertilizer) and the solubility of phosphorus due to the soil's reduced pH caused by the entry of sewage and therefore the increased availability of soluble phosphorus in the soil. Rato Nunes *et al.* achieved similar results in his study [24].

A significant negative correlation was found between Calcium Carbonate Equivalent (CCE) in the soil and Chromium, cadmium, lead and nickel concentrations in the plants. The negative correlation was because lime (CaCO₃) is a soil mineral that can produce a mechanism for metal absorption and retention through the chemical absorption of heavy metals and depositing metal cations and can then reduce the activity of the heavy metals' soluble form, thus making the metals unavailable to the plants. Mozafari *et al.* emphasized the negative effect of lime on the absorption of cadmium by plants [25].

There was a significant positive correlation between the total concentration of metal in the soil and zinc and lead concentrations in plants. A significant positive correlation was also found between DTPA-extractable metals concentrations and Chromium, zinc and cadmium concentrations in plants. Sharma *et al.* also achieved similar results [26].

A significant negative correlation was found between the concentration of clay in the soil and zinc and lead concentrations in plants. A significant negative correlation was also observed between the concentration of sand in the soil and lead and nickel concentrations in plants. A significant positive correlation was observed between the concentration of silt in the soil and Chromium, zinc and nickel concentrations in plants. The negative correlation between the clay concentration and plants is due to the surface absorption of heavy metals by finegrained clay, which reduces the amount of heavy metals available to the roots of the plants. Although sand is larger-grained and has no surface absorbability, and although, in their soluble form, metals can freely be absorbed by plants, there is a negative correlation between sand concentrations and plants, as the presence of greater concentrations of sand in the soil creates a larger pore space in the soil and allows the metals to be easily washed away from the plant roots. The positive correlation between silt concentrations and plants is because silt improves the soil, resulting in better plant growth and increased heavy metal concentrations in the plants. Raj et al. achieved similar results about the effect of soil texture on the absorption of heavy metals [27].

Based on the Kabata-Pendias standards, the entry of raw sewage into the soil examined in the present study has contaminated it and reduced its pH and CCE and increased the soil its EC, OC, available phosphorus and silt. The results of the Kruskal-Wallis test demonstrated that plant species and plant organs affect the absorption of heavy metals. The Pearson correlation test was then used to assess the correlation between the physicochemical properties of soil and the absorption of heavy metals by the plants. The plantabsorption of some metals was found to have increased due to the entry of raw sewage into the soil. Alhagi pseudalhagi (M.B.) Desf., Salvia aristata Aucher ex Benth, Stipa barbata Desf. (Pb), Salsola kali (Pb and Cr), and Peganum harmala L. (Ni), studied for the first time for their phytoremediation capacity, were identified as hyperaccumulators based on the EPA definition. As already found by other researchers, Alhagi persarum Boiss. & Buhse, Bromus Tectorum L. and Peganum harmala L. (Pb), Echinochloa crusgalli (L.) P. Beauv. (Cd) and Hordeum vulgare L. (Cr, Zn, Cd and Pb) were also identified as hyperaccumulators. Compared to the findings of other studies, Pteropyrum Aucheri Jaub. & Spach (Zn and Pb), Astragalus aureus Wild (Pb) and Phragmites australis (Cav) Trin. Ex Steud (Cd and *Pb*) absorbed higher concentrations of heavy metals in the present study due to the high concentrations of environmental pollutants in the area examined and the changes in the physicochemical properties of its soil caused by the entry of raw sewage and are therefore deemed by this study to be hyperaccumulators of these metals. Planting the seeds of these hyperaccumulating plants and the addition of DTPA therefore comprises a suitable strategy for purifying soils through plants' absorption of heavy metals, and the plants themselves can then be harvested, collected and disposed of as regular industrial waste.

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