Vertical Metal Distributions in Brownfield Soils Drive Enzyme Activities and Plant Success

Eshariah N. Dyson
Montclair State University

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Abstract

The distribution of contaminants in polluted soils can vary through depth. We measured the vertical distributions of heavy metals and enzyme activities in the top 10 cm of side-by-side, vegetated and barren brownfield soils. The data showed metals significantly accumulated in the top 2 cm of barren soil and uniformly distributed through vegetated soil. When planted, germination rate was significantly lower in the previously barren soil, with the metal layer intact, compared to mixed barren soil. These data suggest a feedback loop in which plant roots redistribute metals, diluting metal-rich regions, yet germination remains initially limited by a metal cap. We conclude that not only metal concentrations but also their vertical distributions through the top 10 cm of brownfield soil impact soil health and plant success. Published studies on vertical distribution of enzyme activities and metal concentrations have treated the top 10-20 cm as a single cross-section and thus would have missed a thin (< 2 cm thick) metal cap on the surface of the soil. If metals have accumulated on the soil surface, then changing the initial environmental assessment to focus on the top 2 cm can more effectively guide efforts to convert industrial barrens to vegetated environments.

Keywords: brownfield soil, vertical metal distribution, enzyme activities, peroxidase, soil depth, germination, post-industrial soil

Synopsis: We discovered a concentrated <2 cm metal cap on the surface of an industrial barren. Such metal caps may hinder germination at other post-industrial sites.
Vertical metal distributions in brownfield soils drive enzyme activities and plant success

by

Eshariah N. Dyson

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by
Eshariah N. Dyson
Montclair State University
Montclair, NJ
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1. Introduction

Industrial barrens are contaminated, extreme environments that have little to no plant growth due to human activity.¹ These sites are affected by metal and organic contaminants and often remain abandoned for dozens of years. Over time, these areas may adapt to the contaminants and begin to re-green themselves. Industrial barrens present a unique opportunity to study the mechanisms by which contamination impacts soil properties and plant germination and growth. Metal contaminants, which are often present in industrial barrens, can reduce the microbial functioning and primary productivity of brownfield soils.² The presence of metals in the soil can also alter the microbial community structure, and if present in high concentrations, be toxic to plant life.³⁻⁵ The effects of heavy metals may depend not only on their concentrations, but also on how these metals are distributed within a soil.

The relationship between metal concentration and soil depth has been investigated.⁶⁻⁷ These studies typically focus on depths spanning from the soil surface to about 150 cm below the surface and provide valuable information about metal distributions deeper in the soil.⁸⁻⁹ However, a significant fraction of microbial activity, including the decomposition and mineralization of nutrients, occurs in the top 10 cm due in part to accumulation of organic matter, moisture, and soil micro-food-web activity. Prior research studying soil parameters through depth has divided soil into large cross-sections that are greater than 10 cm. Much remains to be learned by evaluating, at a finer level of resolution, the properties of thinner vertical cross-sections in the top 10 cm of the soil, the scale at which most microbial activity is relevant.¹⁰

Liberty State Park (LSP) is an un-remediated brownfield in Jersey City in northern New Jersey, USA. Since its industrial railyard abandonment in the 1960’s, for unknown reasons one site within the park remained an industrial barren while the adjacent site took a trajectory that
resulted in a lush forest. These two sites have similar types of contaminants therefore, the presence of the contaminants alone does not explain why only one of the sites remained barren. Here we studied the relationship between metal concentrations and soil depth within the top 10 cm of the soil collected from the side-by-side vegetated and barren LSP sites. The soil was divided into 2 cm deep vertical cross-sections. The field study design allowed us to compare the distributions of metal concentrations between vegetated and barren side-by-side soils. We also measured the vertical distribution of phosphatase activity, a soil enzyme related to nutrient cycling. Moreover, we investigated germination and primary production of the barren soil in a controlled laboratory study. Here we report results that inform why vegetation does not always spread to all parts of an urban brownfield even decades after abandonment and what factors impact the re-greening process.

2. Methods

2.1 Study site and soil preparation

LSP began as a railyard through which trains transported goods and people between the mid-Atlantic and New York City primarily in the 19th Century. Post abandonment, most of the site was remediated into the urban park present today, but a 100-ha site was closed off from the public allowing for naturally occurring ecological succession. Within the closed off section of the park are sites 25F and 25R (Fig. 1A) which have high heavy metal concentrations that exceed clean up criteria and background metal concentrations at a comparable, forested New Jersey site. A barren site (25R) at LSP has high heavy metal concentrations and poor enzymatic function. Yet these high concentrations have not explained why this site is barren because an
adjacent site (25F) is vegetated with high enzymatic function and with qualitatively similar organic and inorganic contaminant loads.\textsuperscript{11,13}

This study used soils collected from both 25F and 25R in LSP. Three 5 cm diameter soil cores, ten meters apart from one another, were removed from each of the two sites using a 5 cm wide stainless-steel slam bar corer. Each core was divided through depth into five 2 cm cross-sections (0-2 cm, 2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, Fig. 1B). Each cross-section was separated then passed through a 2 mm sieve, bagged, and stored in a refrigerator at 4 °C.
Figure 1. (A) Aerial view of closed off LSP study area with vegetated site (25F) and barren site (25R) shown. Photo credit by Mike Peters/Montclair State University. (B) A photo of a soil core is shown with a cartoon of how the core was divided into cross sections. Experimentally determined soil properties are listed.
2.2 Inorganic elements

To determine metal concentrations of each cross-section we followed the protocol outlined by Hagmann and others (2015). Soil samples (0.5 g) were dried (~100 °C) for 24 hours, ground to a fine powder, then placed in a 50 mL digestion tube. The soils were digested following EPA method 3050B. Briefly, 2.5 ml of a 1:1 nitric acid solution was added to the digestion tube and the sample was refluxed for 15 minutes at 95 °C. After cooling, concentrated nitric acid (5 mL) was added, and the solution refluxed for an additional 30 minutes at 95 °C. While refluxing, the solution was monitored for the formation of brown fumes. Once the fumes stopped forming, the solution was maintained at the same temperature (95 °C) until the volume reduced to 2.5 mL. Cooled solutions were then filtered (1 µm virgin polypropylene DigiFilter, SCP Science, Quebec, Canada) to remove any remaining soil solids, diluted, and analyzed for metal concentrations on an inductively coupled plasma mass spectrometer (ICP-MS, Thermo Fisher Scientific, Bremen, Germany). The metals measured for each soil site were: Pb, Zn, Ni, As, Co, Ba, Cu, and V. A standard curve was determined for each metal in an aqueous dilution from a stock solution.

2.3 Phosphatase

To measure phosphatase activity, 4-methylumbelliferyl-phosphate was used as a substrate. Three substrate wells contained 350 µM of substrate. Soil (0.1 g) was mixed into MES buffer solution (100 mL, 0.1 M, pH = 6.1) and sonicated (Fisherbrand Model 505 Sonic Dismembrator, Fisher Scientific, Parsippany, New Jersey) at 30% amplitude for 3 minutes. Standard curves had 0, 500, 1300, 2000, 3500 pmol of product (4-methylumbelliferone) in four different wells. Phosphatase assays were run at 30.0 °C with an excitation wavelength of 320 nm.
and an emission wavelength of 450 nm. The reaction was run in a black bottom microtiter assay plate for 3 hours on a fluorometer (BioTek Synergy H1 Hybrid Multi-Mode reader, BioTek, Winooski, Vermont), with readings after every 7.5 minutes.

2.4 Peroxidase

To measure peroxidase activity, we followed published protocols by Bach and others (2013). Briefly, an acetate buffer (50 mM, pH 6.1) was prepared, using sodium acetate and glacial acetic acid to adjust the pH. A soil slurry was prepared by combining soil (1 g) with acetate buffer (125 mL). The soil slurries were sonicated at 25 % amplitude and constant pulse for 3 minutes. While the slurry was mixing continuously, aliquots of slurry (200 µL) were added to the wells in a clear 96-well microplate.

The substrate L-3,4-dihydroxyphenylalanine (L-DOPA) was dissolved in hot, deionized (DI) water at about 90 °C to make a 25 mM solution. In addition to the soil slurry, sample wells also contained 0.3% H₂O₂ (10 µL), and of 25 mM L-DOPA (50 µL). To correct for the substrate background, blank wells containing 0.3% H₂O₂ (10 µL), 50 mM acetate buffer (200 µL), and 25 mM L-DOPA (50 µL) were used. To correct for the sample background of each soil sample, negative control wells containing soil slurry (200 µL), 0.3% H₂O₂ (10 µL), 50 mM acetate buffer (50 µL) were used (Fig. S3). Absorbance values measured at 460 nm were obtained using a microplate reader running for 3 hours, with readings after every 7.5 minutes. In order to determine the peroxidase activity, the average absorbance values of the blank and the negative control were subtracted from the average absorbance value of each soil sample and divided by the extinction coefficient of 7.9 mM⁻¹cm⁻¹.
2.5 Plant Growth

To determine the extent to which the metal layer in barren site 25R affects the rate of germination, soil was collected from the site by hammering 2-inch wide polyvinyl chloride (PVC) columns about 15 cm into the ground then removing it intact, thus maintaining integrity of the layered structure. These became the “structured” soil samples. As a control, “mixed” soil samples were assembled by filling parallel PVC columns with stirred soil with no layered structure. Each pot received six premium winter rye grass seeds (*Lolium perenne*), a horticulturally available plant that is naturally available at the site and watered (5 mL, sterile tap water) three times a week for 6 weeks. Pots were kept in an incubator with controlled environmental conditions on a 10.5/13.5-hour day/night cycle. The relative humidity was 65% and the temperature was 24 °C for the day cycle and 16 °C for the night cycle. Photos were taken every week to monitor plant growth in the pots. At the end of six weeks, plant shoots were harvested and germination rates (shoots/seeds sown), shoot heights (cm), dried shoot masses (g) and dried root masses (g), phosphatase activity, and peroxidase activity were determined.

2.6 Data Analysis

Data for soils collected from vegetated site 25F were analyzed separately from data for barren site 25R soils. An analysis of variance (ANOVA) was conducted between cross-sections to compare enzymatic activity, and concentrations of inorganic elements with a significance cut-off value of p < 0.05. Where significant effects were found, we conducted a post-hoc test (Tukey’s HSD). A paired t-test was done to compare the means of the germination rates, shoot heights, shoot masses, and root masses of plants potted in mixed and structured barren 25R soil. An analysis of variance (ANOVA) was conducted to compare phosphatase and peroxidase
activity for mixed and structured barren 25R soil. All statistics were performed using R (version 3.6.2).

3. Results

3.1 Inorganic elements accumulate on the barren soil surface

Metal concentrations in the cores from vegetated site 25F increased with depth from the surface cross-section to a maximum value in the 4-6 cm cross-section and then decreased again (Fig. 2). These differences were not statistically significant, except for V ($F_{4,10} = 5.422$, $p < 0.05$), with the highest metal concentrations in the middle cross-sections. In contrast, at barren site 25R, the concentrations of all eight metals (Pb, Zn, Ni, As, Co, Ba, Cu, V) were significantly higher in the top cross-section (0-2 cm) compared to deeper cross-sections, indicating that metals accumulated on the soil surface (Fig. 2). For example, Pb concentration was significantly higher in the top 0-2 cm cross-section ($16,200 \pm 5,575$ ppm) compared to the bottom cross-section ($301 \pm 114$ ppm) ($F_{4,10} = 7.397$, $p < 0.05$). Fe oxides, potentially originating from historical railyard activity, may have contributed to the formation of a metal cap on the soil surface because other metals can be strongly adsorbed to Fe oxides.\textsuperscript{1,15,16}
Figure 2. Pb, Zn, Ni, As, Co, Ba, Cu, and V concentrations (ppm) in five soil cross-sections from vegetated site 25F (left) and barren site 25R (right). The bars represent averages of triplicate measurements (n=3); standard errors are shown. Note that the x-axis value ranges differ among metals. Means denoted by different letters indicate significant differences among the cross-sections (Tukey’s Test, p < 0.05). Data was collected by Dr. Xiaona Li and Diane Hagmann (Montclair State University, Department of Earth and Environmental Science) and analyzed by me.

This study focused on the top 10 cm of soil where the root density is the highest if plants were present. We found a distinct layer of heavy metals on the top of barren site 25R where no plants grow (Fig. 2). It is surprising that after 60 years the metal cap is still present at the site and has not been diluted or redistributed. It cannot be discerned why the metal cap exists in the soil,
when the cap began to form, or how long it has been present. High metal concentrations can inhibit germination and the high concentration of metals in the top 0-2 cm cross-section of barren site 25R could be the reason why we see no plant growth at this site. Conversely, our data showed a uniform distribution across the cross-sections of vegetated site 25F (Fig. 2). Plant roots can also translocate metals, impacting their concentrations and distributions in the soil. The presence of roots in 25F soil could possibly help facilitate the mixing of the metals into the soil matrix.

3.2 Phosphatase and Peroxidase activity along depth

The top 0-2 cm cross-section of the vegetated site 25F had the highest extracellular phosphatase activity (4.6 ± 0.7 µmol/(hr*dry soil)) (Fig. 3A), followed by a decrease in the deeper cross-sections. Phosphatase activity in vegetated site 25F varied significantly with depth. In the barren site 25R phosphatase activity was lower compared to the vegetated site 25F (Fig. 3B). In the 0-2 cm cross-section of barren site 25R soil the phosphatase activity was low (0.40 ± 0.06 µmol/(hr*dry soil)) and there was not a significant variation of activity with depth (Fig. 3B). Accumulation of leaf litter and subsequent organic inputs at the surface of the soil may have contributed to the high enzyme activities in the top 0-2 cm cross-sections of vegetated 25F soil. Previous studies have also revealed a decrease in enzyme activities with depth. These studies generally examined enzymatic function at larger depths of up to a meter rather than resolving finer scale differences in the top 10 cm of the soil profile.
Figure 3. Extracellular soil phosphatase (A, B) and peroxidase activity (C, D) are shown for five vertical 2 cm soil cross-sections from vegetated (25F, left) and barren (25R, right) sites. All enzymatic activities are reported as the mean in µmol/(hr*g dry soil), n = 3, and standard errors are shown. Means denoted by different letters indicate significant differences between cross-sections (Tukey’s Test, p < 0.05). Phosphatase activity was obtained and analyzed by Cesar Idrovo (Montclair State University, Department of Chemistry and Biochemistry).

Vegetated 25F soil cross-sections had significantly different peroxidase activities (F_{4,10} = 4.612 p < 0.05) (Fig 3C). Starting from the soil surface, peroxidase activity increased with depth to a maximum value (34 ± 3.2 µmol/(hr*g dry)) in the 4-6 cm cross-section followed by a decrease for deeper cross-sections (Fig. 3C). The metal concentrations followed a similar trend among the vegetated 25F cross-sections (Fig. 2 (left side) and Fig. 3C). Spearman correlation analyses show
a significant positive relationship between peroxidase activity and concentrations of Cu (r = 0.45, p < 0.1), As (r = 0.44, p < 0.1), and V (r = 0.54, p < 0.1) in soil 25F cross-sections. Other studies have demonstrated a positive relationship between soil peroxidase activity and metal concentrations. Increased peroxidase activity may be a general plant response to the uptake of toxic metals, for example, peroxidase induction was found to increase with Zn and Cd concentrations in soils. Peroxidase enzymes produced by plants can be exuded into the soil and increased peroxidase activity can be a protective response to oxidative stress, for example, cellular damage caused by active forms of oxygen including hydrogen peroxide.

Unlike the phosphatase activity which is involved in nutrient mineralization, we detected substantial peroxidase activities in barren 25R soil cross-sections (Fig. 3D), for example, the peroxidase activity was 9.5 ± 4.2 µmol/(hr*g<sub>dry soil</sub>) in the 8-10 cm cross-section, which was higher than the phosphatase activity in all cross sections measured at this site. Peroxidase activity from barren site 25R did not vary significantly with soil depth. Although both soils are contaminated with heavy metals, the presence of roots such as in 25F soil, primes the soil such that the capacity of microorganisms to metabolically recycle nutrients or mitigate contaminant stress may be increased.13, 27

3.3 Plant growth in mixed and structured 25R soils

To understand the impacts of the metal layer accumulated on the surface of barren 25R soil, we set up 16 “structured” and 13 “mixed” soils in PVC columns. The “structured” columns were created by hammering empty PVC columns into the soil and extracting them with the soil profiles intact. In contrast, the “mixed” columns were created by placing stirred soil into empty PVC columns. To gain a better understanding of the implications of the metal surface layer on
germination and primary production, seeds (premium winter rye, *Lolium perenne*) were planted in the columns containing structured and mixed 25R soils. Only three plants established across the 16 “structured” replicates while 12 plants grew across the 13 “mixed” replicates. Mixed soils had a significantly higher ($t = 2.753$, df = 15.17, $p < 0.05$) germination rate (17± 4.6%) than structured soils (3.1± 0.78%) (Fig. B). Mixed soils also produced taller shoots and a higher average root masses, though these differences were non-significant (Fig. 4A and 4C). These data together suggest that the high metal concentrations on the surface of barren site 25R inhibit germination. The presence of the “metal cap” and the increased germination in mixed 25R soil explain why across different experiments plants have grown in mixed 25R soils in the incubator even though site 25R is barren.\textsuperscript{13}

**Figure 4:** The shoot height (A), germination rate (B), shoot mass (C), and root mass (D) for structured and mixed PVC columns of 25R soil planted with winter rye seeds. Germination rate was significantly lower in structured compared to mixed PVC columns ($t = 2.753$, df = 15.17, $p < 0.05$). Star (*) indicates a significant difference ($p < 0.05$).
3.4 Enzyme Activity of mixed and structured 25R soils

The extracellular phosphatase and peroxidase activities of the soil from the structured and mixed pots were measured. There were significant differences in phosphatase activities of the soils treated with different conditions ($F_{1,23} = 27.98, p < 0.001$). The phosphatase activities were as follows: structured pots with plants ($0.601 \pm 0.03 \mu \text{mol/(hr*g}}_{\text{drysoil}}$), structured pots without plants ($0.47 \pm 0.04 \mu \text{mol/(hr*g}}_{\text{drysoil}}$), mixed pots with plants ($0.38 \pm 0.04 \mu \text{mol/(hr*g}}_{\text{drysoil}}$), and mixed pots without plants ($0.067 \pm 0.001 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) (Fig. 5A). The average phosphatase activity was found to be higher in pots that germinated a plant ($0.49 \pm 0.03 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) compared to pots that did not germinate a plant ($0.27 \pm 0.02 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) ($F_{1,23} = 16.75, p < 0.001$) (Fig. 5A), regardless of whether the pot contained mixed or structured soil. Vaidya et. al also found that phosphatase activity increased in previously barren soil when plants were grown.\(^{13}\) For the mixed pots alone, soils in pots with plants had a significantly higher mean phosphatase activity ($0.38 \pm 0.07 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) than soils in pots without plants ($0.06 \pm 0.001 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) The presence of plants in contaminated soil may have promoted soil function by increasing soil moisture, providing root exudates as nutrients, distributing heavy metals throughout the soil via roots, and/or altering metal toxicity.\(^{28}\)

Considering those pots that germinated a plant, average phosphatase activity was found to be higher in structured pots ($0.601 \pm 0.028 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) than in the mixed pots ($0.38 \pm 0.07 \mu \text{mol/(hr*g}}_{\text{drysoil}}$) but this difference was not found to be statistically significant. There were only three pots that were structured and germinated a plant and so the number of replicates was low. This is likely due to the concentrated metal load at the top of the structured pots, which may have prevented germination, as discussed in section 3.1. Of all the pots that did not germinate a
plant, the structured pots had a significantly higher phosphatase activity (0.47 ± 0.03 µmol/(hr*g<sub>drysoil</sub>)) than mixed pots (0.063 ± 0.001 µmol/(hr*g<sub>drysoil</sub>)) (Figure 5A). The metal layer in the top 0-2cm of 25R soil may have inhibited plant germination.

![Figure 5](image_url)

**Figure 5**: Phosphatase (A) and peroxidase (B) activity for structured and mixed pots with and without plants. For structured pots with plants n=3 and mixed pots with plants n=7. For
structured pots without plants n=13 and mixed pots without plants n=6. Error bars represent SEs. Stars indicate a significant difference (Tukey’s Test, (**) p < 0.01) and (*** p < 0.001).

Pots that germinated a plant had a significantly lower peroxidase activity (3.03 ± 0.6 µmol/(hr*g_{drysoil})) than pots that did not germinate a plant (24.8 ± 5.0 µmol/(hr*g_{drysoil})) (F_{1,22} = 13.57, p < 0.001), regardless of whether the pot contained mixed or structured soil. The presence of plants in the soil co-occurred with lower soil peroxidase activities and the changes in the soil due to the introduction of plants may have accounted for the lower peroxidase activity in pots that germinated a plant. The peroxidase enzyme in soils serves many different functions such as mitigating oxidative stress and detoxification of phenolics. As the plant and microbial communities of the soil change, so will the peroxidase activity in the soil.\textsuperscript{28}

Overall, there was not a significant difference in peroxidase activity between structured and mixed pots. For all pots that did not germinate a plant, the mean activity of the structured pots without plants (27.2 ± 4.5 µmol/(hr*g_{drysoil})) was similar to the activity of mixed pots without plants (22.4 ± 5.3 µmol/(hr*g_{drysoil})) (Figure 5B). The average peroxidase activity in the structured pots with plants (1.8 ± 0.5 µmol/(hr*g_{drysoil})) was slightly lower than the average peroxidase activity in the mixed pots with plants (4.2 ± 0.7 µmol/(hr*g_{drysoil})) (Fig. 5B). The difference in the peroxidase activity of structured and mixed pots with plants was not statistically significant. This suggests that for peroxidase activity in barren 25R soil, the presence or absence of plants plays a larger role on phosphatase activity than structured or mixed soil structure. When plants and soil interact with each other, complex feedbacks can occur.\textsuperscript{29} The introduction of plants into soil that is contaminated and previously barren causes changes in soil function. Plants
may take-up metals into their roots and shoots. Plants, soil, and contaminants have a dynamic relationship and changes in one factor can influence changes in the other factors.\textsuperscript{30}

To better understand the effect that plants have on the peroxidase activity of previously barren contaminated soil, a follow up experiment has been designed. Twelve pots will be kept in a controlled environment (incubator), six will contain seeds and six will not. The pots will be monitored for plant growth for two months and then soil samples will be collected every few weeks. The goal of this experiment is to obtain more knowledge about how plants affect peroxidase activity and how the activity differs between soil that has not been provided seeds and so that does germinate a plant. As a control, “clean” non-contaminated soil (12 pots, 6 with seeds and 6 without) will also be monitored. Results from these pots will be used to determine if the inhibitory effect of plants on peroxidase activity is unique to contaminated soil or if it can be seen in other soils as well.

4. Conclusion

Introducing vegetation to industrial barrens is desirable because roots can evenly distribute and thus dilute contaminants, emit exudates, and support an active rhizosphere microbial community, all factors that have been found to improve soil function.\textsuperscript{31} Moreover, the presence of plants can stabilize metals and reduce their migration to ground water.\textsuperscript{32} Planting will also lay the foundation for future phytoremediation efforts. In brownfield site investigations, while it is common to measure the concentrations of heavy metals in bulk soil samples, often the vertical distribution of metals is not determined. Here we have shown evidence that in brownfield sites metals can significantly accumulate on the surface of poorly performing brownfield soil. Moreover, the data presented indicate that this concentrated metal layer limits
plant germination. The introduction of plants into contaminated and previously barren soil can override the effects of the metals and effect soil function. We conclude that the vertical distribution through soil depth rather than overall concentration of metals most significantly impacts whether a contaminated site becomes barren or re-greens. To effectively manage industrial barrens, it is necessary to understand factors that prevent plant germination and primary production and how to mitigate them. The data suggest that focusing efforts on the assessment of contaminants in the top two cm of the soil could present a cost-effective option to enable germination and subsequent primary production and increased enzymatic function of such soils.
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